PROCEEDINGS OF THE 12TH ANNUAL SCRI ZEBRA CHIP REPORTING SESSION

F. Workneh, A. Rashed, and C.M. Rush Editors

PROCEEDINGS OF THE 12th ANNUAL 2012 ZEBRA CHIP REPORTING SESSION

F. Workneh, A. Rashed, and C. M. Rush Editors

San Antonio, TX

Oct. 30 – Nov. 2, 2012

PREFACE

Zebra chip of potato (ZC) was first documented from potato fields around Saltillo, Mexico in 1994, and in 2000 it was identified in South Texas. In the USA, the disease initially was considered a regional problem in South Texas, but by 2006 ZC had been identified from all potato production areas in Texas, and also in Arizona, California, Colorado, Kansas, Nebraska, Nevada, and New Mexico. Outside of the USA, ZC has been reported from Guatemala, Honduras, Mexico and New Zealand. Early studies of ZC were hampered by lack of knowledge concerning disease etiology, but in 2007, the potato psyllid, *Bactericera cockerelli*, was definitively associated with ZC and in 2008 two independent studies reported the association of *Candidatus* Liberibacter spp. with ZC. It now has been repeatedly demonstrated that transmission of *Candidatus* Liberibacter solanacearum by the potato psyllid results in diagnostic symptoms of ZC, while infestations by potato psyllids without *Candidatus* Liberibacter solanacearum do not cause ZC. However, questions still exist concerning the effect of pathogen and vector variability on disease severity.

Soon after ZC was first identified in South Texas, representatives from *Frito Lay*, approximately four farmers and two plant pathologists met to discuss how to deal with the new disease. Grower sponsored research projects were initiated the next year, and the same small group met again, after the 2001 harvest, and in an informal setting presented their findings and observations. This meeting constituted the first ZC reporting session. After the disease was identified in potato production regions outside of Texas, the National Potato Council and the US Potato Board recognized the potential danger of this new disease and begin to support additional research. In 2007, the Texas Legislature appropriated $2 million to support research on ZC and in 2009; a multistate, multidisciplinary group of scientists were awarded $6.9 million, from the Federal Specialty Crop Research Initiative (SCRI) Program, to study all aspects of ZC.

On October 30 – November 2, 2012, 135 scientists, farmers, and personnel from agri-industry and potato processing companies, representing five countries, attended the 12th Annual Zebra Chip Reporting Session. Each year, the goal of the meeting is to provide a forum to facilitate collaboration and multidisciplinary research on all aspect of ZC. Those who attend present research results on a wide variety of topics including pathogen detection, vector/pathogen diversity, epidemiology, pest management, breeding for resistance, economics, and disease risk assessment and forecasting. The high quality of information presented in an informal setting to a multidisciplinary group with common interests always makes for an enjoyable, professionally rewarding experience. This volume serves as a record of information presented at our most recent meeting and represents the first published Proceedings of the ZC Reporting Session. It is hoped that the information presented in this Proceedings will be useful to all those interested in ZC.

Charlie Rush
ZC SCRI Program Director

ACKNOWLEDGEMENTS

The publication of this Proceedings and the research reported herein was made possible through a Federal grant from the United States Department of Agriculture-National Institute of Food and Agriculture-Specialty Crop Research Initiative (USDA-NIFA-SCRI) Program, Grant #2009-51181-20176 and through the Texas Department of Agriculture.

The organizers of this meeting would like to express their gratitude to Ms. Patty Garrett for facilitating local arrangements for this meeting. We also would like to acknowledge Bayer Crop Science, Frito Lay, Syngenta, DuPont, Nichino America and Dow AgroSciences for covering expenses for the Welcome Reception and Hospitality events. Finally, we appreciate the assistance of Jerri Hamar, and the efforts of Kay Ledbetter and Donnie Parrack in recording interviews with all speakers and Advisory Board members for posting on the SCRI ZC Website.

ADVISORY BOARD

MODERATORS

Psyllid Monitoring and Sampling Strategies #1
Gerhard Bester – Session I

Post-Harvest Effects of ZC
Jessica Dohmen-Vereijssen – Session II

Vector Management
John Nordgaard – Session III

Poster Session
Open Viewing – Session IV

Psyllid Monitoring and Sampling Strategies #2
Don Henne – Session V

Potato Psyllid/ZC Ecology and Epidemiology
Arash Rashed – Session VI

Physiological Responses to Lso/Psyllid
Chris Wallis – Session VII

Pathogen Detection/Molecular Analysis
Blake Bextine – Session VIII

Genotype Development/Cultivar Resistance
Bob Harveson – Session IX

TABLE OF CONTENTS

Preface i
Acknowledgements ii
Moderators iii
Table of Contents iv
Authors Index 209

Psyllid Monitoring and Sampling Strategies #1

Overview of the 2011-2012 Potato Psyllid Areawide Monitoring Program
Henne, D., Aniciso, J., Bradshaw, J., Whipple, S., Carpio, L., Schuster, G., Richmond, J., Walker, S., Porter, P., Barrett, S., Willett, J., Mathews, J.M., Deroo, K., Seger, P., Crosslin, J., Hamlin, L., and Goolsby, J. 1

A Tale of 30,000 Psyllids; and What Did They Tell Us?
Crosslin, J.M., Henne, D.C. and Goolsby, J.A. 5

Monitoring of potato psyllids, *Candidatus* Liberibacter solanacearum, and zebra chip in Idaho during the 2012 growing season
Wenninger, E.J., Olsen, N., Thornton, M., Nolte, P., and Karasev, A. 10

Population Dynamics of Tomato and Potato Psyllid (*Bactericera cockerelli*) in New Zealand
Tran, L.T., Worner, S.P., Vereijssen, J., and Teulon, D.A.J. 13

Post-Harvest Effects of ZC

Impact of late season psyllid infestation on Potato Seed Quality
Rush, C., Workneh F., Rashed, A., and Paetzold, L. 18

Effects of Zebra Chip Disease on Potato Postharvest
Munyaneza, J.E., Buchman, J.L., and Henne, D.C. 23

Yield and Quality Consequences of TPP and Lso: Year 2 of a New Zealand Study
Pitman, A.R., Berry, N., Thompson, S., Taylor, N., Wright, P., Shah, F., Walker, M., Read, S., Beard, S., Jorgensen, N., Butler, R., and Thompson, S. 26

Vector Management

Insect Resistance and Other Factors Affecting Neonicotinoids in Potatoes
Trumble, J.T., Prager, S.M., and Kund, G. 31

Introducing DuPont™ Benevia™ and Verimark™ New Insect Control Products for the Management of Potato Psyllid and Zebra Chip Disease and Other Key Potato Pests in the US
Portillo, H.E., Brister, C.D., Castner, E.P., Medlin, C.R., McKinley, N.D., Wilson, G.D., Kral, C.W., Ramirez, H.T., Alvarez, J.M., and Annan, I.B. 36

Torac® 15EC: A New Tool for the Management of Potato Psyllids
Ludwig, S., Samler, J., Walston, A., and Adams, J. 40

Management of Zebra Chip of Potato with Alternative Chemistries
French-Monar, R.D., Patton III, A.F., and Serrato-Diaz, L.M. 43

Psyllid Monitoring and Sampling Strategies #2

Area Wide Sampling for Potato Psyllids: Comparisons of Distributions and Scouting Strategies on Potatoes, Tomatoes, and Peppers
Prager, S.M., Butler, C., and Trumble, J.T. 48

Comparing Sampling Techniques and Challenges for Potato Psyllids monitoring in the Columbia Basin of Oregon and Washington
Rondon, S.I. and Murphy, A.F. 53

Potato Psyllid Population Fluctuations in Natural Vegetation and in Relation to Potato Emergence Time Across Texas
Rashed, A., Paetzold, L., Archer, C., Pattison, S., Bralley, J.B., Gray, J., Workneh, F., and Rush, C.M. 58

Overwintering of the Potato Psyllid in the Northwest on *Solanum dulcamara*
Jensen, A.S., Rondon, S.I., Murphy, A.F., and Echegaray, E. 63

Potato Psyllid/ZC Ecology and Epidemiology

Assessing the Associations of Management and Environmental Factors with Regional Psyllid Abundance and Zebra Chip Intensity
Workneh, F., Henne, D.C., Goolsby, J., Crosslin, J.M., Whipple, S., Bradshaw, J.D., Rashed, A., Paetzold, L., Harveson, R., and Rush, C.M. 65

Zebra Chip Update in the Lower Columbia Basin of Oregon and Washington: First year Retrospective toward Managing the Disease
Rondon, S.I. 70

Tomato/Potato Psyllid Phenology in a Temperate Climate
Vereijssen, C., Jorgensen, J., Taylor, N., Read, N.S., and Berry, N.A. 74

Spatial-temporal Distribution of '*Candidatus* Liberibacter solanacearum' Haplotypes in the United States
Wen, A., Johnson, C., and Gudmestad, N.C. 79

Physiological Responses to Lso/Psyllid

Plant Defenses against Psyllids and *Liberibacter*
Tamborindeguy, C., Huot, O., and Levy, J. 84

Different Potato Cultivars May Vary in Zebra Chip Symptoms and Associated Tuber Physiological Changes When Infected by '*Candidatus* Liberibacter solanacearum'
Wallis, C.M., Rashed, A., Wallingford, A., and Rush, C.M. 89

Proteomic and Physiological Characterizations of Potato Plants in Response to Zebra Chip Disease
Nwugo, C.C. and Lin, H. 94

Pathogen Detection/Molecular Analysis

Genome Sequencing of '*Candidatus* Liberibacter solanacearum' Haplotype A and Comparison to the Haplotype B Genome CLso-ZC1
Johnson, C.P., Duan, Y.P., Wen, A., and Gudmestad, N.C. 99

Comparison and Evaluation of '*Candidatus* Liberibacter solanacearum' Haplotyping Markers
Wen, A., Johnson, C., and Gudmestad, N.C. 104

Haplotyping Studies of the Potato Psyllid by High Resolution Melting Analysis
Swisher, K.D., Munyaneza, J.E., and Crosslin, J.M. 109

Relationships of Potato Psyllid Populations Based on Total Microbial Community Composition
Bextine, B. and Arp, A. 114

Genotype Development/Cultivar Resistance

Breeding Clones Derived from Wild Potato Species Exhibit Resistance to Potato Psyllid and Possible Resistance to Zebra Chip Disease
Novy, R.G., Trumble, J.T., Butler, C.D., Diaz-Montano, J., Gonzalez, B., Manjunath, K.L., Lee, R.F., and Munyaneza, J. 118

Update on the Potato Variety Screening For Zebra Chip Resistance Trial
Munyaneza, J.E., Buchman, J.L., Bester, G., Novy, R., Miller, C., Van Hest, P., Nordgaard, J., Yilma, S., and Wallis, C. 122

Investigations on Putative ZC Tolerant Potato Selections
Lévy, J., Scheuring, D.C., Koym, J.W., Henne, D.C., Tamborindeguy, C., Pierson, E. and Miller, J.C., Jr. 125

POSTER SESSION

Vector/ZC Management: Chemicals and Alternative Measures

Investigations of Potato Psyllid Repellents
Prager, S.M., Kund, G., and Trumble, J.T. 130

Potato Psyllid Insecticide Evaluation Trials in the Lower Rio Grande Valley of Texas
Henne, D.C. 135

Evaluating the Efficacy of Insecticides and Insecticide Regimes against Potato Psyllid to Control Zebra Chip
Lewis, M., Michels, J., Bible, J., Jones, E., Lange, R., Footle, D., Brazille, J., Crites, S., and Carroll, B. 140

Effects of Planting Dates on ZC Incidence on Four Varieties in Pearsall, TX
Schuster, G., Trevino, J., Arp, A., and Bextine, B. 145

2010-2012 Research Advances on Integrated Management of Potato Psyllid *Bactericera Cockerelli* (Sulc) in Chihuahua, Mexico
Galaviz, R., Barrera, M., Espinoza, G., Cedillo, C., Cerezo, G., González, J., and Durazo, F. 148

A Global Perspective on the Management of Tomato-Potato Psyllid, Zebra Chip and Other Key Potato Pests with the Use of the New DuPont™ Benevia™ and Verimark™ Insecticides
Alvarez, J.M., Portillo, H.E., Annan, I.B., Cameron, R.A., Brister, C.D., and Munoz, J.R. 153

Extension and Economics

Bugspotting Psyllids: Developing a Web-Based "Speed Scouting" and Survey Tool for Potato/Tomato Psyllids
Bradshaw, J.D., Whipple, S.D., Henne, D.C., and McCornack, B.P. 158

Psyllids, Whiteflies and Environmental Factors Affecting Potato Trials in S. Texas: 2010-2012
Villanueva, R.T. and Esparza-Diaz, G. 163

Zebra Chip Economics
Guenthner, J., Greenway, G., Goolsby, J. and Henne, D. 168

Extension Activities for the Zebra Chip SCRI Program 2010-2012
Harveson, R., Bradshaw, J., Whipple, S., French, R., Schuster, G., and Appel, D. 173

Molecular and Technological Advances

Scanning Electron Microscopy of "*Candidatus* Liberibacter solanacearum" in Infected Tomato Phloem Tissue
Chen, J. 175

New Methods for Streamlining the DNA Extraction Process for Detection of "*Candidatus* Liberibacter solanacearum" from Insect Vectors
Levy, J.G., Hancock, J., Ravindran, A., Gross, D.C., Tamborindeguy, C., and Pierson, E.A. 179

A Fast-Track Breeding Methodology for Developing ZC Tolerant Potato Cultivars
Miller, J.C., Jr.' Henne,' D.C., Levy, J.G., Koym, J.W., and Scheuring, D.C. 184

The Potato Psyllid (*Bactericera cockerelli*) Genome Project
Hail, D.A., Hanson, J.D., Hunter, W.B., Tamborindeguy, C., Johnson, C.P., Gudmestad, N.C., and Bextine, B. 186

Vector Ecology

Cold tolerance in potato psyllids
Whipple, S.D., Bradshaw, J.D., and Harveson, R.M. 191

Can Carrot Psyllid (*Trioza apicalis*) Transmit '*Candidatus* Liberibacter solanacearum' to Potato?
Nissinen, A., Latvala, S., Rastas, M., Lemmetty, A., Hannukkala, A., and Pirhonen, M. 194

Host Selection by the Potato Psyllid, *Bactericera Cockerelli* (Sulc) (Hemiptera: Triozidae)
Thinakaran, J., Tamborindeguy, C., Pierson, E., and Henne, D. 199

Tritrophic Interactions between Psyllids, *Liberibacter,* and Solanaceous Plants: Effects on Aphids and *Hippodamia Convergens*
Kay, S. and Tamborindeguy, C. 204

Attendees of the
2012 SCRI Zebra Chip Annual
Reporting Session
Oct. 30 - Nov. 2, 2012

2012 SCRI ZC ANNUAL REPORTING SESSION

OCT. 30–
NOV. 2, 2012

SCRI Zębra Chip

Overview of the 2011-2012 Potato Psyllid Areawide Monitoring Program

Henne, D.[1], Aniciso, J.[1], Bradshaw, J.[2], Whipple, S.[2], Carpio, L.[3], Schuster, G.[4], Richmond, J.[4], Walker, S.[5], Porter, P.[6], Barrett, S.[7], Willett, J.[8], Mathews, J.M.[9], Deroo, K.[10], Seger, P.[11], Crosslin, J.[12], Hamlin, L.[12], and Goolsby, J.[13]. [1]Texas A&M AgriLife Research and Extension, Weslaco, TX ([5]Uvalde, TX, [6]Lubbock, TX); [2]Univ. of Nebraska, Scottsbluff; [3]CSS Farms, Dalhart, TX; [4]Texas A&M Kingsville, TX; [7]Barrett Farms, Springlake, TX; [8]Agro Engineering, Alamosa, CO; 9 Walther Farms, Wray, CO; [10]Premium Source, Garden City, KS; [11]R.D. Offutt, O'Neill, NE; [12]USDA-ARS, Prosser, WA; [13]USDA-ARS, Edinburg, TX.

Abstract

Potato psyllids (*Bactericera cockerelli*) have been monitored in a number of central U.S. states for several years now. Monitoring potato psyllid activity over this time has provided a broad and unique perspective of their local and regional population dynamics. Gathering this information is needed for the eventual development of predictive analytical tools, and to provide commercial potato growers with feedback on their management practices. Despite unusually elevated potato psyllid activity throughout the central U.S. during the 2011-2012 monitoring season, this did not translate into a similarly elevated incidence of zebra chip (ZC) disease. A south-north declining trend in percentage of adult psyllids testing positive for the ZC pathogen was noted. Potato psyllid activity in the central U.S. was bimodal, characterized by regionally synchronized populations in south Texas (Lower Rio Grande Valley and Pearsall) comprising the winter season population cycle, and another in the Colorado/Nebraska region comprising the summer population cycle. Historical activity in areas of Texas that have been monitored since 2008 is presented. Distinct year-year population variability occurs and it is argued that environmental drivers need to be identified before predictive ability can be achieved.

Introduction

A critical aspect of managing the potato psyllid (*Bactericera cockerelli*) (Hemiptera: Triozidae) includes the routine monitoring of their populations in commercial potato fields over local and regional scales. Because the potato psyllid is the sole known vector of '*Candidatus* Liberibacter solanacearum' (Lso), it is important to gather information about vector population dynamics and the incidence of Lso in these populations. Additionally, no early warning system exists to alert growers of potential risk of Lso, nor is their any capability to predict in advance future psyllid population behavior. Nevertheless, commercial potato growers benefit from information gathered by the monitoring program in a number of ways, including measures of psyllid pressure in their area, and feedback on their management practices. In addition, live material obtained from a broad geographic region is potentially useful for those interested in genetic analyses of psyllid population structure. Here, we present information gathered from the psyllid monitoring program that was run from December 2011 to September 2012 within the central region of the U.S., and also present annual historical psyllid population patterns from four areas of Texas dating back to at least 2008-2010.

Materials and Methods

The psyllid monitoring program is comprised of a network of locations in multiple states (and one Canadian Province): Texas, Nebraska, Kansas, Colorado, North Dakota, Minnesota, Wisconsin, and Manitoba, Canada (Fig. 1). In Texas, commercial potato fields near McAllen, Pearsall, Olton, Springlake, and Dalhart were sampled. In other states, locations near Scottsbluff and O'Neill, Nebraska; Alamosa, Fort Morgan, and Wray, Colorado; Garden City, Kansas, and numerous locations in North

Dakota and Minnesota were sampled. Untreated potato plots were maintained at several locations (Weslaco, Pearsall, Halfway, Texas) close as possible to commercial fields. At each commercial field sampling location, five yellow sticky cards (Pherocon® AM No-Bait Traps, Trécé, Inc., Adair, Oklahoma, USA, Product Code 3306-00) were deployed every 200′ along a transect from near the southern edge of fields inward to the center. In the same fields, 100 compound leaves were collected (10 from each of 10 equidistant locations along the field perimeter) and placed in labeled plastic bags. Every week, leaves and sticky traps were shipped to the Weslaco laboratory, where adults on sticky traps were counted using a stereomicroscope, removed and sorted, and then shipped to Prosser, Washington for Lso determination. Leaf samples were processed under a stereomicroscope to determine counts of psyllid eggs, small nymphs (instars 1-3), and large nymphs (instars 4-5). Counts were summarized by state region and compiled into a report that was distributed by email to growers, scientists, industry, and students interested in the results (approximately 180 recipients). Preseason surveys were performed at most locations to gauge psyllid activity prior to planting, by deploying transects of 20-100 yellow sticky traps. Historical adult activity for the Lower Rio Grande Valley (LRGV), Pearsall, Olton, and Dalhart, Texas sample regions were compiled from psyllid adult count data as far back as 2008. The data were standardized by time of year and all counts were square root-transformed to dampen extreme differences in magnitude of numbers within and between years.

Results and Discussion

Lso incidence (Fig. 2): Despite psyllid activity occurring in all areas sampled, Lso-positive psyllids were present only in the LRGV, Pearsall, and Olton, Texas. A definite south-north trend of declining %-positive adult psyllids was found. In general, the highest percentage of Lso-positive adults originated in the LRGV (~10%), followed by Pearsall (~5%), and Olton (<1%). With the exception of one adult from O'Neill, Nebraska that was a very weak Lso-positive, no other adult potato psyllids outside of Texas tested positive for Lso.

2011-2012 Summary (Fig. 3): Potato psyllid activity in the central U.S. was characterized by a bimodal population trend, with psyllids in south Texas (LRGV and Pearsall) comprising the winter season population cycle, and the Colorado/Nebraska location comprising the summer population cycle. Very good synchrony exists in these regions, which is not surprising given the geographical proximity of these populations. The populations in the Olton/Dalhart region of Texas, and Garden City, Nebraska have no distinct pattern. However, it is noted here that this region is intermediate geographically between the south Texas and Colorado/Nebraska populations, and may be a bridge population between the southern and northern central U.S. populations. *a) LRGV, Texas*: Potato psyllids were first detected in the LRGV at the end of November-early December 2011. At this time, 39 adults were trapped on the early-season trapline located near Edinburg, 13% of which were Lso-positive. This suggests that a population of Lso-positive adults migrated into the LRGV from another area that remains unknown at this time. Adult activity peaked during the latter half of March and continued through mid-April. Activity ceased by late May-early June. Percent ZC in commercial fields in the LRGV was <1-3%, but was as high as 40-50% in untreated plots located at Weslaco. *b) Pearsall, Texas*: Pearsall had a very active potato psyllid season with some of the highest trap counts ever recorded (200-300 adults/trap) which occurred during mid-April. It was noted that large nymph activity was elevated during late February-early March, which was approximately one month before the elevated adult activity occurred. Clearly, widespread potato psyllid reproduction was likely occurring within fields from the very start of the season. Percent ZC was <4% in sampled fields. *c) Olton and Dalhart, Texas; Garden City, Kansas:* Substantial pre-season psyllid activity was noted at both Texas locations. Activity of all stages was

erratic throughout the growing season and no specific pattern emerged. However, it was noted that adult abundance at both locations slowly climbed throughout the season. No ZC was found in any tuber samples sent to Weslaco. Similar activity was documented in the Garden City, Kansas region as well. *d) Colorado and Nebraska*: Both states had very similar patterns of potato psyllid activity. Adult activity was not detected until late May-early June, and peaked from late July into late August. No ZC was found in any tuber samples sent to Weslaco.

Recent historical psyllid population dynamics (Figs. 4-7): Potato psyllid activity does not follow annually predictable trends. In the LRGV, psyllid activity in 2008-2010 was more or less consistent, but 2011 and 2012 were not (in the case of 2011, perturbations due to late season freezes were one factor). Activity in the Pearsall region of Texas has shown a continually increasing abundance of adult psyllid activity annually from 2008-2012. In the Texas Panhandle, adult psyllid activity fluctuated erratically throughout each season, but abundance appears to have decreased in recent years. It should be noted that insect populations are driven by environmental factors such as temperature, rainfall, host quality, etc., both locally and regionally. For example, the role that El Niño/La Niña events are influencing psyllid population dynamics needs to be investigated. In years with a mild winter occurring in a large geographic area (such as 2011-2012) allow overwintering psyllids to build up larger populations than usual.

Acknowledgements
Thanks to Bill Warfield, Adrian Silva, Mayra Oyervides, and Abel Villareal for assistance with processing samples. This research was supported by grants from SCRI (2009-34381-20036) and the Texas Department of Agriculture. Thanks also to the numerous growers who participated in this effort and offered support and encouragement.

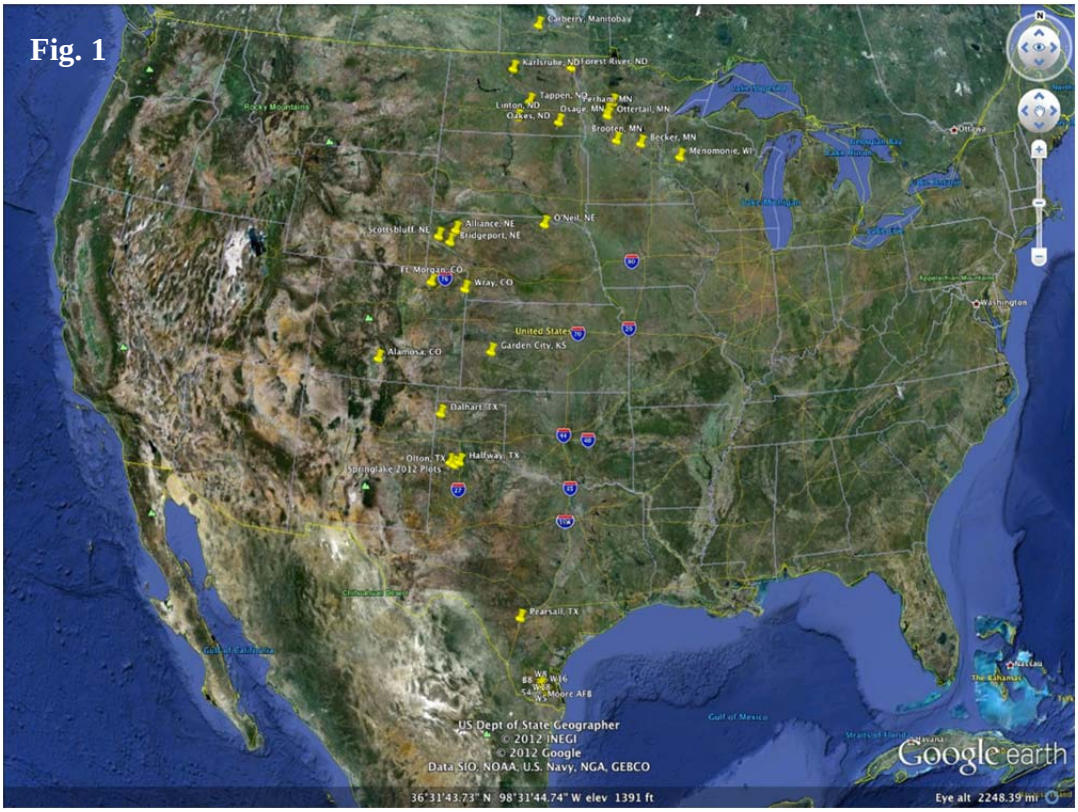


Fig. 2

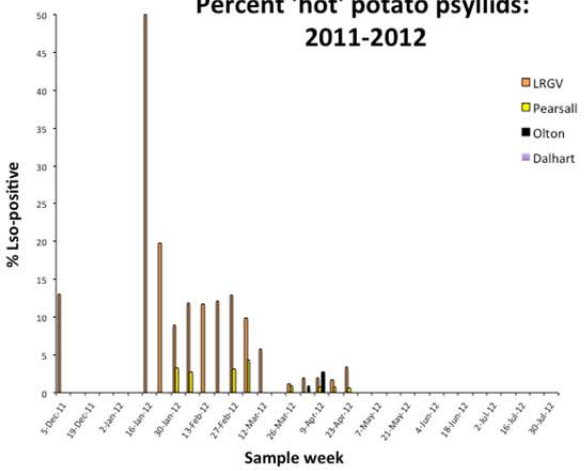


Fig. 3

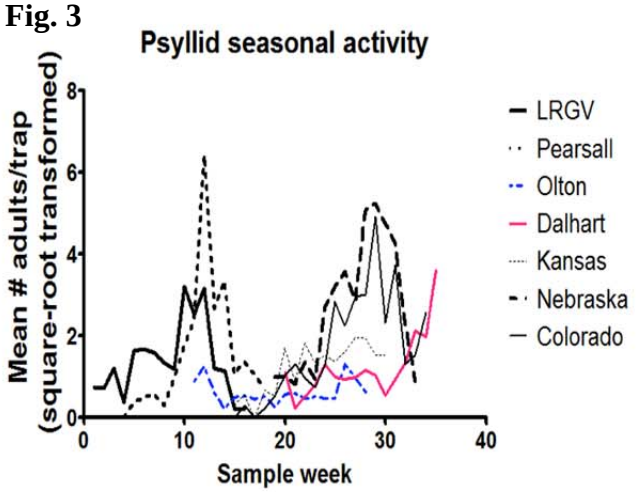


Fig. 4

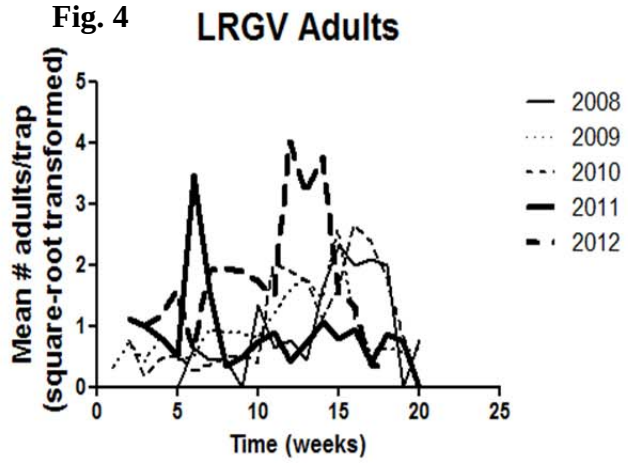


Fig. 5

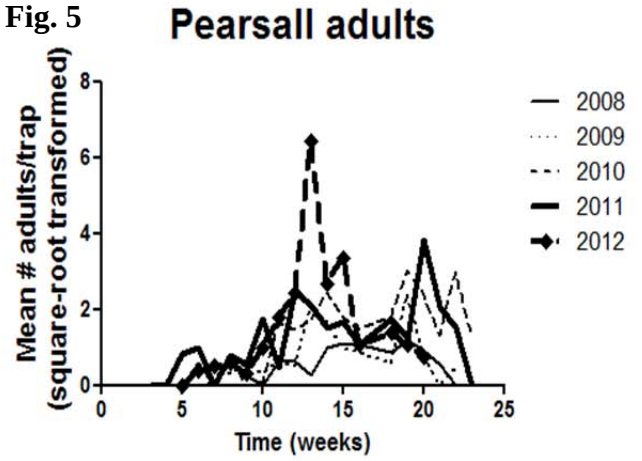


Fig. 6

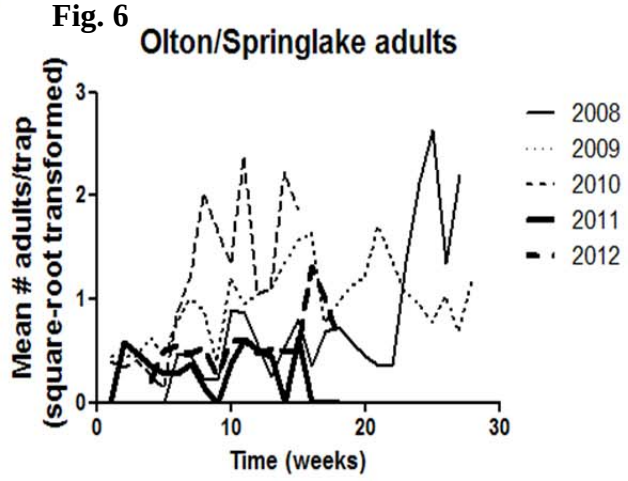
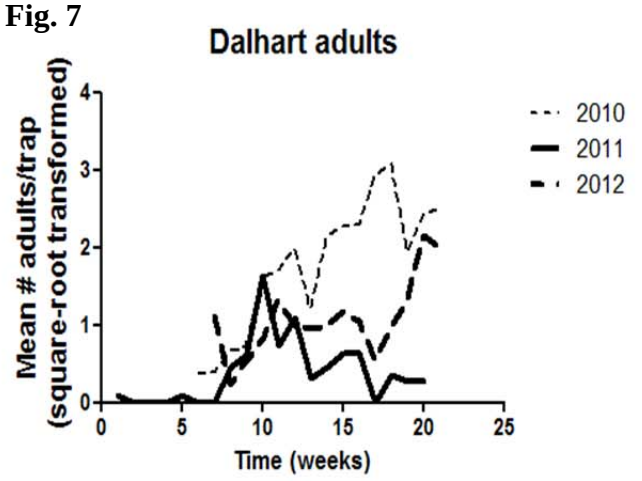


Fig. 7



A Tale of 30,000 Psyllids; and What Did They Tell Us?

[1]Crosslin, J.M., [2]Henne, D.C. and [3]Goolsby, J.A. [1]USDA-ARS, Prosser, WA 99350, USA; [2]Texas AgriLife Research, Weslaco, TX 78596, USA; [3]USDA-ARS, Weslaco, TX 78596, USA.

Abstract

In the 2009, 2010, 2011, and 2012 seasons 2,386, 7,440, 1,483, and 10,888 psyllids, respectively, were received from TX, KS, NE, CO and a few other locations. These were tested for the presence of *Candidatus* Liberibacter solanacearum (Lso), the bacterium associated with the zebra chip disease of potatoes. Lso infection rates in 2009, 2010, 2011, and 2012 were 2.1, 1.0, 5.1, and 1.4%, respectively. Additionally, during this period, 5,395 psyllids were collected from a second set of locations in the greater NE and CO areas. Of these, only 12 (0.2%) were Lso positive. The average Lso infection rate over all sites and years was 1.4%.

Introduction

The potato zebra chip disease (ZC) is a serious and emerging disease of potatoes. In the last few years the disease has been associated with the presence of the potato psyllid (*Bactericera cockerelli*) within the affected fields (Munyaneza et al. 2007a; 2007b). Even more recently, the putative causal agent of ZC was identified as a newly described alpha-proteobacterium, *Candidatus* Liberibacter solanacearum (Lso) (a.k.a. *Ca*. L. psyllaurous; Hansen et al. 2008; Liefting et al. 2009). The bacterium is transmitted to potatoes by the potato psyllids and within about three weeks symptoms of the disease begin to develop and include chlorosis, leaf scorch, aerial tuber formation, and wilt (Crosslin and Munyaneza 2009; Crosslin et al. 2010; Liefting et al. 2009). Recently, a thorough review of research on psyllids and ZC was published (Munyaneza 2012). Because of the association of the psyllid with the bacterium and subsequent development of ZC disease, a program to monitor the incidence of Lso in populations of psyllids collected in Texas, Nebraska, and Kansas was begun in January of 2009. Previous work has shown that not all populations of the psyllid harbor Lso (Munyaneza et al. 2008). Data on the incidence of psyllids in the 2011 season was presented at last years' ZC meeting. Here we report a summary of the 2009-2012 psyllid testing program and present some thoughts about the psyllid and the ZC disease situation.

Materials and Methods

Beginning approximately in October in each of 2008, 2009, 2010, and 2011, yellow sticky traps were placed in and near potato production fields in five general areas of the south central US: Lower Rio Grande Valley (McAllen, Weslaco), Pearsall vicinity, Texas panhandle (Dalhart area), southwestern Kansas (Garden City area), parts of Colorado, and southwestern Nebraska. In 2012, additional trapping sites included Minnesota, Wisconsin, North Dakota, and Manitoba, Canada. John Goolsby was in charge of the trapping network in 2009-11, and Don Henne took over responsibility for the network in late 2011 through 2012. Sticky traps from the various locations were collected weekly and sent to their laboratories in Weslaco, TX. Insects were identified, counted, and psyllids were removed from traps,

placed into vials and shipped to Prosser, WA for molecular testing (PCR) for the presence of the bacterium. A number of additional psyllid samples were collected by Steve Marquardt from the Nebraska-Colorado area. A few additional insect samples were received from Mexico and New Mexico and similarly tested for Lso. More specific information on the trapping sites in 2012 and methods can be found in Don Henne's report. DNA was extracted from individual psyllids using previously published procedures (Crosslin et al. 2006). Insect extracts were subjected to PCR analysis using previously published primers OA2/OI2c (Crosslin and Munyaneza 2009; Liefting et al. 2009). PCR products were analyzed by agarose gel electrophoresis and presence of the predicted ~1,160 base pair amplified fragment indicated an Lso-positive sample.

Results and Discussion

In the 2009, 2010, 2011, and 2012 seasons 2,386, 7,440, 1,483, and 10,888 psyllids, respectively, were received and tested for Lso (Fig. 1). Most of the samples that tested positive for Lso were collected between February and April of each year and the majority of these were collected from untreated control plots, especially those in the Lower Rio Grande Valley (LRGV). In general, a low incidence of Lso was observed in most years and most locations. An additional 5,395 psyllids were tested from the trapping network coordinated by Steve Marquardt in 2009-2012 (Fig. 2). Only 12 of these insects (0.2%) tested positive for Lso. A general summary of psyllids tested and collection sites is shown in Fig. 3.

Topics presented for discussion included: (1) The relatively high incidence of Lso in psyllids in LRGV in Feb-Apr, followed by low incidence of Lso in Northern TX, KS, CO, NE later, suggests psyllids DO NOT move via long distance migration. If they did, they would take the Lso with them. It is not known at this time if psyllids must have access to an Lso-infected host in order to maintain a significant infection level or not. (2) What led to the ZC outbreak in the PNW in 2011? Why were psyllids in some areas Lso infected (McNary, Hermiston), and others in relatively close proximity (Prosser, Moxee) were not infected? (3) Testing of a zillion psyllids really doesn't tell us much. What's the difference in ZC incidence between 0.1 and 3.0% Lso infected psyllids in a given area? I don't think that the data is there to tell us. Do you wait to start spraying until you detect an Lso-infected psyllid? When do you STOP spraying? And lastly, (4), if psyllid testing for Lso IS of value, what are the testing priorities? How many insects should be tested? You can't test psyllids in every potato field in the US!

Acknowledgements

I thank John Goolsby, formerly of USDA-ARS, Weslaco, TX, and Don Henne, Texas A & M, Weslaco, TX for providing most of the psyllids used in this research. Additional insects were kindly provided by Steve Marquardt, Nebraska Potato Certification, and Joe Munyaneza, USDA-ARS, Wapato, WA. I gratefully acknowledge Launa Hamlin, Mary Sue Roster, Eric Krohn, Kylie Swisher, and Rich Quick for the large amount of work required for extraction and testing of the psyllids, and the data management. Financial support was provided by the USDA-SCRI Project # 2009-51181-20176 and the USDA-RAMP Project # 2009-51101-05892.

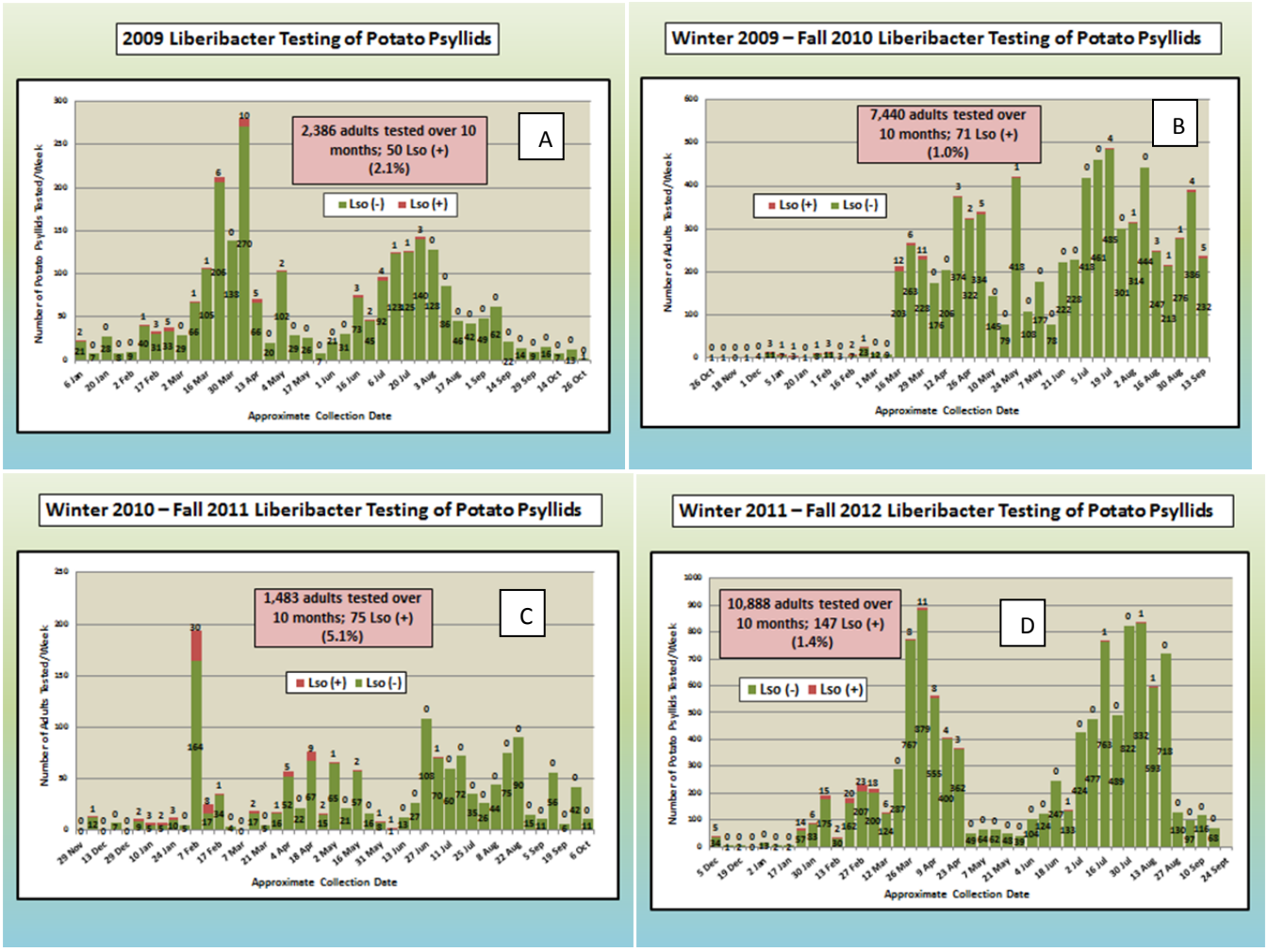


Figure 1. Weekly breakdown of number of psyllids received and tested for *Candidatus* Liberibacter solanacearum (Lso) in seasons corresponding to: A, 2009; B, 2010; C, 2011 and D, 2012. Number of insects testing positive for Lso are shown as "caps" and numbers on top of columns.

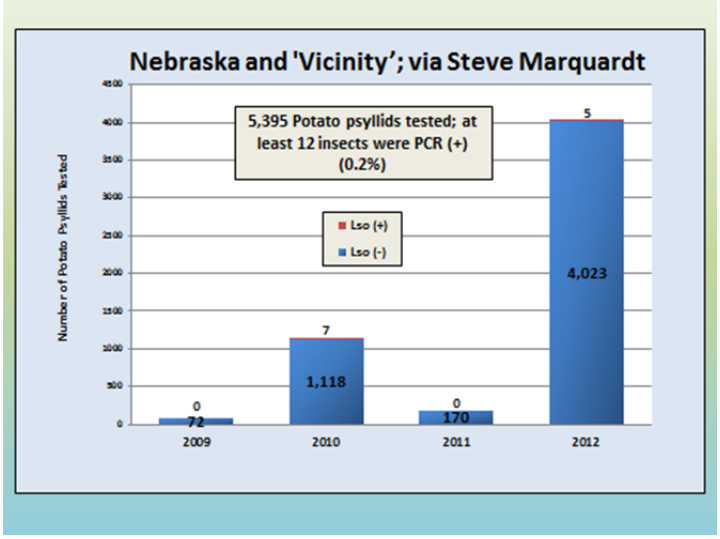


Figure 2. Psyllids received from the trapping network of Steve Marquardt (Nebraska-Colorado vicinity) in the 2009 to 2012 seasons. Number of insects testing positive for Lso is shown on top of columns.

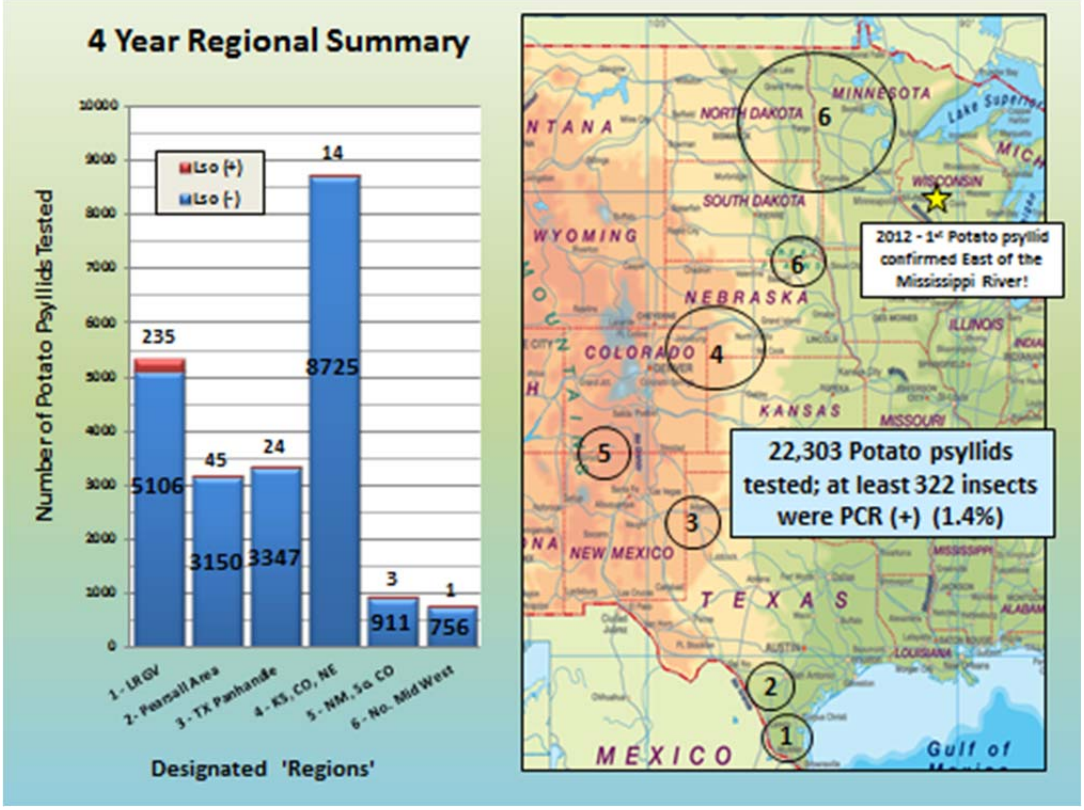


Figure 3. Four-year summary of insects received and tested for Lso. General locations of the various trapping areas are shown. The number of insects testing positive for Lso is shown on top of columns. Note that most positive samples originated in the Lower Rio Grande Valley (LRGV).

References

Crosslin, J.M. and Munyaneza, J.E. 2009. Evidence that the zebra chip disease and the putative causal agent can be maintained in potatoes by grafting and *in vitro*. Am. J. Pot. Res. 86:183-187.

Crosslin, J.M., Munyaneza, J.E., Brown, J.K. and Liefting, L.W. 2010. Potato zebra chip disease: a phytopathological tale. Online. Plant Health Progress doi: 10.1094/PHP-2010-0317-01-RV.

Crosslin, J.M., Vandemark, G.J. and Munyaneza, J.E. 2006. Development of a real-time, quantitative PCR for detection of the Columbia Basin potato purple top phytoplasma in plants and beet leafhoppers. Plant Dis. 90:663-667.

Hansen, A.K., Trumble, J.T., Stouthamer, R. and Paine, T.D. 2008. A new huanglongbing species, "*Candidatus* Liberibacter psyllaurous" found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). App. Env. Micro. 74:5862-5865.

Liefting, L.W., Sutherland, P.W., Ward, L.I., Paice, K.L., Weir, B.S. and Clover, G.R.G. 2009. A new '*Candidatus* Liberibacter' species associated with diseases of Solanaceous crops. Plant Dis. 93:208-214.

Munyaneza, J.E. 2012. Zebra chip disease of potato: biology, epidemiology, and management. Am. J. Pot. Res. 89:329-350.

Munyaneza, J.E., Buchman, J.L., Upton, J.E., Goolsby, J.A., Crosslin, J.M., Bester, G., Miles, G.P. and Sengoda, V.G. 2008. Impact of different potato psyllid populations on zebra chip disease incidence, severity, and potato yield. Subtrop. Plant Sci. 60:27-37.

Munyaneza, J.E., Crosslin, J.M. and Upton, J.E. 2007a. Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "Zebra Chip", a new potato disease in southwestern United States and Mexico. J. Econ. Entomol. 100:656-663.

Munyaneza, J.E., Goolsby, J.A., Crosslin, J.M. and Upton, J.E. 2007b. Further evidence that zebra chip potato disease in the lower Rio Grande Valley of Texas is associated with *Bactericera cockerelli*. Subtropical Plant Science 59:30-37.

Monitoring of potato psyllids, *Candidatus* Liberibacter solanacearum, and zebra chip in Idaho during the 2012 growing season

Wenninger, E. J., Olsen, N., Thornton, M., Nolte, P., and Karasev, A. University of Idaho, ID, USA

Abstract
Caused by the bacterium (*Candidatus* Liberibacter solanacearum [Lso]) and vectored by the potato psyllid *(Bactericera cockerelli*), zebra chip (ZC) is an emerging disease of potatoes that causes millions of dollars in losses annually to growers in the southwestern United States. Despite occurrence of the potato psyllid in the Pacific Northwest over many decades, ZC was thought to not occur in this region. However, during September 2011, ZC was widely reported in Idaho, Washington, and Oregon. Because of the potential threat of this disease to Idaho's potato industry, we initiated a monitoring program in Idaho to clarify the distribution and abundance of potato psyllids, ZC, and Lso, throughout the potato production areas in the state. Psyllids were abundant only in the Magic Valley region of south-central Idaho. Numbers of psyllids gradually increased over the season until vine kill. Yellow sticky cards trapped more psyllids than did vacuum samples. Few sites showed colonization by immature psyllids. Incidence of Lso was relatively high (30-50%) in adult psyllids; however, samples from later in the season remain to be tested. Sampling of tubers from fields indicated that ZC was present at low levels on all sites, and ZC rating did not necessarily relate with psyllid numbers trapped at each site. Our observations show that continuation of the monitoring program during the next season is warranted.

Introduction
Zebra chip (ZC) is an emerging disease of potatoes that causes millions of dollars in losses annually to growers in the southwestern United States. The causative agent is a bacterium (*Candidatus* Liberibacter solanacearum [Lso]) transmitted by the potato psyllid *(Bactericera cockerelli*). ZC-infected potato produces tubers with striped necrotic patterns that are more pronounced when tubers are fried, making chips and fries unmarketable.

The potato psyllid is thought to be native to the southwestern United States and northern Mexico, which is where the insect has been thought to overwinter. Assisted by wind currents, psyllids appear to migrate annually to northern regions during late spring as temperatures warm in the overwintering areas. Potato psyllids had been reported to occur from the central plains states west to Idaho and Alberta, Canada as early as the 1940s and 50s, and were recently found to occur during three consecutive seasons in Washington state. The annual arrival period of the psyllid into Washington appears to be in early to late July. However, that interval is likely to vary geographically and from year-to-year.

Despite occurrence of the potato psyllid in the Pacific Northwest, ZC was thought to not occur in this region. However, that consensus changed in September 2011, when ZC was widely reported in Idaho, Washington, and Oregon. ZC could pose a substantial threat to Idaho's potato industry; however, the extent and severity of the potential threat is unknown. Knowledge of the distribution and abundance of potato psyllids in Idaho is critical to developing effective management strategies to mitigate the threat of ZC in Idaho. We initiated a monitoring program in Idaho to more fully understand the distribution and abundance of potato psyllids, ZC, and Lso, throughout the potato production areas in the state.

Materials and Method

Potato psyllids were monitored in 14 commercial potato fields in each of three growing areas of Idaho: Treasure Valley (western Idaho; 4 sites), Magic Valley (south-central Idaho; 6 sites), and eastern Idaho (6 sites). All fields were 'Russet Burbank' except for one 'Ranger Russet' field. All fields were treated with an insecticide program targeting potato psyllids. In addition, monitoring was conducted on a ca. 0.33 acre block of potatoes at the Kimberly Research & Extension Center (in the Magic Valley) that received minimal insecticide inputs. Monitoring began during the week of May 14 and continued weekly for each field until vine kill. Within each field around the perimeter (about 9 feet from the edge), 10 yellow sticky traps were deployed; traps were replaced weekly and adult potato psyllids on each trap were counted. Within the vicinity of each of the 10 sticky trap locations, 10 leaf samples were collected each week (total of 100 leaf samples from each field each week); leaf samples were returned to the lab and eggs and nymphs of potato psyllids found on leaves were counted. In addition, each week a leaf blower with a vacuum attachment was used to sample insects from potato foliage. The windward side of each field was sampled for 5 minutes at each site each week, and samples were returned to the lab to count adult potato psyllids collected.

Adult potato psyllids were tested for the presence of Lso by PCR, using the methodology of Crosslin et al. (2011). Initially psyllids were bulk sampled to test for Lso, but in subsequent samples each psyllid (up to 50 per site) was individually tested for Lso.

Tubers were collected and assessed for zebra chip (ZC) symptoms only for the Magic Valley sites. All of the tubers from three plants in the vicinity of each of the 10 sticky trap locations were collected from each site and tubers from an additional six plants (three plants from each of two locations in each field) were collected as well. Raw tubers were rated using a 0-3 rating scale (0 = healthy tuber; 3 = severe ZC symptoms).

Results and Discussion

Very few psyllids were collected on sticky traps outside of those deployed in fields in the Magic Valley. A total of three adults was collected on sticky traps in eastern Idaho during the week of July 23. In western Idaho, one adult was collected during the week of August 20 and two adults during the week of August 27. No psyllids were collected in western or eastern Idaho in vacuum or leaf samples. Numbers of psyllids collected from Magic Valley fields were considerably higher than those observed in western and eastern Idaho. Adult psyllids were first observed on June 18 in Twin Falls Country, and numbers gradually increased over the

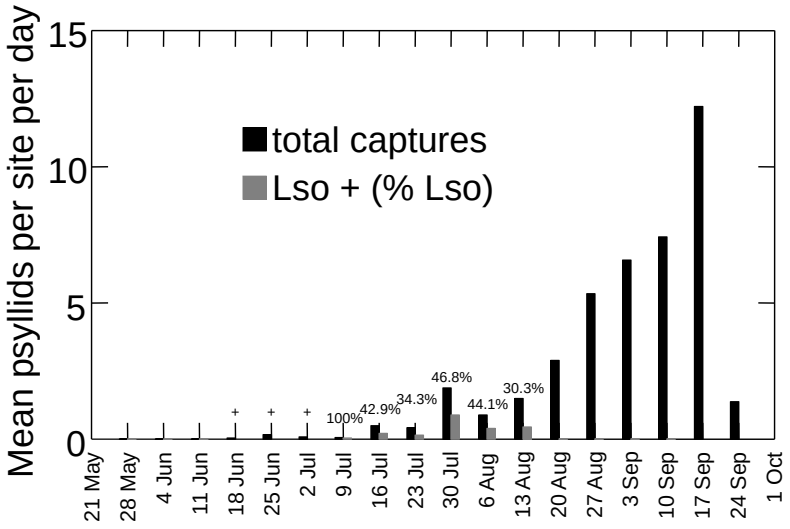


Figure 1. Mean number of adult potato psyllids (and % positive for Lso) collected on sticky traps across Magic Valley sites during 2012. Psyllids were bulk sampled for the first three weeks and all samples were positive for Lso.

season until vine kill began (September 24; Figure 1). Patterns were similar with vacuum samples, though overall numbers were lower and the first psyllids were not observed until July 9 (data not shown). This gradual increase in numbers of psyllids sampled over the season is consistent with patterns observed in Washington state (Munyaneza et al. 2009). Nymphs were not observed in leaf samples until August 27; numbers of nymphs collected in leaf samples gradually increased until vine kill (Figure 2). The percentage of Lso+ psyllids ranged from ca. 30-50% through mid-August (Figure 1); samples from subsequent dates remain to be tested for Lso. This incidence of Lso is relatively high compared to observations in other parts of the country, so it will be interesting to see if Lso incidence remains this high in samples from later in the season.

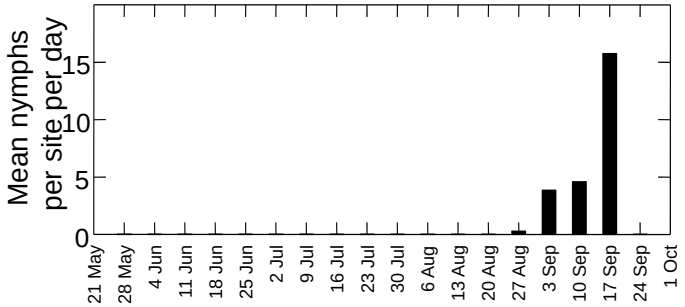


Figure 2. Mean number of potato psyllid nymphs collected from leaf samples across Magic Valley sites during 2012.

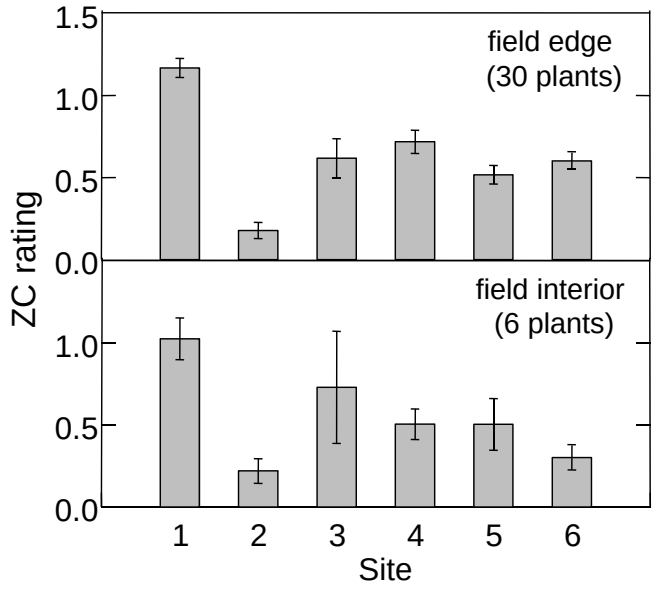


Figure 3. Mean (+/- SEM) ZC rating compared among the six commercial Magic Valley fields.

ZC ratings tended to be similar between samples from field edges and the interior (Figure 3). This was not expected given the more obvious foliar symptoms of ZC observed along field margins (Wenninger and Olsen, personal observation). Sites 3 and 4 had the most adult psyllids over the season, but did not necessarily exhibit the highest ZC ratings. Site 1 had very few adults collected, but was the first site to show colonization by nymphs and tended to show the highest ZC ratings. This might indicate ineffective control of nymphs with the insecticide program on this field.

References

Crosslin, J.M., Lin, H., and Munyaneza, J.E. 2011. Detection of '*Candidatus* Liberibacter solanacearum' in the potato psyllid, *Bactericera cockerelli* (Sulc), by conventional and real-time PCR. Southwest. Entomol. 36:125-135.

Munyaneza, J.E., Crosslin, J.M., and Buchman, J.L. 2009. Seasonal occurrence and abundance of the potato psyllid, *Bactericera cockerelli*, in south central Washington. Am. J. Pot. Res. 86:513-518.

Pavlista, A.D. 2002. Potato (tomato) psyllids. Nebraska Potato Eyes 14. 4 pg.

Pletsch, D.J. 1947. The potato psyllid *Paratrioza cockerelli* (Sulc) its biology and control. Mont. Agric. Exp. Stn. Bull. 446.

Wallis, R.L. 1955. Ecological studies on the potato psyllid as a pest of potatoes. USDA Tech. Bull. 1107:1-24.

Population Dynamics of Tomato and Potato Psyllid (*Bactericera cockerelli*) in New Zealand

Tran, L. T.[1], Worner, S. P.[1], Vereijssen, J.[2], and Teulon, D. A. J. [1,2]

[1]Bio- Protection Research Centre, PO Box 84, Lincoln University 7674, Christchurch, New Zealand
[2]The New Zealand Institute for Plant & Food Research Limited Lincoln, Private Bag 4704, Christchurch Mail Centre, Christchurch 8140, New Zealand

Corresponding author: tranl@lincoln.ac.nz; tranlucfuv@yahoo.com

Abstract
The tomato/potato psyllid (TPP), *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), invaded New Zealand in early 2006 and has now spread throughout most of the North and South Island. Laboratory and field experiments were conducted to determine the population development, phenology of TPP and to investigate aspects of its control, particularly in relation to using developmental parameters for preliminary forecasting models and investigate the efficacy and impact of predatory bug *Orius vicinus* (Ribaut) feeding on TPP by functional response and prey preference.

Introduction
The tomato/potato psyllid (TPP), *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) is an economically important crop pest that not only causes damage through its feeding but also transmits the bacterium, "*Candidatus* Liberibacter solanacearum", which causes zebra chip disease in potato. TPP and Liberibacter have been responsible for substantial economic losses across a wide geographic range, including North America and New Zealand. The recent arrival and the economic importance of TPP and Liberibacter in New Zealand and interest in the biology and management of these organisms makes TPP a very suitable target for fundamental research leading to applied outcomes. Understanding how the lifecycle of this pest insect is essential to study its population ecology, forecast population development and subsequently timing of pest management.

In addition, chemical suppression of TPP populations requires frequent applications of insecticides (Goolsby *et al*. 2007) with highly variable results (Gharalari *et al*. 2009, Berry *et al*. 2009). Furthermore, there is an increasing trend for TPP to become resistant to insecticides at least in parts of North America (Liu and Trumble 2005, 2006) and there is concern that the same will happen in New Zealand (Walker *et al*. 2012). This means we cannot rely only on insecticides to manage TPP populations and therefore investigations of potential biological control agents for TPP are very important. We investigated *Orius vicinus*, a potential natural enemy that occurs naturally within New Zealand, to determine its potential to reduce TPP populations primarily through functional response and prey preference.

Our objectives were to: 1) determine important developmental parameters that describe population growth of TPP in relation to temperature; 2) determine the efficacy of a selected natural enemy, *O. vicinus* for regulation of TPP populations, and particularly in the presence of alternative prey; and 3) develop and compare forecasting models based on the relationship between the psyllid and climate to predict population phenology, seasonal timing and potential abundance.

Materials and Methods

Developmental parameters. Egg, nymph and adult development times were determined at constant temperatures of 8°C, 10°C, 15°C, 20°C, 23°C, 27°C, and 31°C (±1). Relative humidity of approximately 50-60% and a constant photoperiod of 16:8 L: D were maintained at each temperature. Eggs and nymphs were checked daily. One linear and two nonlinear (Lactin 1995 and Briere 1999) models were used to estimate important developmental parameters.

Functional response of *O. vicinus* feeding on TPP and prey preference. Five densities (2, 4, 8, 16, and 32) of TPP eggs, nymphal instars 1 and 2, nymphal instars 3 and 4, and nymphal instar 5 were randomly selected and tested. Each adult *O. vicinus* (5-10 days old) was tested once only, and then discarded. For each density, TPP eggs or nymphs were arranged evenly on the upper surface of a potato leaf which was subsequently placed in a 9 cm Petri dish fitted with a mesh lid. The *Orius* predators were starved for 24h and added singly to each Petri dish with different prey densities and different prey stages. After 24h each predator was withdrawn from the Petri dish and the unconsumed prey was counted. Each treatment density had 10 replicates (*i.e.* 10 individual *O. vicinus* tested at each density) and five controls without predators.

In an additional experiment, four density combinations of TPP (nymph 1-2) and thrips (nymph 1-2) at density ratios of 4:4, 8:8, 16:16, and 32:32 were prepared. Each adult *O. vicinus* (5-10 days old) was used once and then discarded. TPP and thrips nymphs were arranged on a potato leaf in a plastic cylinder (8 cm diameter, 9 cm high). The lower surface of the cylinder was sealed with fine mesh for ventilation and the upper surface was covered by parafilm. The predators were starved for 24h and added singly to a cylinder with a different prey density combination. After 24h, predators were withdrawn from each cylinder and any unconsumed prey was counted. Each density combination had 15 replicates and four controls without predators.

Forecasting models. The relationship between the cumulative percentage of TPP caught on yellow sticky traps in unsprayed fields and degree days was modeled using a Weibull function and a bimodal equation. Data from the trap catches from 2009 to 2012 in New Zealand were converted to cumulative proportions. Models were parameterized on the weekly cumulative trap catches (dependent variable) and cumulative degree days (DD) (independent variable) calculated from the biofix (November 1st). Because of year to year variability in population numbers the weekly trap catch data were normalized within each year by calculating the proportion of weekly trap catches of the total trap catches within the year. Parameters of models were estimated using nonlinear regression functions.

Results & Discussion

Developmental parameters. The developmental durations of all immature stages and total development decreased in relation to temperature as it increased from 8°C to around 27°C. At temperatures above 27°C the developmental times started to increase for all stages. One linear and two nonlinear models were used to determine the relationship between developmental rate and temperature. These models fitted the data well, as evidenced by high values of R^2 and small values of Residual Sum of Squares (RSS) and Akaike Information Criterion (AIC). The linear model was used to estimate a lower temperature threshold and thermal constant. The lower threshold temperatures of egg, nymph, and total development were 7.9, 4.2, and 7.1°C, respectively. The thermal requirement for total development of TPP is 358 degree days. The optimum and upper temperature threshold were estimated by nonlinear models. In this study, the Briere nonlinear model was found to provide a slightly better fit than the

Lactin nonlinear model with optimum temperatures of 26.9°C. Upper temperature threshold was estimated as 33.9°C (Tran *et al*. 2012). These lower and upper temperature thresholds are key developmental parameters that, along with degree day requirements, are used to develop temperature-based phenology models. The phenology models have long been used as part of decision support systems to help growers timing insecticide sprays or begin pest scouting (Welch *et al*. 1978, Worner and Penman1983).

Functional response of *O. vicinus* feeding on TPP and prey preference. The number of prey at different stages consumed by the predator decreased significantly as prey size increased from egg to nymph 5. The number of prey consumed by the predator was negatively correlated with the prey densities offered. *O. vicinus* clearly exhibited Type II functional response which means that the predation rate or the proportion of the prey population predated decreases with an increase in prey density.

Manly's measure of preference was greater than 0.5 at all prey densities although only at two combinations of high prey densities (16:16 and 32:32) were thrips was significantly more preferred than psyllids. Results showed that the functional response and the effects of alternative prey of *O. vicinus* alone may only give an indicative predictive value in determining effectiveness of the predator in a biological control program of TPP. However, the responses did indicate that this predator will consume both TPP and thrips. This means that the predators can remain in the crop and maintain themselves on thrips (or similar non-target prey) if the TPP population is at low density. While laboratory experiments with single-prey and two-prey systems may not give completely confident predictions about the effectiveness of *O. vicinus* for augmentative biological control of TPP in a greenhouse or field, nevertheless, the functional responses of *O. vicinus* feeding on TPP and prey preference of this predator on thrips and TPP could serve as a useful guideline for estimating the potential impact of this predator on both thrips and TPP populations and for the design of further studies.

Forecasting models. TPP was monitored at weekly intervals during the growing season for three years (2009-2010, 2010-2011, and 2011-2012) using yellow sticky traps in unsprayed potato crops in various locations in New Zealand. The phenological pattern of TPP indicated that adult TPP have two peaks in abundance during a potato growing season with the first peak occurring from 722 to 749 DD, and the second peak from about 1189 to 1264 DD after biofix. The time of the first peak has important implications for timing of control measures as the peak egg infestation of TPP in a crop was reported to be related to the trend of adult trap catches (Walker *et al*. 2011). On the whole, the bimodal function described the observed data better than the Weibull function and had a smaller AIC and slightly higher R^2 than the Weibull model.

The research in this study contributes new knowledge about the life cycle of the tomato/potato psyllid and has identified areas in the knowledge base for further research. In addition to continuing to increase understanding of TPP biology and ecology, future research needs to develop action thresholds for the control of this species appropriate for the various crop management systems. Also, accumulating more detailed knowledge about the interactions of the psyllid, the disease it vectors and its host plants and natural enemies will be important. Such research should lead to more sustainable management of this pest. For example, in the present study we estimated the developmental threshold of *B. cockerelli* to be 7.1ºC reared on potato with a thermal budget of 358 degree days. Using this data we can estimate approximately how many generations of *B. cockerelli* might occur per year for specific sites throughout

New Zealand. Figure 1 shows the number of generations of TPP in different potato growing regions in New Zealand. For early crop potatoes at Pukekohe only 0.8 generations occur from July 1st until potato emergence (Fig. 1) and 1.8 generations on the crop (emergence to harvest). For main crop potatoes at Pukekohe, 2 generations occur from July 1st until potato emergence and 5.2 generations on the crop. In contrast, for the main crop in the cooler temperatures in Canterbury only 0.7 generations occur before potato emergence, and only 3.8 generations on the crop. Note that Pukekohe has potato hosts available for a much longer period than in Canterbury due to an early crop in addition to main crop.

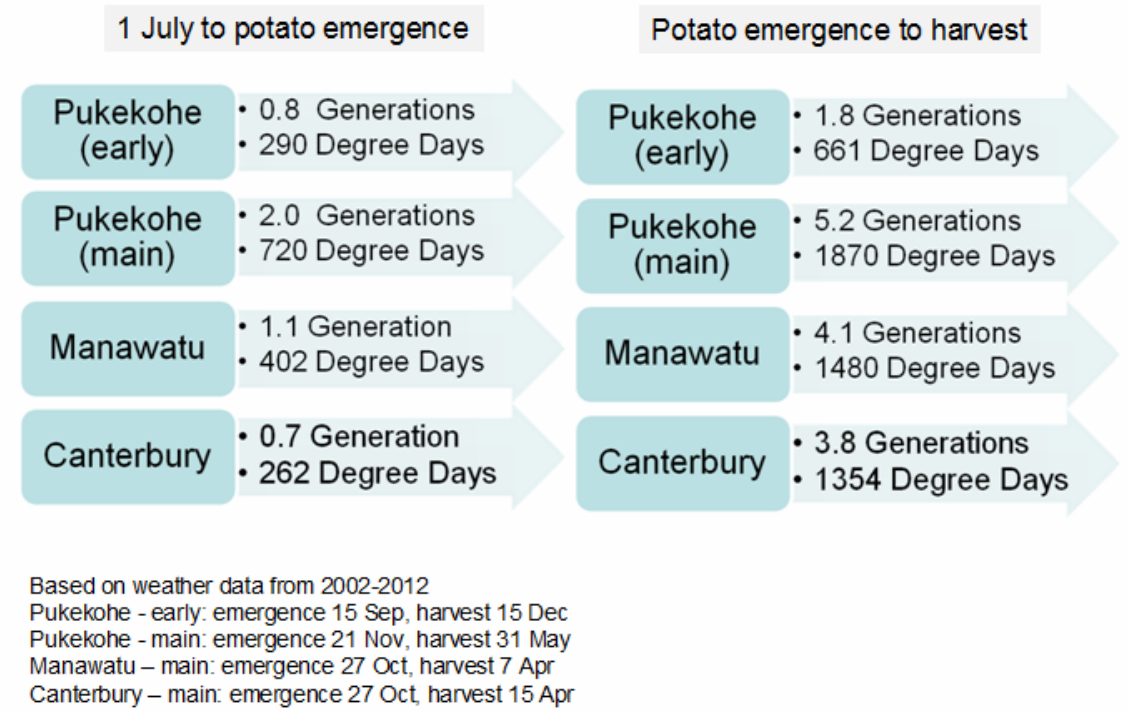


Figure 1: Degree days available and number of generations of TPP on potato crops at different sites in New Zealand. Degree days are calculated from July 1st.

References

Berry, N.A., Walker, M.A., and Butler, R.C. 2009. Laboratory studies to determine the efficacy of selected insecticides on tomato/potato psyllid. N. Z. Plant Protect. 62: 145-151.

Briere, J., Pracros, P., Roux, A.L., and Pierre, J. 1999. A novel model of temperature-dependent development for arthropods. Environ. Entomol. 28: 22–29.

Gharalari, A.H., Nansen, C., Lawson, D.S., Gilley, J., Munyaneza, J.E., and Vaughn, K. 2009. Knockdown mortality, repellency, and residual effects of insecticides for control of adult *Bactericera cockerelli* (Hemiptera:Psyllidae). J. Econ. Entomol. 102: 1032-1038.

Goolsby, J.A., Adamczyk, J., Bextine, B., Lin, D., Munyaneza, J.E., and Bester, G. 2007. Development of an IPM Program for management of the potato psyllid to reduce incidence of Zebra chip disorder in potatoes. Subtrop. Plant Sci. 59: 85-94.

Lactin, D. J., Holliday, N.J., Johnson, D.L., and Craigen, R. 1995. Improved rate model of temperature-dependent development by arthropods. Environ. Entomol. 24: 68–75.

Liu, D., and Trumble, J.T. 2005. Interaction of plant resistance and insecticides on the development and survival of *Bactericera cockerelli* [Sulc] (Homoptera:Psyllidae). Crop Prot. 24(2): 111-117.

Liu, D.G., and Trumble, J.T. 2006. Ovipositional preferences, damage thresholds, and detection of the tomato-potato psyllid *Bactericera cockerelli* (Homoptera: Psyllidae) on selected tomato accessions. Bull. Entomol. Res. 96: 197-204.

Tran, L.T., Worner, S.P., Hale, R.J., and Teulon, D.A.J. 2012. Estimating development rate and thermal requirements of *Bactericera cockerelli* (Hemiptera: Triozidae) reared on potato and tomato by using linear and nonlinear models. Environ. Entomol. 41: 1190-1198.

Walker, G.P., MacDonald, F.H., Puketapu, A.J., Fergusson, H.A., Connolly, P.G., Wright, P.J., and Anderson, J.A.D. 2012. A field trial to assess damage by *Bactericera cockerelli* to early potatoes at Pukekohe. N. Z. Plant Protect. 65: 148-154.

Welch, S.M., Croft, B.A., Brunner, J.F., and Michels, M.F. 1978. PETE: an extension phenology modeling system for management of multi-species pest complex. Environ. Entomol. 7: 482-494.

Worner, S.P., and Penman, D.R. 1983. Analysis of thermal summation models. Pp.250-254. Proceedings of the 46th New Zealand Weed and Pest Conference, Palmerston North, August 1983.

Impact of Late Season Psyllid Infestations on Potato Seed Quality

Rush, C. M., Workneh, F., Rashed, A., and Paetzold, L. Texas A&M AgriLife Research, 6500 Amarillo Boulevard. West, Amarillo, TX. 79106

Abstract

Infection of potatoes by '*Candidatus* Liberibacter solanacearum' (Lso), vectored by the potato psyllid, *Bactericera cockerelli* Sulc (Hemiptera: Triozidae), causes the disease zebra chip (ZC). Infected tubers exhibit dark brown medullary rays, an accumulation of phenolic compounds, amino acids and reducing sugars, and poor germination and emergence when planted. These symptoms are most severe when plants are infected early in the growing season, but the impact of late season infection on tuber quality was unknown. Potato plants were infested with bacteriliaferous potato psyllids weekly during the growing season, starting one week after plant emergence and continuing until one week before harvest. At harvest, tubers were dug, scored for ZC symptom severity, examined by qPCR for Lso titer and placed in cold storage at 42 F. After nine months in storage, tubers were planted in the field, emergence was recorded and plants were tested for Lso. In a second study, tubers from plants infested either one or two weeks before harvest were taken from cold storage after two months, held at room temperature and tested weekly for Lso for five weeks. In the field germination study, a total of 636 tubers were planted, 199 (31%) emerged but only 16 (8%) tested positive for Lso. Seventy five percent of the plants that emerged and tested positive for Lso came from tubers infested late in the season, i.e., one or two weeks before harvest. Our storage study indicated that pathogen-tuber interaction continues post-harvest. Percentage of detected Lso-positive tubers increased during cold storage. After removal from the storage both percent positive detection and Lso titers increased over time.

Introduction

Zebra chip (ZC) of potato is putatively caused by the fastidious bacterium '*Candidatus* Liberibacter solanacearum' (Lso), which is vectored by the potato psyllid. The disease reduces both yield and quality in all market classes of potato, and has caused significant losses each year, since it was first reported in the lower Rio Grande Valley in 2000. Since the etiology of ZC was first identified, there has been concern that infected tubers might be a source of primary inoculum, if used as seed. Henne et. al., (2011) were the first to study this question and reported that only a low percentage of Lso-infected seed tubers germinated and emerged. An even lower percentage of plants from infected tubers tested positive for Lso, and those that did were typically stunted and frequently died soon after emergence. They concluded that although Lso-infected seed could potentially serve as a source of primary inoculum, the likelihood for this was very low, and from an epidemiological standpoint, seed tubers with ZC would be insignificant. However, researchers in New Zealand (Pitman, et. al., 2011) had different results and reported that a high percentage of Lso-infected seed potatoes emerged as relatively normal-sized plants that tested positive for Lso. The different results from these studies only served to increase concern among farmers and seed producers.

Subsequent to these studies, it was reported that a number of plant metabolites were significantly associated with ZC (Wallis, et. al., 2012; Rashed, et. al., 2013). Surprisingly, most of these changes were not significantly correlated to Lso titer but rather were correlated to ZC symptom severity. Furthermore, it was found that duration of infection impacted both Lso titer and symptom expression, biochemical changes were not evenly distributed throughout the infected tuber and that late season infections were often asymptomatic. These results clearly demonstrated that duration of infection impacted tuber quality and raised the possibility that duration of infection could be associated with germination and emergence of Lso-infected seed potatoes. We hypothesized that seed potatoes from

plants infected late in the growing season would have greater emergence and be more likely to produce Lso-infected seedlings. Studies were then initiated to evaluate the impact of cold storage on seed potato quality infested late during growing season.

Materials and Methods

Field Germination Study. The study was conducted at the Texas A&M AgriLife Research and Extension Center, Bushland. Seed potatoes of FL 1867 cultivar were planted in the field in April 2011 and covered with 100x100x100-cm mesh cages prior to emergence. Following emergence plants were thinned down to 4, such that one plant was located at each corner of the cages.

Potato psyllids from an Lso-positive colony were used to infest plants in the experimental cages. Infestations started approximately 10 days post emergence and continued weekly, until one week before harvest (9 infestation events). Each cage was infested by releasing 30 potato psyllids at the base of a plant in each cage. Insects were allowed to feed for one week, and then were sprayed with insecticides. At harvest, a thin slice, approximately 5mm thick, was removed from the stolon attachment end and tubers were scored for symptom severity on a 0-3 scale (Rashed et al. 2013). All tubers from plants on which the insects were originally released were sampled for pathogen quantification by qPCR (see below). Tubers obtained from the rest of the plants were sampled prior to planting the following year (see below). Samples were stored in a -80 freezer until DNA extractions were performed. After the visual scoring and sampling, tubers were stored at 4C for 9 months.

There were three germination cage experiments. Tubers were removed from cold storage and placed at room temperature for 4 days. Seed tubers were then planted in 4 rows, approximately every 40-cm, during April and May 2012, and covered with mesh cages. The first experimental cage was planted April 4 and contained tubers from plants on which the psyllids were initially released. These plants were the first to develop foliar ZC symptoms in 2011. The second cage was planted on May 9 and contained tubers collected from the second plant in each of the 2011 cages that developed foliar symptoms of ZC. The third cage was planted on May 17 and included tubers of the remaining plants in each cage from the last 2 infestation treatments in 2011, infested 1 and 2 weeks before harvest (infestations 8 and 9, respectively).

Each cage was monitored every 3 days for up to two months and emergence status was recorded for each planted tuber. At the end of each experiment, stem tissue of the emerged plants were tested for Lso by qPCR.

Laboratory Storage Study. Tubers of the potato cultivar FL1867 were planted in April 2012 and covered with mesh cages before emergence. Each cage contained 4 plants. Plants in four of these cages were infested two weeks before harvest and plants in the remaining cages were infested one week before harvest. In this study each plant was infested by releasing 7 Lso-positive psyllids at the base of its stem. At harvest, tubers were sampled, at the stolon attachment point, for Lso quantification and stored at 4C.

After 2 months, 64 tubers were removed from cold storage (32 from plants infested 2 weeks before harvest and 32 from plants infested 1 week before harvest) and placed at room temperature. These tubers were sampled weekly, for five consecutive weeks, to quantify Lso titer, starting the day they were removed from cold storage. Samples were taken from around the stolon attachment point (next to at-harvest sampling point) to evaluate changes in Lso titer over time. All samples were maintained in a -80 freezer until they were extracted.

DNA extraction and Lso quantification. Tuber extractions were performed following a slightly modified DNeasy® Plant Mini Kit (Qiagen, Valencia, CA, USA) protocol. Immediately following

removal from -80C storage, finely chopped tuber samples (100-200 mg) were placed in liquid nitrogen and then ground in a Hard Tissue Grinder (VWR, Sugar Land, TX, USA) for 3 min (2x), using a 5mm ball bearing bead. Final DNA product was eluted in 100µl of the elusion buffer.

Comparative Ct method, also known as ΔΔCt, was performed to quantify Lso titers using an Applied Biosystems (Carlsbad, CA, USA) 7500 Real-Time PCR System. The reaction mix contained TaqMan Universal Master Mix (Applied Biosystems), 0.3 µM forward primer LsoF (Li et al 2009), 0.3 µM reverse primer HLBr (Li et al. 2006), and 0.25 µM HLBp TaqMan probe. Eukaryotic 18S rRNA (VIC/MGB probe, primer limited, Applied Biosystems) was our endogenous control. The amount of target was normalized to the Lso endogenous reference and quantified in relation to a calibrator which was created by amplification of a 1168bp fragment of the 16s rDNA region of Lso using OA2/OI2C primer set (Liefting et al. 2008). The amplicon was cloned using a TA cloning Kit (Invitrogen, CA, USA). The calibrator was created by serial dilution of quantified plasmid DNA with healthy plant DNA to contain 50,000 genome copies. Multiple plates were analyzed at the same time, using the plate study function of ABI default analysis software (see Rashed et al. (2013) for details).

Results and Discussion

Field germination study. A total of 636 seed potatoes collected from plants which were infested at different times throughout the field season were planted. Emergence success was estimated to be 31.3% (N = 199). Eight percent of plants emerged from these infected tubers (2.5 percent of total tubers planted) tested positive for the pathogen. However, the majority of these infected plants remained small and exhibited severe foliar ZC symptoms. Twelve of the 16 emerged plants (75%) sprouted from tubers of plants infested 1 and 2 weeks before harvest. This finding indicates that seed potatoes from the late-season infected plants are most likely to germinate and produce Lso-positive plants. Although time to emergence did not differ between non-infected controls and seed potatoes from the last infestation date, tubers from plants infested relatively earlier in the season had delayed emergence (Figure 1).

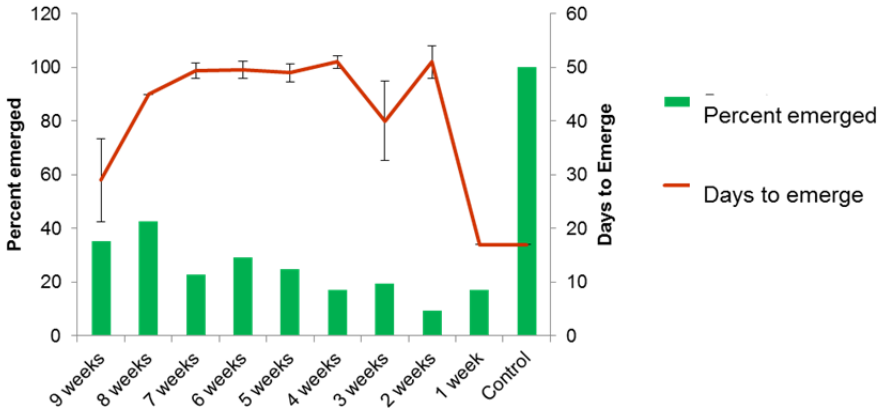


Figure 1: Illustration of the percent emergence and time to emergence in tubers of the plants infested on different weeks before harvest. Error bars represent ±SE.

The low sprouting rate of the infected tubers, coupled with delayed emergence of plants from these infected tubers suggest that there would be no significant epidemiological threat by seed-borne ZC in potato fields. This is because by the time infected plants emerge and grow to appropriate size, to be exposed to psyllids, healthy plants would have already been established and out grew these diseased seedlings. However, it may be a different scenario when it comes to volunteer potatoes. The sporadic emergence and presence of these non-cultivated plants would expose them to potato psyllids more effectively. Therefore, infected volunteer potatoes may act as a source for the pathogen in the absence of the cultivated crop. This is of a particular importance in light of recent surveys that reported peaks in psyllid numbers in natural vegetation as well as volunteers prior to crop emergence.

Rashed et al. (2011) demonstrated that all of the tubers from plants which had been infected for one week remained asymptomatic and tested negative for Lso. Although, only a small percentage of tubers from plants which had been infected for 2 weeks developed ZC symptoms, majority of them tested positive for the pathogen at harvest. Despite lack of symptoms in these late infestation dates and undetectable levels of Lso in plants infected for one week, the germination rates in both treatments were impacted in the following year. This indicates that pathogen-potato tuber interactions continues post-harvest and perhaps during storage.

Laboratory storage study. To evaluate this possibility we proceeded with our storage study which was conducted on tubers from plants which were infested 1 and 2 weeks before harvest. A higher percentage of tubers were Lso-positive after two months of storage in 42F, than those just after harvest (Figure 2a,b). This finding was despite the fact that we consistently found higher Lso titers at the point of stolon attachment, which was sampled at harvest, than other points around stolon attachment area sampled over time (Figure 3).

The percentage of the tubers that tested positive showed more than 150% increase in tubers of plants which were infested two weeks before harvest. Lso titer increased sharply over time when the infected tubers were moved into the room temperature (Figure 2a), a pattern confirmed by a significant positive correlation between Lso titer levels and sampling time (Spearman Rho corr. coef. = 0.56, $P < 0.001$). A similar pattern was observed in tubers of plants which were infected for 1? week. In this treatment, while all tubers tested negative for Lso at harvest, pathogen was detected in more than 3 percent of the tubers when they were tested after two months of storage. Pathogen titers peaked after 20 days of being placed in the room temperature and there was a positive correlation between Lso titer and sampling time following tuber removal from the storage (Spearman Rho corr. coef. = 0.47, $P < 0.001$). In both treatments percentage of tubers that tested positive for the pathogen did not show a significant change after 3 weeks of placement in the room temperature.

Overall, we showed that pathogen may arrive to tuber tissue as early as 6 days post plant infestation with the infective psyllids. However,

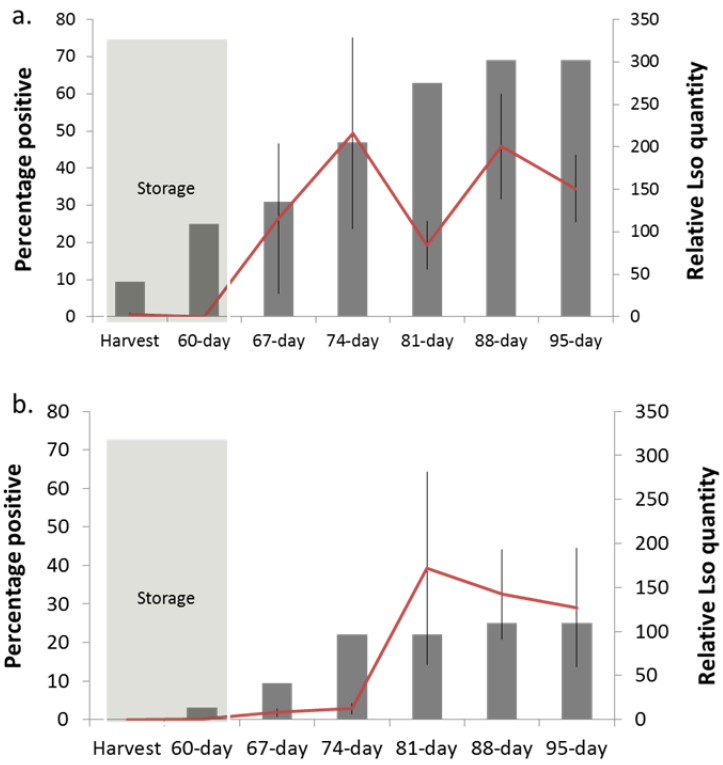


Figure 2: Changes in Lso titer (line) and percentage positive tubers (bars) in tubers of plants infested two weeks (a), and one week (b) before harvest. Shaded area highlights changes during storage and the rest of the graph illustrate variations at room temperature (73 F). Error bars represent ±SE.

Position	RQ Value
Stolon attach.	103.47
Inner 1	6.40
Inner 2	4.06
Side 1	6.16
Side 2	5.02

Figure 3: schematic representation of different sampling location and an example of their relative Lso quantification (RQ)

Lso remains at undetectable levels at harvest, an observation which suggests that seed certification at harvest may be more complicated in localities with high psyllid pressure late in the season, than previously thought. Since Lso titer increases significantly over time at room temperature, in these high risk areas Lso assessments of potato tubers could be conducted on a subset of tubers that have been placed in room temperature for at least 2 weeks. Transporting tubers to the storage immediately would delay pathogen development in late-season infections. This study highlights the importance of psyllid/Lso monitoring throughout the potato field season.

Acknowledgements
Thanks to our lab members for their help with different aspects of this project. Funding for this project was provided by USDA-SCRI (# 2009-511-20176).

Literature

Henne, D.C., Workneh, F., Wen, A., Price, J.A., Pasche, J.S., Gudmestad, N.C., and Rush, C.M. 2010. Characterization and epidemiological significance of potato grown from seed tubers affected by zebra chip disease. Plant Dis. 94: 659-665.

Li, W., Abad, J.A., French-Monar, R.D., Rascoe, J., Wen, A., Gudmestad, N.C., Secor, G.A., Lee, I., Duan, Y., and Levy, L. 2009. Multiplex real-time PCR for detection, identification and quantification of 'Candidatus Liberibacter solanacearum' in potato plants with zebra chip. J. Microbiol. Meth. 78: 59-65.

Li, W., Hartung, J. S., and Levy, L. 2006. Quantitative real-time PCR for detection and identification of *Candidatus* Liberibacter species associated with citrus huanglongbin. J. Microbiol. Meth. 66:104-115.

Liefting, L. W., Perez-Egusquiza, Z. C., Clover, G. R. G., and Anderson, J. A. D. 2008. A new '*Candidatus* Liberibacter' species in *Solanum tuberosum* in New Zealand. Plant Dis. 92:1474.

Munyaneza, J.E., J.L. Buchman, B.E. Heilman, V.G. Sengoda, and D.C. Henne. 2011b. Effects of zebra chip and potato psyllid on potato seed quality, pp. 37–40. In *Proceedings of the 11th Annual Zebra Chip Reporting Session*, eds. F. Workneh, A Rashed, and C.M. Rush. Fredric Printing, Aurora, CO.

Pitman, A., Drayton, G., Kraberger, S., Genet, R., Scott, I., Tuber transmission of `*Candidatus* Liberibacter solanacearum' and its association with zebra chip on potato in New Zealand. Eur J Plant Pathol. 129: 389-398

Rashed, A., Wallis, C.M., Paetzold, L., Workneh, F., and Rush, C.M. 2013. Zebra Chip disease and potato biochemistry: Tuber physiological changes in response to '*Candidatus* Liberibacter solanacearum' infection over time. Phytopathology 103, *in press*.

Rashed, A., Workneh, F., Paetzold, L., Gray, J., Wallis, C., and Rush, C.M. 2012. Zebra chip disease severity and '*Candidatus* Liberibacter solanacearum' titer load of the potato hosts inoculated throughout the field season. In *Proceedings of the 11th Annual Zebra Chip Reporting Session*, eds. F. Workneh, A Rashed, and C.M. Rush. Fredric Printing, Aurora, CO.

Wallis, C. M., Chen, J., and Civerolo, E. L. 2012. Zebra chip-diseased potato tubers are characterized by increased levels of host phenolics, amino acids, and defense–related proteins. Physiol. Mol. Plant Pathol. 78: 66-72.

Effects of Zebra Chip Disease on Potato Postharvest

Munyaneza, J.E. [1], Buchman, J.L. [2], and Henne, D.C. [3]

[1]USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951, USA
[2]Frito-Lay Inc., Rhinelander, WI 54501, USA
[3]Texas AgriLife Research, Weslaco, TX 78596, USA

Abstract
Zebra chip (ZC), an emerging and economically important disease of potato in the United States, Mexico, Central America, and New Zealand, is causing losses of millions of dollars to the potato industry, occasionally leading to abandonment of entire fields. The disease is associated with the bacterium "*Candidatus* liberibacter solanacearum" (Lso), transmitted to potato by the potato psyllid, *Bactericera cokerelli*. Little is known on the risk of ZC developing in tubers while in storage. This is especially a major concern for the Pacific Northwest, where over 50% of U.S. potatoes are produced and majority of which go into storage and where fields are subjected to Lso late infection because of the psyllid arrival into the potato fields late in the season. It has been determined that it takes about three weeks after Lso inoculation for ZC symptoms to develop in potato plants and tubers. Plants exposed to Lso-infected psyllids less than three weeks before harvest generally produce tubers without ZC symptoms. It is not known whether these symptomless tubers will eventually develop ZC in storage. Results of preliminary studies we conducted in 2010 and 2011 indicated that 20 and 60% of Atlantic tubers developed ZC symptoms after two and three months in storage, respectively. A follow up study in 2012 with Russet Burbank, Ranger Russet, Umatilla Russet, Russet Norkotah, Alturas, Atlantic, Pike, FL1867, and FL 1879 showed no significant ZC symptom development in any of the tested cultivars during five months of storage. These results suggest that although ZC may develop in storage, the problem may not be as serious as believed and further investigation is warranted.

Introduction
Zebra chip (ZC), a new and important disease of potato in the Americas and New Zealand, is causing losses of millions of dollars to the potato industry, often leading to abandonment of entire fields. This disease severely affects potatoes produced for both processing and fresh markets. The Pacific Northwest had so far been spared from ZC despite occurrence of the potato psyllid in this important potato producing region of the US. However, in September 2011, the disease was for the first time reported in Idaho and the Columbia Basin of Washington and Oregon, threatening more than 50% of the US potato production (Crosslin et al. 2012a,b). Although the impact of ZC has been substantially documented for processing and table potatoes, little is known of the risk of ZC developing in potato tubers while in storage. This is especially a major concern for potatoes produced in the Pacific Northwest, the majority of which go into storage and where potato fields are subjected to Lso late infection because of the psyllid colonizing the potato fields late in the season. It has been determined that it takes about three weeks after Lso inoculation for ZC symptoms to develop in potato plants and tubers (Buchman et al., 2012). Plants exposed to Lso-infected psyllids less than three weeks before harvest generally produce tubers without ZC symptoms. It is not known whether these symptomless tubers will eventually develop ZC symptoms in storage. In 2010 and 2011, we collected symptomless Atlantic potato tubers from a controlled late Lso infection study we conducted in both TX and WA. The tubers were stored under standard commercial conditions at the USDA-ARS facility in Wapato, WA and Frito-Lay facility in WI.

Samples of potato tubers were pulled from storage every 30 days and checked for ZC symptoms in raw and fried chips. Results indicated that 20 and 60% of the tubers developed ZC symptoms after two and three months in storage, respectively. However, other potato cultivars were not evaluated during these trials and Atlantic is not a storage variety.

The objective of this study was to further assess the risk of ZC developing into storage, especially for varieties commonly grown and stored in the Pacific Northwest where ZC in stored potatoes may become a serious issue.

Materials and Methods

A follow up study was conducted at both the Texas AgriLife research farm in Weslaco, TX and USDA-ARS research farm near Yakima in WA in the spring and summer of 2012, respectively to further assess the risk of ZC developing into storage, especially for varieties commonly grown in the Pacific Northwest. The potato varieties evaluated at each location included Russet Burbank, Ranger Russet, Umatilla Russet, Russet Norkotah, Alturas (fry varieties), Atlantic, Pike, FL1867, and FL 1879 (chipping cultivars). Atlantic was included to serve as a control as it had shown to develop ZC symptoms in storage in our preliminary study. The same field experimental design was used at both locations. Tubers were planted in cages each consisting of a tent-like narrow cage (6 ft wide X 15 ft long) designed to cover a single row of eight potato plants and made of fiberglass tree stakes for frame and USGR insect screen fabric as previously describe by Munyaneza et al. (2008) and Buchman et al. (2011a,b). Irrigation of the plants was accomplished by a drip tape that was buried in the hill just after planting. Cages were covered immediately after potato planting and the bottoms of the cages were buried in the ground to exclude unwanted insects. A pre-plant herbicide was used to control weeds in the cages. There were eight cages for each variety and plants in four cages were exposed to Lso-infected psyllids 2-3 weeks before harvest, whereas the remaining cages were not exposed to psyllids and served as controls. After harvest, tubers were checked for ZC symptoms and stored following standard commercial conditions at the USDA-ARS Wapato in WA. Potato samples from both plants exposed to psyllids and control plants were collected from storage every 30 days and assessed for ZC symptom development in raw and fried chips or fries.

Results and Discussion

No significant ZC symptoms were observed in any of the tested cultivars during five months of storage of potatoes grown in Texas, following harvest in May. No disease symptoms have been observed in the tubers collected from the WA trial and harvested in September, three months into the storage; the processing of the tubers at monthly intervals is continuing. These preliminary results suggest that although ZC may develop in storage, the problem may not be as serious as believed and a further investigation is warranted. We will repeat the study in 2013 to confirm the 2012 results.

Acknowledgements

We are grateful to Blaine Heilman, Francisco de la Rosa, Millie Heidt, Jacob Dixon, Jerry Gefre, and Venkat Sengoda for their invaluable technical assistance. Financial support for this work was partially provided by USDA-CSREES-SCRI (Project #2009-51181-20176).

References

Buchman, J.L., Heilman, B.E. and Munyaneza, J.E. 2011a. Effects of *Bactericera cokerelli* (Hemipetra: Triozidae) density on zebra chip potato disease incidence, potato yield, and tuber processing quality. J. Econ. Entomol. 104:1783-1792.

Buchman, J.L., V.G. Sengoda, V.G., and and Munyaneza, J.E. 2011b. Vector transmission efficiency of liberibacter by *Bactericera cokerelli* (Hemiptera: Triozidae) in zebra chip potato disease: effects of psyllid life stage and inoculation access period. J. Econ. Entomol. 104:1486-1495.

Buchman, J.L., Fisher, T.W., Sengoda, V.G., and Munyaneza, J.E. 2012. Zebra chip progression: from inoculation of potato plants with liberibacter to development of disease symptoms in tubers. Am. J. Pot. Res. 89:159-168.

Crosslin, J.M., Hamm, P.B., Eggers, Rondon, S.I., Sengoda, V.G., and Munyaneza, J.E. 2012a. First report of zebra chip disease and "*Candidatus* Liberibacter solanacearum" on potatoes in Oregon and Washington State. Plant Dis. 96:452.

Crosslin, J.M., Olsen, N., and Nolte, P. 2012b. First report of zebra chip disease and "*Candidatus* Liberibacter solanacearum" on potatoes in Idaho. Plant Dis. 96:453.

Munyaneza, J.E., Buchman, J.L., Upton, J.E., Goolsby, J.A., Crosslin, J.M., Bester, G., Miles, G.P., and Sengoda, V.G. 2008. Impact of different potato psyllid populations on zebra chip disease incidence, severity, and potato yield. Subtr. Plant Sci. 60:27-37.

Munyaneza, J.E., Buchman, J.L., Sengoda, V.G., Fisher, T.W., and Pearson, C.C. 2011. Susceptibility of selected potato varieties to zebra chip potato disease. Am. J. Pot. Res. 88:435-440.

Yield and Quality Consequences of TPP and Lso: Year 2 of a New Zealand Study

Pitman, A.R., Berry, N., Thompson, S., Taylor, N., Wright, P., Shah, F., Walker, M., Read, S., Beard, S., Jorgensen, N., Butler, R., and Thompson, S. New Zealand Institute for Plant & Food Research Ltd, Private Bag 4704, Christchurch, New Zealand.

Summary
Significant variation has been observed in the viability of seed tubers of potato due to tuber-borne inoculum of *Candidatus* Liberibacter solanacearum (Lso). In the United States, infected seed tubers usually fail to sprout whereas trials in New Zealand have shown a high frequency of emergence and subsequent growth of progeny plants. Here, results from Year 2 of field trials in New Zealand are presented, which confirm the high rate of emergence (>85%) from Lso-positive seed tubers and the subsequent production of progeny tubers of marketable quality. However, both the rate of emergence and growth of progeny plants were slower than from Lso-negative seed tubers, resulting in a reduction (> 50%) in marketable yield. Although no impact on specific gravity (SG) or zebra chip was associated with seed tuber inoculum, exposure to Lso-positive tomato potato psyllid (TPP) during the season had a dramatic impact on SG and increased zebra chip symptoms in harvested tubers upon frying.

Introduction
Lso is an unculturable phloem-limited bacterium that is widely accepted as the causal agent of economically important diseases in solanaceous plants, including zebra chip of potato in New Zealand (Liefting et al. 2009). Zebra chip is characterized by dark flecking throughout affected tubers that result in non-marketable tubers. Although Lso can be successfully transmitted to potato plants by TPP, *B. cockerelli* (Munyanaeza et al. 2007), the impact of seed tuber-borne inoculum on potato production appears to vary from report to report. In the United States, tubers with symptoms of zebra chip are generally unable to sprout (Henne et al. 2010). Recent estimates indicate that up to only 44% of ZC-affected seed tubers remain viable. In contrast, tubers infected with Lso in New Zealand frequently fail to develop characteristic zebra chip symptoms and are often able to sprout the following season (Pitman et al. 2011).

Here, we describe data from Year 2 of replicated trials undertaken in Pukekohe (North Island, New Zealand) and in Lincoln (South Island, New Zealand) to study further the translocation of Lso from infected seed tubers into the emerging crop and the impact of this inoculum on potato production. In contrast to Year 1 of the trial (in which Lso-negative TPP were added to cages), Lso-positive TPP were also added to cages during the season to compare the role of infected and non-infected TPP in development of disease symptoms.

Materials and Methods
Detection of Lso in seed tubers
Seed tubers (‘Moonlight’) collected from a field affected by TPP in Pukekohe were tested for infection with Lso (Haplotype A) using a single-tube seminested conventional PCR assay (Beard et al, 2012). A total of 300 Lso-negative and 300 Lso-positive seed tubers were used for planting in the field trials.

Trial design
Replicate trials were planted at Pukekohe and Lincoln in early November 2011. Each trial comprised 60 cages in an adapted row-column design (12 rows x 5 columns), which included five replicates of the following treatments using either Lso-positive seed tubers or Lso-negative seed tubers: No TPP (double-replicated), 25 Lso-positive TPP added to the cage for one week, 90 Lso-positive TPP added to the cage for one week, 25 Lso-positive TPP added to the cage for the entire experiment, 90 Lso-positive TPP added to the cage for the entire experiment. TPP was added in mid-January, during flowering, when insect numbers were at their highest in potato crops and conditions were considered conducive to proliferation of populations.

Measuring growth parameters and disease incidence
After planting, stem emergence, stem number and maximum stem height were measured weekly for 42 days (d). Stunting (defined by plants smaller than 20 cm) or death of plants was also recorded after 42 d to capture those emerged plants that had prematurely senesced or had growth defects. At harvest the total and marketable (including tubers >50 gm) yield (weight and number of tubers) for each plant was measured. The effect of the treatments on the dry matter of progeny tubers was determined by calculating the SG of tubers and the level of zebra chip in progeny tubers for each plant was calculated by measuring the discoloration of each tuber upon fry testing using a 1-10 scale.

Statistical analyses
The percentage of tubers not emerging was analysed using a Bernoulli Generalized linear model, with a separate analysis for each site. Marketable yields and numbers of tubers were analysed with analysis of variance (data for each site was treated separately). Before analysis, tuber numbers were transformed using sqrt(Num +0.5) to stabilise the variances. In the graphs, the axes are spaced to reflect this transformation, but are labeled on the back-transformed (count, score) scale, and the LSD bars relate to the means on the transformed scale, not the back-transformed scale.

Results and Discussion
Year 2 of the trial confirmed that the presence of Lso inoculum in seed tubers results in a significantly higher proportion of plants that fail to emerge when compared to Lso-negative seed tubers ($p<0.001$). The percentage that did not emerge was similar at both trial sites ($p=0.764$), and resulted in an average emergence rate of 84% from Lso-positive tubers versus 98% from Lso-negative tubers (Figure 1).

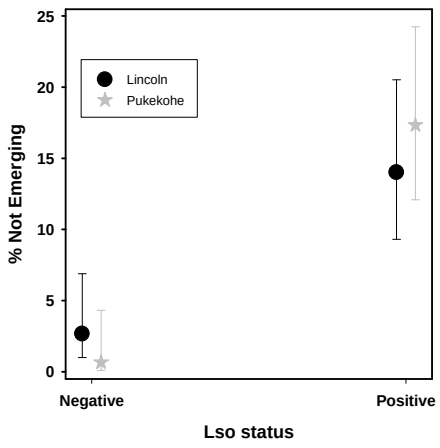


Figure 1. Percentage of tubers that had not emerged 42 d after planting. Negative, Lso-negative seed tubers; Positive, Lso-positive seed tubers.

Similarly, consistent with Year 1 of the trial, Lso-positive seed tubers showed delayed emergence ($p<0.001$). Furthermore, the growth of plants emerging from Lso-positive seed tubers was retarded compared to that of plants developing from Lso-negative seed tubers (Figure 2).

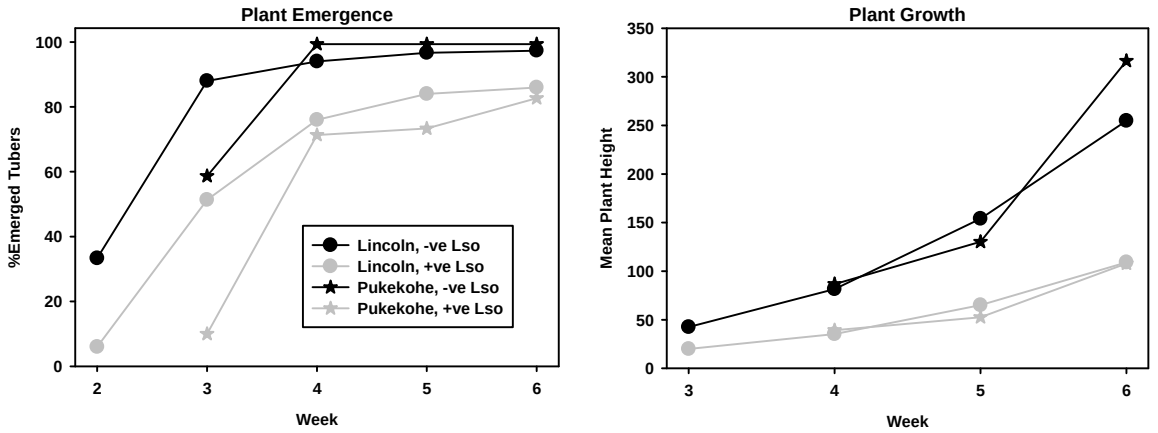


Figure 2. Stem emergence (%) and stem growth (mm) from potato seed tubers for a period of 42 d after planting.

Marketable yield (Figure 3) and marketable numbers of tubers (data not shown) were significantly higher for plants grown from Lso-negative seed tubers than from Lso-positive seed tubers, at both sites ($p<0.001$ in both cases).

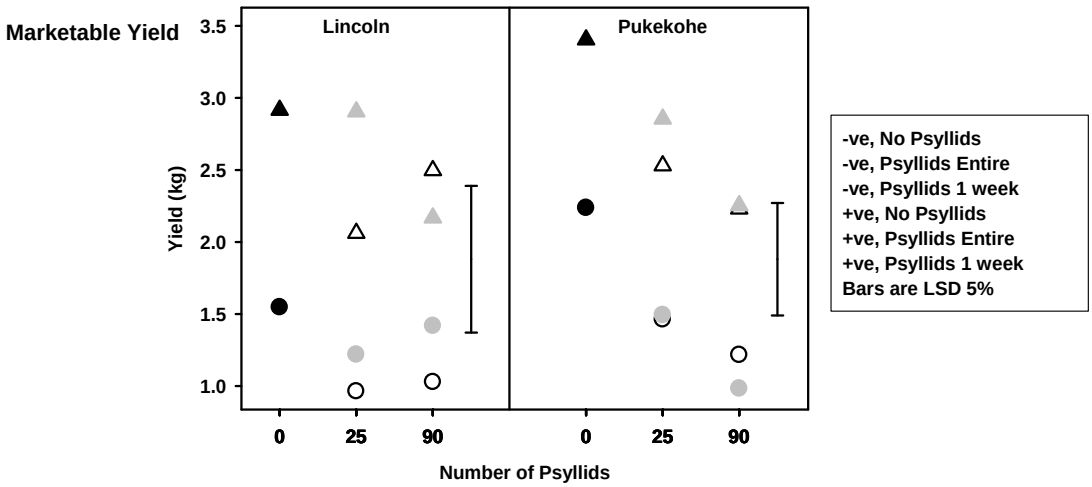


Figure 3. Marketable yield and number of tubers produced from Lso-positive seed tubers and Lso-negative tubers upon exposure to different densities of Lso-positive TPP for different durations.

Tuber numbers were twice or more as high for Lso-negative seed tubers as for Lso-positive seed tubers. Marketable yields were also double at Lincoln, and almost double at Pukekohe. The Lso-positive TPP treatment had relatively little effect on these measurements at Lincoln ($0.1<p<1$ for the overall effect and the interaction with tuber infection), but there were some differences relating to the TPP treatment at Pukekohe for both measurements ($p<0.001$ and $p=0.018$, respectively). The TPP treatment effect at Pukekohe was reasonably similar for both Lso-positive and Lso-negative tubers ($p>0.07$ for the interaction between tuber infection and psyllid treatment). Tuber numbers and yields were greatest for

the no TPP treatments, and tended to decrease with increasing psyllid numbers, with relatively less effect of exposure time.

At Lincoln, SG of harvested tubers did not vary strongly with Lso PCR status ($p>0.5$ for the overall effect and the interaction with TPP treatment), and there was also relatively little difference relating to the psyllid treatments ($p=0.135$). In contrast, at Pukekohe, SG varied with TPP treatment ($p<0.001$), and the treatment effects varied with PCR status ($p=0.004$ for the interaction). SG tended to decrease with increasing psyllid numbers. However, whilst for the no TPP and 25 TPP treatments SG was very similar for both Lso-positive and Lso-negative tubers, for the 90 TPP treatments SG was lower (at about 1.055) for Lso-positive tubers than for Lso-negative tubers (SG of 1.070) (Figure 4).

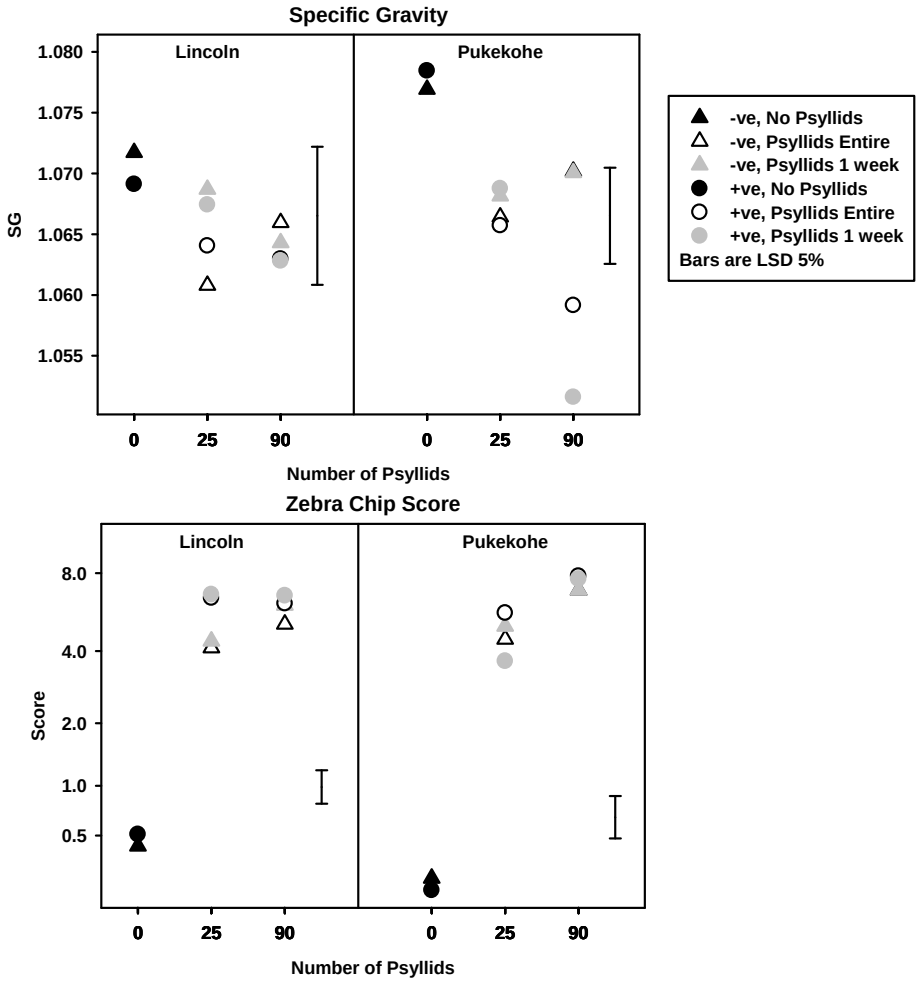


Figure 4. SG and zebra chip symptoms of marketable size tubers produced from Lso-positive seed tubers and Lso-negative tubers upon exposure to different densities of Lso-positive TPP for different durations.

Mean plant zebra chip score varied with PCR status at both sites ($p<0.001$), and also with TPP treatment ($p<0.001$ and $p=0.002$ at Lincoln and Pukekohe respectively) (Figure 4). The effect of PCR status was relatively similar regardless of TPP treatment ($p>0.2$ for the interactions). At both sites, zebra chip score was greater on average from Lso-positive seed tubers than from Lso-negative tubers, with a larger difference at Lincoln than at Pukekohe. For both sites, zebra chip score was much larger on average for

tubers from plots with psyllids, with ZC scores for No psyllid plots less than 0.6, but those from plots with psyllids having an average score above 3.6. Scores tended to be greater at Pukekohe upon treatment with 90 TPP than with 25 TPP. At Lincoln, there was a slightly lower zebra chip score for 25 psyllids with 1 week exposure than for the other psyllid treatments.

In summary, the presence of Lso inoculum (Haplotype A) in seed tubers was shown to reduce both emergence of tubers and the subsequent growth of plants during the season. This resulted in a lower yield of marketable tubers. These data are consistent with the first year of the trial. However, the high level of emergence remains in contrast to data for seed tuber sprouting in the United States. The reasons remain unknown for the differences observed, but may be related to titre or genotype of Lso, cultivar or environment.

In addition to the effect of seed tuber inoculum, exposure to Lso-positive TPP reduced yield (to a lesser extent), caused significant changes to SG and increased the incidence and severity of zebra chip. Previously, exposure to Lso-negative TPP had shown no significant impact on either SG or zebra chip of tubers. Neither was ‘psyllid yellows’ observed on plants exposed to Lso-negative TPP, suggesting that feeding of TPP alone does not play a role in expression of foliar or tuber symptoms associated with zebra chip. Given that raw tubers show little or no symptoms associated with infection by Lso and the impact of seed tuber inoculum observed in these field trials, seed certification (via diagnostics) will be critical to minimize the impact of Lso on potato production in New Zealand.

Acknowledgements
The authors would like to acknowledge the New Zealand Institute for Plant & Food Research Ltd for funding this project. This research was supported by grants from SCRI (2009-34381-20036) and the Texas Department of Agriculture.

References
Beard, S. S., Pitman, A. R., Kraberger, S., Scott, I. A. W. (2012). SYBR Green real-time quantitative PCR for the specific detection and quantification of '*Candidatus* Liberibacter solanacearum' in field samples from New Zealand". *European Journal of Plant Pathology* In press (DOI: 10.1007/s10658-012-0156-5).

Henne, D. C., Workneh, F., Wen, A., Price, J. A., Pasche, J. S., Gudmestad, N. C., Rush, C. M. (2010). Characterization and epidemiological significance of potato plants grown from seed tubers affected by Zebra chip disease. *Plant Disease* 94, 659-665.

Liefting L. W., Sutherland P. W., Ward L. I., Paice K. L., Weir B. S. & Clover G. R. G. (2009). A New '*Candidatus* Liberibacter' Species Associated with Diseases of Solanaceous Crops. Plant Disease, 93, 208-214. DOI: 10.1094/Pdis-93-3-0208.

Munyaneza, J. E., Crosslin, J. M., & Upton, J. E. (2007). Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with “zebra chip”, a new potato disease in Southwestern United States and Mexico. *Journal of Economic Entomology* 100, 656-663.

Pitman, A. R., Drayton, G. M., Kraberger, S. J., Genet, R. A., Scott, I. A. W. (2011). Tuber transmission of ‘*Candidatus* Liberibacter solanacearum’ and its association with zebra chip in potato. *European Journal of Plant Pathology* 129, 389-398.

Werren J. H., Windsor D. & Guo L. R. (1995). Distribution of Wolbachia among Neotropical Arthropods. Proceedings of the Royal Society of London Series B-Biological Sciences, 262, 197-204.

Insect Resistance and Other Factors Affecting Neonicotinoids in Potatoes

Trumble, J.T., Prager, S.M., and Kund, G. Department of Entomology, University of California, Riverside, Riverside, CA

Abstract

Neonicotinoid pesticides are used profusely in the management of potato psyllids (*Bactericera cockerelli*). However, certain factors raise concern about their use, including effect of irrigation regimes and potential resistance. We found that watering regime has a significant influence on both thiamethoxam and imidacloprid concentrations in plant tissue, with more water leading to reduced concentrations of insecticide in plant tissue. We tested for resistance to imidacloprid and found that newly collected populations from southern Texas require significantly higher doses to achieve 50% mortality than those colonies maintained in the lab. Moreover, we found that there is no longer a behavioral response to imidacloprid at any dose, which suggests that the previously observed repellent effects have been lost. In response to these findings, we suggest that use of neonicotinoid pesticides needs to be carefully managed.

Introduction

Current potato psyllid management relies nearly entirely on the application of a small group of pesticides. This group includes two neonicotinoid compounds, imidacloprid (Admire) and thiamethoxam (Platinum). Typically, the neonicotinoid compounds are applied at or around planting in order to provide early protection against the psyllid. In Texas, imidacloprid is ubiquitous; Guenther et al. (2012) report its use on over 90% of fields in 2011. High rates of use were also reported for Kansas, Nebraska where it was applied to 50% of fields and California where, in 2010, it was used on approximately 42% of planted potato acreage. While reported use of thiamethoxam is less than that of imidacloprid, it fills a similar niche and is also the insecticidal component of at least one common seed treatment (CruiserMaxx). Additionally, thiamethoxam belongs to the same IRAC mode of action group (4-A) as imidacloprid, which suggests a substantial chance of cross-resistance. High usage of neonicotinoid insecticides is also reported for tomatoes in California. In 2010, for instance, over 20,000 acres were treated with a neonicotinoid compounds. Additionally, there was a large increase (22%) of imidacloprid use between 2009 and 2010.

Previous work by Liu and Trumble (2007) demonstrated differences between the concentrations of imidacloprid required to kill 50% of *B. cockerelli* nymphs (LC_{50}) collected in California and Texas (Fig 1.). Treatment with imidacloprid has also been shown to alter behaviors including probing, cleaning, resting and walking in colonies collected in Texas but maintained in the lab for an extended period of time (Butler et al. 2011). Similarly, *B. cockerelli* have been shown to exhibit behavioral responses to imidacloprid (Butler et al., 2011 and 2012). In particular, Butler et al. (2011, 2012) report a reduction in feeding on imidacloprid treated plants.

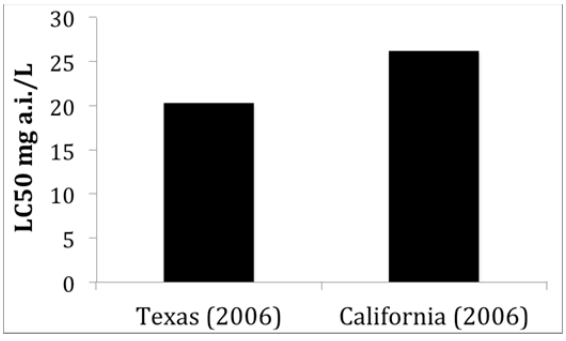


Fig 1. LC_{50} at 10 days for *B. cockerelli* nymphs from a Texas and a California colony after 10 days of exposure to imidacloprid.

Materials and Methods

We examined how the amount of water applied to soil influences retention of imidacloprid and thiamethoxam in plant tissue. Potatoes were planted in pots and treated with 550 ml of either imidacloprid or thiamethoxam. Following treatment, plants were maintained for 70 days and systematically watered at one of three volumes (250, 500 and 1000 ml). Following the 70-day period, insecticide concentrations were measured using commercially available ELISA kits.

Since nymphs do not fly, they are more suited for constant exposure bioassays. Moreover, previous work has been conducted on nymphs, and so testing nymphs allows for comparisons among studies. The Trumble group has an established protocol for performing mortality bioassays and calculating LC_{50} values in potato psyllid nymphs. We used that same protocol in to compare LC_{50} values between the previously examined colony of psyllids collected in Texas, and a colony established from psyllids collected in the Texas in the spring of 2012. In the testing protocol, tomato plants were treated with multiple rates of thiamethoxam (24, 48, 96, 192 ml/L) including the equivalent of maximum field rate via soil drench. As a control, plants were also treated with water. Following application, we allowed the pesticide to distribute throughout the plant for 5 days before beginning bioassays. After 5 days, we hand transferred 20-25 2^{nd} or 3^{rd} instar nymphs to plants using a paintbrush. Once nymphs were transferred, plants were examined daily, and the numbers of live nymphs, dead nymphs, and adults (when applicable) were recorded.

In order to test if the behavioral response of potato psyllids to imidacloprid previously observed (Butler et. al, 2011 and 2012) was still present in the 2012 Texas population, we conducted behavioral assays. Assays were performed using the methods of Liu and Trumble (2004). Again, this consistency among methods allowed for comparisons with previous studies. In summary, assays were conducted on whole intact leaves treated with rates to match LC_{50} assays. Leaves were placed in arenas created by layering a Plexiglas rectangle, the test leaflet, a foam insert with a hole cut in it, and a clear plastic cover. Newly emerged adult females were introduced into the arena, and allowed to adjust to the microenvironment for 5 minutes. Observations began and continued for 15 minutes during which the behaviors cleaning, feeding, jumping, resting, off leaf (exiting or abandoning the leaf surface), walking, and probing were recording using the Noldus® Observer program.

Results and Discussion

Retention experiments suggest that there is a significant effect of both amount of water and time since application on imidacloprid levels in plant tissue (Water: $F_{2, 153}=22.7$, $p<0.001$; Time: $F_{7, 146}=9.9$, $p<0.001$; Time*Water: $F_{7, 146}=4.1$, $p<0.001$) (Fig 2). The lower watering rates were not significantly different, but the 500 ml differed from 1000 ml, and the 1000 ml rate differed from 250ml. A similar overall trend was observed with thiamethoxam (Fig 2), although, no statistical analysis was possible. In comparison, it appears that thiamethoxam levels decrease more substantially over time than levels of imidacloprid. This confirms the idea that thiamethoxam is more mobile in the soil. However, with both insecticides it is clearly important not to over-irrigate for risk of lowering concentrations below effective levels.

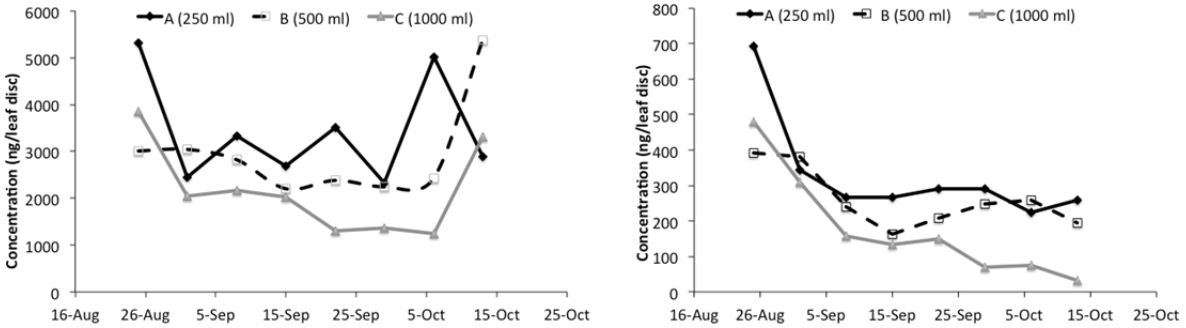


Fig 2. Mean concentration of thiamethoxam (left) or imidacloprid (right) in plant tissue over time when watered at three different rates.

We conducted bioassays to determine if resistance to imidacloprid has developed in populations of potato psyllids from Texas. This was accomplished by comparing a colony of potato psyllids originally collected near Weslaco, Texas but maintained in the lab for over six years, to a colony newly collected from the same area (Edinburgh, TX). We first examined LC_{50} values for the two colonies and found that a substantially higher dose was required to kill 50% of nymphs in the colony from 2012 than from the lab maintained colony (Fig 3). Moreover, the percent mortality differed at each dose ($F_{3, 72}$=14.68, p<0.001) and at every dose the mortality was lower in the lab maintained colony (Fig 4). The colonies also differed with respect to mortality over time (Fig. 5). As expected, mortality increased over time ($\chi^2_{1,78}$=191.14, p<0.001). Most important, there was a significant difference between colonies ($\chi^2_{1,78}$=373.79, p<0.001) and an interaction of dose and colony ($\chi^2_{3,76}$=78.99, p<0.001). Collectively, this suggests that the colony collected in 2012 has developed resistance to imidacloprid. This interpretation is also supported by the observation that the Texas 2012 colony also had a higher LC_{50} than psyllids collected from Texas when tested in 2006. Interestingly, the laboratory colony appears to have retained whatever level of resistance it previously possessed, as the LC_{50} when tested in 2012 is similar to that from 2006. Finally, we conducted behavioral bioassays to determine whether the previously observed responses to imidacloprid were present in the colonies collected in 2012, and also compared these results to laboratory colony to determine if the reduced mortality in the 2012 colony was due to behavior (Table 1). We found that the 2012 colony fed less often, although for similar duration, than the lab maintained colony. Additionally, there were more instances of cleaning and leaving the leaf on the newly collected colony. However, there was no dose effect with respect to any of these behaviors. It is therefore likely that while some behaviors may differ among populations of *B. cockerelli*, the repellent effects of imidacloprid have been lost.

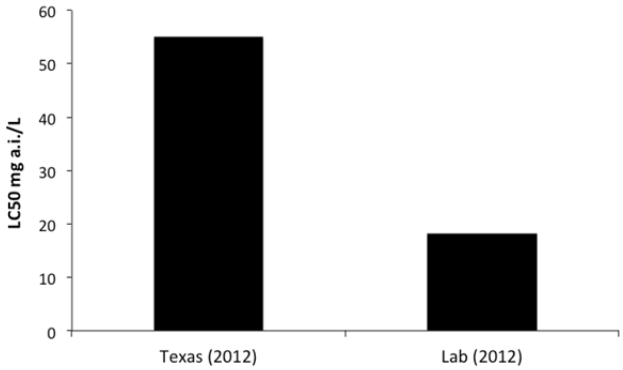


Fig 3. LC_{50} at 10 days for *B. cockerelli* nymphs from a Texas colony collected in 2012 and a colony collected in Texas, but maintained in the lab for over 5 years. Based on mortality at 10 days of exposure to imidacloprid.

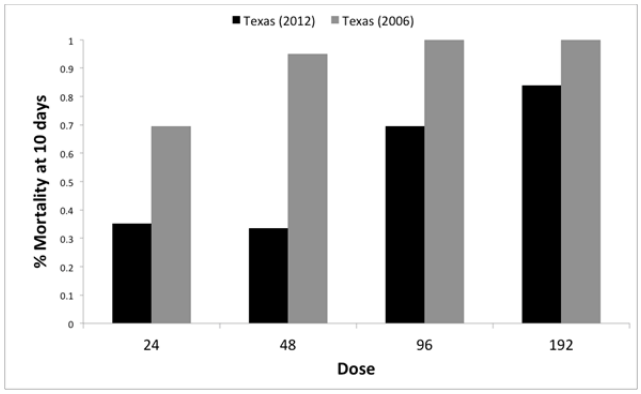


Fig 4. Percentage of mortality (number of dead nymphs) at 10 days on plants treated with a given dose of imidacloprid. Black bars are nymphs collected from the field in Texas in 2012. Grey bars are nymphs from colonies collected in Texas and maintained in the lab for over 6 years.

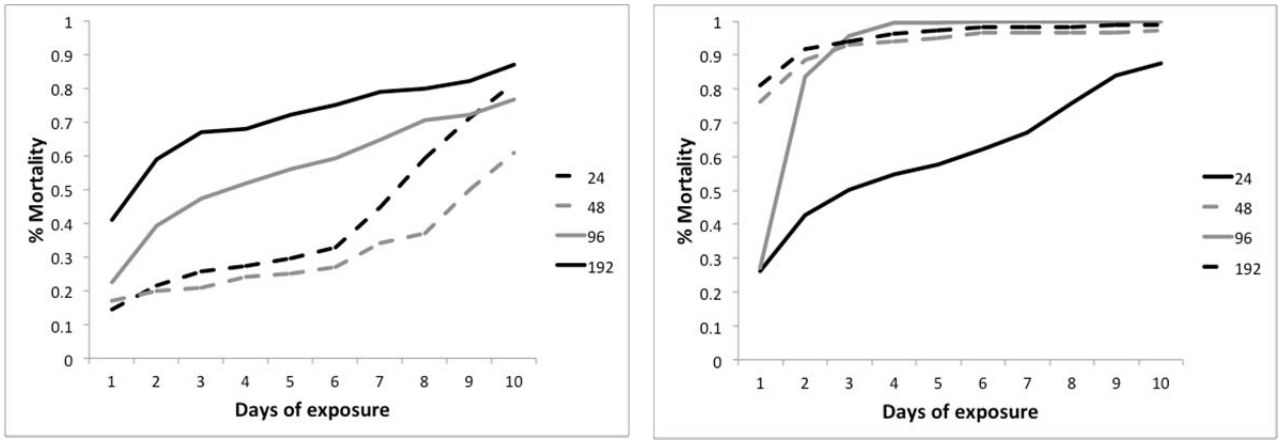


Fig 5. Mortality over time for potato psyllid nymphs from a colony of maintained in the lab for over 6 years (left) and from a colony collected in 2012 (right) on plants treated with one of four rates of imidacloprid.

Table 1: Comparison of potato psyllid behaviors between colonies (lab maintained and collected in 2012) and with respect to various dosages of imidacloprid. Significant differences at $p<0.05$.

Behavior	Dose effect (Frequency)	Colony effect (Frequency)	Dose effect (Duration)	Colony effect (Duration)
Walking	None	None	None	None
Resting	None	None	None	None
Probing/Feeding	None	Less in 2012	None	None
Cleaning	None	More in 2012	None	More in 2012
Jumping	None	None	None	Yes
Off leaf	None	More in 2012	None	More in 2012

In conclusion, neonicotinoid insecticides are frequently used in management of potato psyllids. However, their use must be carefully considered. First, overwatering is likely to reduce concentrations in plant tissue, possibly below useful levels. Second, we have found substantial evidence that resistance

to imidacloprid is developing in psyllids in southern Texas. Consequently, imidacloprid use will have to be strictly managed, including applying highest allowable rates and avoid foliar applications. This is especially the case in California and the northwest where resistance may not yet be present, and the zebra chip is only now becoming an issue. Finally, we do not know how application methods influence efficacy of neonicotinoid insecticides, but this is a topic we are currently investigating.

Acknowledgements
We thank S. Gilbert, N. Drew and B. Vindiola for assistance in conducting assays and in the greenhouse.

References
Butler, C.D., F. Byrne, M. L. Keremane, R.F. Lee, and J. T. Trumble. 2011. Effects of insecticides on behavior of adult Bactericera cockerelli (Hemiptera: Psyllidae) and transmission of *Candidatus* Liberibacter psyllaurous. J. Economic Entomology 104:586-594.

Butler, C.D., G.P. Walker and J.T. Trumble. 2012. Feeding disruption of potato psyllid, *Bactericera cockerelli*, by imidacloprid as measured by electrical penetration graphs. Entomologia Experimentalis et applicata 142: 247-257.

Liu, D. and J. T. Trumble. 2004. Tomato psyllid behavioral responses to tomato plant lines and interactions of plant lines with insecticides. J. of Economic Entomology 97: 1078-1085.

Liu, D. and J. T. Trumble. 2007. Comparative fitness of invasive and native populations of the potato psyllid (*Bactericera cockerelli*). Entomologica Experimentalis et applicata 123: 35-42.

Guenthner, J., Goolsby, J., Greenway, G. Use and Cost of Insecticides to Control Potato Psyllids and Zebra Chip on Potatoes. Southwestern Entomologist. 37(3): 263-270

Introducing DuPont™ Benevia™ and Verimark™ New Insect Control Products for the Management of Potato Psyllid and Zebra Chip Disease and Other Key Potato Pests in the US

Portillo, H.E., Brister, C.D., Castner, E.P., Medlin, C.R., McKinley, N.D., Wilson, G.D., Kral, C.W., Ramirez, H.T., Alvarez, J.M., and Annan, I.B. DuPont Crop Protection, Newark DE 19702

Abstract

DuPont™ Benevia™ and Verimark™ new Insect Control products contain Cyazypyr™ (aka Cyantraniliprole, DPX-HGW86), which is the second active ingredient in the anthranilic diamide class of chemistry, but the first to control a cross-spectrum of chewing and sucking pests, including whiteflies, leaf-feeding beetles, leafminers, fruit flies, psyllids, and lepidopteran pests. Benevia™ and Verimark™ have a unique mode of action activating ryanodine receptors (RyR) in insect muscle cells, which play a critical role in muscle function resulting in rapid feeding cessation. Cyazypyr™ has been assigned to group 28 (ryanodine receptor modulators) of the IRAC Mode of Action classification. Benevia™ and Verimark™ are reduced-risk insecticides, with a very low toxicity to vertebrates and non-target organisms. Cyazypyr™ moves in the plant xylem, providing root systemic and foliar translaminar activity. Benevia™ has been designed for foliar applications and optimized for leaf penetration and locally systemic movement. Verimark™ has been designed for soil applications and optimized for root uptake and crop safety to tender roots and shoots, protecting both older leaves and new growth. Multiple trials in the field and laboratory/greenhouse in Texas and Oregon over the last 5 years showed that Benevia™ applied at 0.088-0.134 lb ai/A provide superior potato yields and significant reduction in zebra chip disease (*Candidatus* Liberibacter solanacearum (psyllaurous)) as a result of excellent control of potato psyllid (*Bactericera cockerelli* Ŝulc, Hemiptera: Triozidae) adults and nymphs, being comparable to or better than current standards. Preliminary testing in 2012 with Verimark™ in-furrow applications at 0.134 lb ai/A alone or in tank mix with Vydate® at 2 lb ai/A in the US indicates that when used in a potato psyllid program, Verimark™ contributes to higher yields, potato psyllid control and lower zebra chip disease incidence. These results indicate that Cyazypyr™ will be a key component of potato psyllid and zebra chip management programs in potatoes. As of this reporting Cyazypyr™ is not registered for sale in the United States, registrations for use on potatoes as well as many other crops such as vegetables, fruit and row crops are pending and anticipated in 2013.

Introduction

Zebra chip (ZC) is an economically important disease of potato (*Solanum tuberosum* L.), caused by the bacterium *Candidatus* Liberibacter solanacearum (psyllaurous)) which is vectored by the potato psyllid, *Bactericera cockerelli* (Hansen et al. 2008). The disease was first documented in the United States in the year 2000 in potato fields in southern Texas (Munyaneza et al. 2007a, b). Infected potato fields have since been documented in several other states, including Nebraska, Colorado, Kansas, Wyoming, New Mexico, Arizona, Nevada, and California, Idaho, Oregon, and Washington ((Munyaneza 2010, Hamm et al. 2011). Plants infected with ZC produce tubers with discoloration, the symptoms become more pronounced when potatoes are fried, rendering those potatoes unmarketable (Munyaneza et al. 2007a, b). Rejection of potatoes by processors can be triggered when infected tubers are present at levels as low as 5%, however South Korea set a precedent for zero tolerance when banning potatoes produced in the Pacific Northwest after ZC was detected there in 2011 (Capital press, August 2012, Hamm et al. 2011). Vector management using insecticides is key to producing potatoes with acceptable levels of ZC in areas where the vector and pathogen are present. DuPont™ Benevia™ and Verimark™ new Insect Control products contain Cyazypyr™ (aka Cyantraniliprole, DPX-HGW86), which is the second active ingredient in the anthranilic diamide class of chemistry (Lahm et al 2005, Cordova et al. 2005, Lahm et

al 2007), but the first to control a cross-spectrum of chewing and sucking pests, including whiteflies, leaf-feeding beetles, leafminers, fruit flies, psyllids, and lepidopteran pests (Portillo et al. 2009, Annan et al. 2010, Stansly et al 2010). Benevia™ and Verimark™ have a unique mode of action activating ryanodine receptors (RyR) in insect muscle cells, which play a critical role in muscle function resulting in rapid feeding cessation (Cordova et al. 2005). Cyazypyr™ has been assigned to group 28 (ryanodine receptor modulators) of the IRAC Mode of Action classification (www.irac-online.org, IRAC 2012). Benevia™ and Verimark™ have very low toxicity to vertebrates, pose low risk to the environment and has low impact on non-target organisms (Dinter et al. 2012), the US Environmental Protection Agency (EPA) granted reduced-risk status to all candidate Cyazypyr™ products in May 2012. Cyazypyr™ moves in the plant xylem, providing root systemic and foliar translaminar activity. Benevia™ has been designed for foliar applications and optimized for leaf penetration and locally systemic movement. Verimark™ has been designed for soil applications and optimized for root uptake and crop safety to tender roots and shoots, protecting both older leaves and new growth. We report results with DuPont™ Benevia™ and Verimark™ new Insect Control products that show these new insecticides will be a key component of ZC management programs and results in superior yield and quality in potatoes in the Unites States.

Materials and Methods

Field trials to determine the efficacy of Benevia™ and Verimark™ were conducted at various locations in Texas and Oregon between 2009 and 2012. Trials were conducted by DuPont research personnel at the DuPont Rio Grande Research Station in Donna TX as well as by University researchers at the Research Farm, Texas AgriLife Research and Extension Center at Weslaco, TX and by private researchers in Hermiston, OR. Common local cultural practices used to grow potatoes in south Texas and Oregon were used, including irrigation, fertilization and fungicides and herbicide maintenance sprays. The chipping potato variety Atlantic was used in all trials in Texas, whereas a Russet variety was used in Oregon. The experimental design was a randomized complete block with 3-4 replications in all trials, plot size ranged from two 40” wide rows by 15’ long to four 40”-rows by 75’ long. Insecticide applications were made using back pack or tractor-mounted sprayers. At planting in-furrow applications were delivered using 20-30 gallons per acre, foliar spray applications were delivered at 30-40 gallons per acre. Potato psyllid evaluations were made by sampling 10-100 leaves per plot and counting number of eggs, nymphs and adults, typically 7 days after each foliar application or 48-60 days after the in-furrow application. Tubers were evaluated at harvest for ZC by slicing 25-100 tubers per plot/replication and visually inspecting for symptoms. Damage was recorded as percent incidence. Although not reported here, tubers were sliced and fried in selected trials and an additional raw tuber evaluation was also planned in some trials after tubers are stored for 3 months after harvest. Yield data was recorded by weighing tubers in 15-100 ft of row/plot or by weighing 50-100 randomly collected per plot. Data was analyzed using ANOVA and means separated using the Least Significant Difference method at p=0.05.

Results and Discussion

Multiple trials in the field and laboratory/greenhouse in Texas and Oregon over the last 5 years showed that Benevia™ applied at 0.088-0.134 lb ai/A (13.5-20.5 oz product/A) provided superior potato yields and significant reduction in zebra chip disease as a result of excellent control of potato psyllid adults and nymphs, being comparable to or better than current standards. Direct comparisons of Benevia™ with Radiant at 6 oz/A or Movento at 5 oz/A showed that although potato psyllid control was similar, plots treated with Benevia™ resulted in significantly or numerically lower zebra chip levels, indicating Benevia™ may be providing faster control or preventing adult psyllids from feeding long enough to

transmit the bacterium. Cyazypyr™ has demonstrated excellent reduction in transmission of other insect vectored diseases such as tomato spotted wilt (TSWV) virus vectored by tobacco thrips (*Frankliniella occidentalis*) and Western flower thrips (*F. occidentalis*) (Jacobson and Kennedy, 2011) and Cucurbit yellow stunting disorder virus (CYSDV) in melons and Tomato yellow leaf curl virus (TYLCV) in tomatoes vectored by the silverleaf whitefly (*Bemisia tabaci*), among others (Castle et al 2009; Portillo et al., 2009; Schuster et al., 2009; Stansly et al., 2010). Use of Benevia™ as the first two applications of a season long program showed that programs with Benevia™ resulted in higher yields and lower ZC incidence versus similar programs where Benevia™ was substituted by Movento. Preliminary testing in 2012 with Verimark™ in-furrow applications at 0.134 lb ai/A (13.5 oz product/A) alone or in tank mix with Vydate® at 2 lb ai/A in the US indicates that when used in a potato psyllid program, Verimark™ contributes to higher yields, potato psyllid control and lower zebra chip disease incidence. Cyazypyr™ products have a novel mode of action on sucking pests and has been found to lack cross resistance on key pests such as green peach aphid (*Myzus persicae*), cotton aphid (*Aphis gossypii*) (Foster et al 2011) and silverleaf whitefly (*Bemisia tabaci*) (Caballero et al. 2013). These results indicate that Cyazypyr™ will be a key component of potato psyllid and zebra chip management programs in potatoes. As of this reporting Cyazypyr™ is not registered for sale in the United States, registrations for use on potatoes as well as many other crops such as vegetables, fruit and row crops are pending and anticipated in 2013.

Acknowledgments

We thank the following researchers and organizations that contributed to some of the work presented here or that have helped our understanding of Cyazypyr™ : Dr. T. X. Liu, formerly with Texas A&M Agrilife Research Station, Weslaco TX, Dr. John Goolsby, formerly with Texas A&M Agrilife Research Station, Weslaco TX, Dr. Don Henne, Texas A&M Agrilife Research Station, Weslaco TX, Dr. Raul Villanueva, Texas A&M Agrilife Research Station, Weslaco TX, Dr. John Trumble and team, University of California, Riverside, USA, Dr. Silvia Rondon, Oregon State University, Hermiston OR, Dr. Joseph E. Munyaneza, USDA-ARS, Yakima Agricultural Research Laboratory Dr. Erik Wenninger, University of, Idaho and all DuPont employees that worked with Cyazypyr™ insecticide around the world.

References

Annan, I.B., Portillo, H.E., Lund, A.E., and Thompson, M.E. 2010. DuPont Cyazypyr™ insecticide (DPX-HGW86, Cyantraniliprole): unique product for premium pest control and agronomic plant protection, in Entomological Society of America National Meeting, December 14, San Diego, CA, Entomological Society of America, Lanham, MD.

Caballero, R., Cyman, S., Schuster, D.J., Portillo, H.E. and Slater, R. 2013. Baseline susceptibility of *Bemisia tabaci* (Genn.) biotype B in southern Florida to Cyantraniliprole. Crop Protection 44, 104-108.

Castle, S., Palumbo, J., Prabhakerc, N. 2009. Newer insecticides for plant virus disease management. Virus Research 141, 131–139.

Cordova, D., Benner E.A., Sacher M.D., Raul J.J., Sopa J.S., Lahm G.P. 2006. Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. Pestic Biochem Physiol 84:196–214.

Dinter, A., Samel A., Frost N.M., and Groya F.L. 2012. Cyantraniliprole (DPX-HGW86, DuPont™ Cyazypyr™) – a novel DuPont insecticide with selectivity towards beneficial non-target arthropods. Pesticides and Beneficial Organisms, IOBC-WPRS Bulletin Vol. 82, 2012 pp. 9-14.

Foster, S.P., Denholm, I., Rison, J.L., Portillo, H.E., Margaritopoulis, J., and Slater, R. 2012. Susceptibility of standard clones and European field populations of the green peach aphid, *Myzus persicae*, and the cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae), to the novel anthranilic diamide insecticide Cyantraniliprole. Pest Management Science vol. 68 issue 4. p. 629-633.

IRAC. 2012. IRAC MoA Classification Scheme, Version 7.2. IRAC International MoA Working Group. http://www.irac-online.org/content/uploads/MoA-classification.pdf. 23 p., (5 October 2012).

Jacobson, A.L. and Kennedy, G.G. 2011. The effect of three rates of Cyantraniliprole on the transmission of tomato spotted wilt virus by *Frankliniella occidentalis* and *Frankliniella fusca* (Thysanoptera: Thripidae) to *Capsicum annuum*. Crop Prot. 30, 512-515.

Hamm, P.B., S.I. Rondon, J.M. Crosslin, and J.E. Munyaneza. 2011. A new threat in the Columbia Basin of Oregon and Washington: zebra chip, pp. 1–5. In Proceedings of the 11th Annual Zebra Chip Reporting Session, ed. F. Workneh, A. Rashed, and C.M. Rush. San Antonio, TX (November 6–9, 2011).

Hansen, A.K., J.T. Trumble, R. Stouthamer, and T.D. Paine. 2008. A new huanglongbing species, '*Candidatus* Liberibacter psyllaurous' found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). Applied and Environmental Microbiology 74: 5862–5865.

Lahm, G.P., Selby T.P., Freudenberger J.H., Stevenson T.M., Myers B.J., Seburyamo G. 2005. Insecticidal anthranilic diamides: a new class of potent ryanodine receptor activators. Bioorg Med Chem Lett 15:4898–4906.

Lahm, G.P., Stevenson T.M., Selby T.P., Freudenberger J.H., Cordover D., Flexner L. 2007. Rynaxypyr™: a new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor activator. BioorgMed Chem Lett 17:6274–6279.

Munyaneza, J.E., J.M. Crosslin, and J.E. Upton. 2007a. Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "zebra chip", a new potato disease in southwestern United States and Mexico. Journal of Economic Entomology 100: 656–663.

Munyaneza, J.E., J.A. Goolsby, J.M. Crosslin, and J.E. Upton. 2007b. Further evidence that zebra chip potato disease in the lower Rio Grande Valley of Texas is associated with *Bactericera cockerelli*. Subtropical Plant Science 59: 30–37.

Munyaneza, J.E. 2010. Psyllids as vectors of emerging bacterial diseases of annual crops. Southwestern Entomologist 35: 417–477.

Portillo, H.E., Annan, I.B., and Marcon, P.C. 2009. Cyazypyr™ (DPX-HGW86, Cyantraniliprole): a novel anthranilic diamide insecticide for control of whiteflies and other important arthropod pests. 5th International Bemisia Workshop, November 9–12, Guangzhou, China.

Stansly, P.A., Kostyk, B., and Riefer, R. 2010. Effect of rate and application method of Cyazypyr (HGW86) on control of silverfleaf whitefly and southern armyworm in staked tomato, 2009. Arthropod Manage. Tests, 35, E43.

Torac® 15EC: A New Tool for the Management of Potato Psyllids

Ludwig, S.[1], Samler, J.[2], Walston A.[3], and Adams, J.[3]. [1]Nichino America, Arp, TX; [2] Nichino America, Kennewick, WA 99337; [3]Nichino America, Wilmington, DE 19808

Abstract

Torac® 15EC will provide potato growers with a new mode of action for the management of many potato pests including: potato psyllids, Colorado potato beetle, and aphids. Efficacy trials conducted in south Texas have shown that Torac provides rapid knockdown of potato psyllids, which resulted in lower rates of zebra chip symptoms in potato tubers.

Introduction

In 2013, Nichino America will be introducing Torac® 15EC (tolfenpyrad) in US agriculture production. Torac has broad-spectrum activity against several economically important potato pests including potato psyllids, Colorado potato beetle, and aphids. Tolfenpyrad, the active ingredient in Torac, has been classified by the Insecticide Resistance Action Committee (IRAC) as Group 21A, which are the Mitochondrial Complex I Electron Transport Inhibitors (METI). These compounds work by inhibiting cellular respiration in the mitochondria. As a result, Torac causes rapid death of the target pests.

Torac is a contact, foliar insecticide. Direct application to the target pest is critical for maximum control. The product should be applied with an agricultural adjuvant in sufficient spray volume to achieve maximum results.

Torac has been in potato psyllid efficacy trials in south Texas since 2008. In only about half those years have the psyllid populations cooperated and enabled the Texas AgriLife cooperators to obtain usable data. We will summarize the results from efficacy trials conducted in 2008 and 2009 by Dr. Tong-Xian (T.-X.) Liu at the Texas A&M AgriLife Research and Extension Center at Weslaco.

Materials and Method

Dr. T.-X. Liu conducted small plot efficacy trials in 2008 and 2009 to screen insecticides against potato psyllids and the reduction of zebra chip in tubers. Both trials were conducted at the Texas A&M AgriLife Research and Extension Center at Weslaco using Atlantic potatoes. Trials were replicated 4 times.

In 2008, potatoes were planted on 18 December 2007. Foliar insecticides were applied 24 January, 7, 14, and 22 February 2008. Sprays were applied using 30 gallons per acre. Plants were sampled for the number of eggs and psyllid nymphs on 23 January, 6, 13, 21 February, and 3, 10, 24 March. To sample the psyllid population the number of eggs and nymphs from 10 leaves per plot were recorded. Plots were harvested 28 May.

In 2009, potatoes were planted on 09 December 2008. Foliar insecticides were applied 27 January, 11, 22 February, and 17 March 2009. Sprays were applied using 20 gallons per acre. Plants were sampled for the number of eggs and psyllid nymphs on 26 January, 10, 26 February, and 16, 30 March. To sample the psyllid population the number of eggs and nymphs from 10 leaves per plot were recorded. Plots were harvested 2 June.

Results and Discussion

In both the 2008 and 2009 efficacy trials potatoes treated with Torac had the fewest number of potato psyllids. Torac provided a greater level of control than Fulfill, Oberon, and Leverage in 2008 (Fig. 1a) and AgriMek, Oberon, and Leverage in 2009 (Fig. 2a).

In 2008, 100% of the potatoes harvested from the untreated plots showed Zebra chip symptoms. Torac treated potatoes had the lowest level of symptoms at 36% (Fig. 1b). The Fulfill and Leverage treatments had statistically higher levels of tubers with zebra chip symptoms than the Torac treatment.

In 2009, 96% of the potatoes harvested from the untreated plots showed Zebra chip symptoms. Eight percent of the tubers from the Torac treated plants exhibited zebra chip symptom (Fig. 2b). The Oberon treatment was the only insecticide treatment with significantly high levels of zebra chip symptoms.

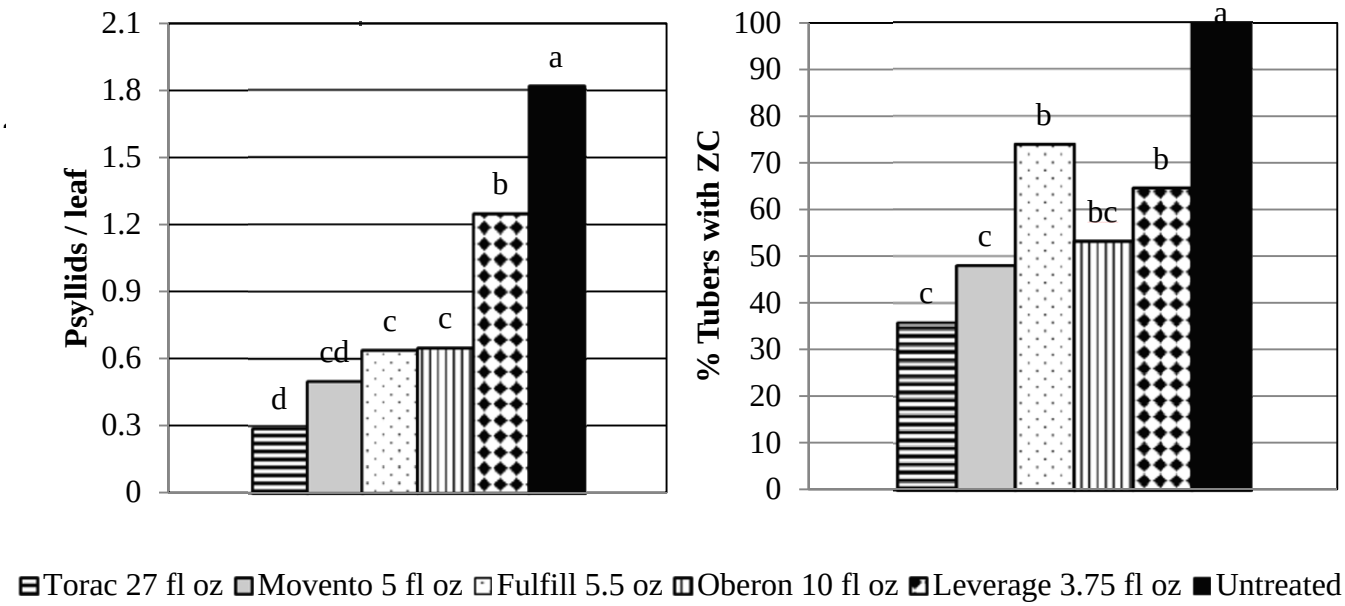


Figure 1. (a) Overall mean number of potato psyllid eggs and nymphs per leaf in the 2008 efficacy trial. (b) Percent of tubers showing zebra chip symptoms at harvest. Means followed by the same letters are not significantly different (Tukey's, P=0.05).

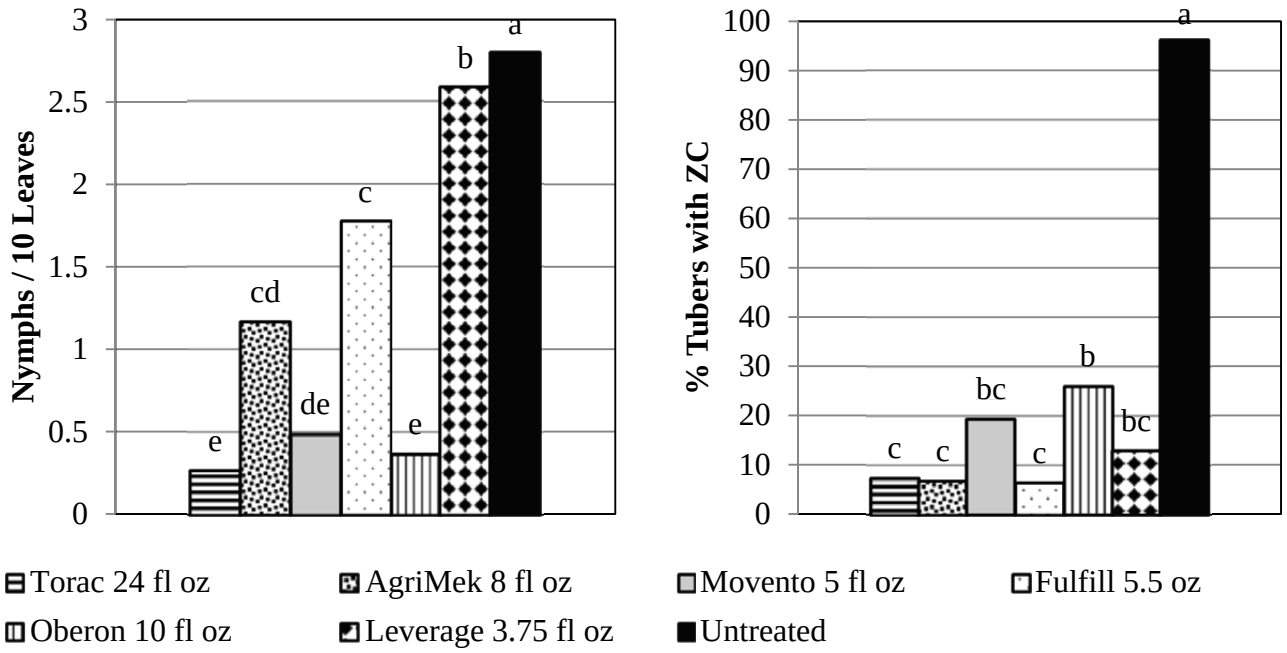


Figure 2. (a) Overall mean number of potato psyllid eggs and nymphs per leaf in the 2008 efficacy trial. (b) Percent of tubers showing zebra chip symptoms at harvest. Means followed by the same letters are not significantly different (Tukey's, P=0.05).

Acknowledgements

We would like to thank all of the cooperators who have conducted efficacy trials with Torac on potato psyllids: Tong-Xian Liu, Raul Villanueva, Don Henne, Jerry Michels, Tim Waters and Silvia Rondon. The trials presented in this report were funded by the IR-4 Project.

References

Liu, T-X. 2008. IR-4 Potato Zebra Chip Trial, Spring 2008. http://www.ir4.rutgers.edu/FoodUse/PerfData/2279.pdf

Liu, T-X. 2009. IR-4 Potato Zebra Chip Trial, Spring 2009. http://www.ir4.rutgers.edu/FoodUse/PerfData/2712.pdf

Management of Zebra Chip of Potato with Alternative Chemistries

French-Monar, R. D., Patton, A. F. III, and Serrato-Diaz, Luz M.

Department of Plant Pathology and Microbiology, Texas A&M AgriLife Extension Service, Amarillo, TX 79106, USA

Abstract
Zebra chip (ZC) is a potato disease caused by the bacterium '*Candidatus* Liberibacter solanacearum' and vectored by the potato or tomato psyllid, *Bactericera cockerelli*. Currently, the only effective approach to managing ZC is to apply insecticides. The focus of this study was to look at three non-insecticide alternatives: 1) target the pathogen by using antibiotics or chemistries that could end up being bactericidal; 2) trigger a plant defense response similar to a systemic acquired resistance (SAR) or induced systemic resistance (ISR) by applying chemistries with such potential; and 3) utilize nutrient supplements to offset the effects of ZC by supplying key nutrients lost or unavailable to the plant. Based on results from this year's study the potential exists for streptomycin sulfate, SAR compounds, and nutrient supplements, alone or in combination, to play a role in plant disease or stress management.

Introduction
Zebra chip, a disease causing economic losses to the potato industry in countries such as the United States, Mexico, Honduras, and New Zealand, is caused by the non-culturable, phloem-limited bacterium '*Candidatus* Liberibacter solanacearum' (Abad et al., 2009; Liefting et al, 2008; Liefting et al., 2009; Rehman et al, 2010). The potato psyllid, *Bactericera cockerelli*, is associated with ZC by vectoring the bacterium and causing psyllid yellows (Munyaneza et al., 2007; Hansen et al., 2008).

Efforts to manage ZC have centered on managing the potato psyllid through the use of insecticides. Efforts are underway to understand if planting date, tolerant varieties, repellents, and other alternatives may be suitable additions or alternatives to manage this disease. This study looked at three non-insecticide alternatives: 1) target the pathogen by using antibiotics or chemistries that could end up being bactericidal; 2) trigger a plant defense response similar to a systemic acquired resistance (SAR) or induced systemic resistance (ISR) by applying chemistries with such potential; and 3) utilize nutrient supplements to offset the effects of ZC by supplying key nutrients lost or unavailable to the plant.

In citrus greening (huanglongbing or HLB) caused by three other species of '*Ca*. Liberibacter'), leaf symptoms were reduced with the antibiotic tetracycline hydrochloride in several countries and a relatively successful control or management was obtained with penicillin carbendazin (Abdullah et al, 2009). Plant activators, such as acibenzolar-s-methyl, have been documented to suppress viruses such as *Tomato spotted wilt virus*, the oomycete *Pythium ultimum*, and the bacterium *Xanthomonas campestris* pv. *pruni* (Csinos et al, 2001). Enhanced nutrition programs are currently being used against HLB in Florida, with mixed results or anecdotal success.

For this study, the use of streptomycin sulfate (antibiotic), plant activators (ISR/SAR), potentially bactericidal compounds, and nutrient supplements were evaluated in on-farm experiments on potatoes against ZC and/or the causal agent, *Ca*. Liberibacter solanacearum.

Materials and Methods
Field Site. Field trial was conducted from 29 March to 31 July near Springlake, Texas, on a commercial potato production farm. This site had a previous history with both potato psyllids and *Ca*. Liberibacter solanacearum, and similar trials were conducted in 2009, 2010, and 2011. The soil was Tivoli fine sand.

A total of 10 treatments were arranged in a randomized complete block design and were replicated 6 times. Each replicate consisted of 2-row plots of 15 potato plants per row, for a total of 30 plants per replicate. Potato seed tubers 'Russet Norkotah 278' were used for this study. Potatoes were planted on 24 March, vines were killed on 23 July, and tubers harvested on 31 August.

Field Treatments. For this study, the treatment numbers and treatments were: 1) Untreated (Grower Standard); 2) Streptomycin Sulfate; 3) Potassium salts of phosphorous acid (K-Phite); 4) Acibenzolar-s-methyl (Actigard); 5) Salicylic acid (SAver), 6) KPX-B1 (Experimental micronutrients), 7) Renew (Potassium Polyphosphate and Phosphite, Salicylic acid, other nutrients); 8) Actigard + SAver; 9) Streptomycin Sulfate + SAver; and 10) KPX-B1 + Renew. Treatments were applied as foliar sprays on 5 June, 19 June, 3 July, and 18 July. All chemistries were applied with a backpack CO_2 sprayer at 35 psi using a 3 and/or 4 nozzle aluminum spray boom. Rates were consistent with label, adapted from label, or according to manufacturer recommendation. All other chemistries were applied by the producer as needed and consistent with commercial production practices. Herbicides applied were Sencor, Roundup, and Treflan. Insecticides applied were: Movento, Platinum, Epimek, and Fulfill. No foliar fungicides were applied. Seed was treated with Cruiser Maxx Potatoes (Insecticide and Fungicide).

Assays for Detection of Zebra chip or *Ca*. Liberibacter solanacearum. Zebra chip was confirmed by visual ratings of cut, raw tubers at harvest, and by tuber frying.

Results and Discussion

Field observations. Field plots were subjected to below average precipitation for the entire growing season, except for the second week in July. High temperatures were 10 degrees Fahrenheit higher and low temperatures were 7 degrees Fahrenheit higher than normal in April and May.

Yield. For overall total yield of all tubers harvested, no treatments had significantly higher yields than the untreated control. Numerically, all treatments except for Renew (treatment 7), had higher yields than the untreated control (Table 1). Untreated control yielded 243.2 cwt/A, the highest yield was 299.9 cwt/A for KPX-B1 + Renew (treatment 10), and lowest yield was 240.2 for Renew (treatment 7).

For 10-18 oz. tubers, there were two treatments with significantly higher yields than the untreated control: KPX-B1 + Renew (treatment 10) and Streptomycin Sulfate + SAver (treatment 9). For total yield of 4-18 oz. tubers (U.S. No. 1), only one treatment had a significant higher yield than the untreated control: KPX-B1 + Renew (treatment 10) (Table 1).

For tubers less than 4 oz., there were no significant differences amongst any of the treatments in comparison with the untreated control. Although six treatments (including the untreated control) had tubers over 18 oz. in weight, it was not significantly different due to low numerical values for such yields. Culls had no significant differences amongst treatments.

Zebra chip and *Ca*. Liberibacter solanacearum was present in these field plots (data not included). KPX-B1 + Renew (Treatment 10), Streptomycin Sulfate (treatment 2), Streptomycin Sulfate + SAver (treatment 9), SAver (treatment 5), Actigard + Salicylic acid (treatment 8), and the untreated control had ZC confirmed by the frying method (chipping). Other treatments were not positive but that may have been a result of sampling number rather than disease escape by these treatments that did not test positive.

Unlike the previous two years, both psyllid populations and zebra chip present in the field. ZC foliar symptoms were not observed which could indicate there was less stress on the plants this year, even with

the presence of the potato psyllid, the bacterium, and ZC on tubers. Other factors such as below average precipitation for almost all season long, high temperatures 10° F higher than normal in April and May, and lower temperatures that were 7° F higher than normal in April and May, may have contributed to a lesser tuber production in the untreated control or loss in nutrient availability or presence. Some of these chemistries may have allowed for potatoes to better withstand weather and other environmental pressures that did affect the untreated control.

The only treatment with higher significant yields for all U.S. No. 1 (4-18 oz.) tubers, in comparison with the untreated control, was KPX-B1 + Renew (treatment 10). This treatment consisted of micronutrients plus potassium polyphosphate and phosphite, salicylic acid, other nutrients. Individually, KPX-B1 (treatment 6), Renew (treatment 7) and SAver (treatment 5) did not perform significantly better than the untreated control. However, all three treatments are "included" in KPX-B1 + Renew (treatment 10) and only then were significantly higher yields observed in comparison with the control.

In trials conducted in 2009 with similar chemistries, applications of streptomycin sulfate gave significantly higher yields for total U.S. No. 1 tubers (4-18 oz.) in comparison to the control. That was not the case for 2012, although Streptomycin sulfate (treatment 2) was, numerically, the second highest yielding treatment for all U.S. No. 1 tubers. For tubers 10-18 oz. in weight, both KPX-B1 plus Renew (treatment 10) and streptomycin sulfate plus SAver (treatment 9) were significantly higher yielding than the untreated control. It would seem that the antibiotic streptomycin sulfate in combination with salicylic acid may also be a potentially viable treatment against ZC as it may target both the bacterium and the plant's ability to defend itself via SAR or ISR. Individually that was not the case for 10-18 oz. tubers.

Chemistries for this study were applied as foliar sprays and would be an ideal method for application of chemistries in the field especially if they can be translocated to the phloem. Unlike antibiotics and fungicides that can be applied to trees as injections, this is not an option for potatoes. For the first time, treatments were replicated replicating six times which may have allowed for a more sound statistical analysis. Although it may be difficult to label an antibiotic for foliar applications in potato field production systems, streptomycin sulfate is already labeled as a seed treatment on potato. This antibiotic is labeled for foliar use against fire blight on apples and pears.

Gottwald et al. (2012) consider the use of nutritional treatments as "inconsequential" for control of citrus huanglongbing. However, recent but preliminary research would indicate that a "nutrient cocktail" can rejuvenate an HLB-infected tree (Boyd, 2012). This foliar nutrition approach may need to be further looked at as a viable option for citrus growers in Florida affected by HLB (Spann et al., 2011) and may substantiate data obtained in this study.

The judicious use of insecticides in combination with plant tolerance or resistance, other practices such as those targeting the pathogen, activating a plant defense response, or improving plant health, could prove useful in an integrated disease control approach for ZC disease management in the near future.

Table 1. Total Yield, total yield of U.S. No. 1, yield of under 4 ounce and yield for culls/No.2 potatoes for 10 treatments

Treatment # and Description	Total Yield Cwt/A	U.S. No. 1 Cwt. Per Acre						
		Total Yield	4-6 oz	6-10 oz	10-18 oz	Over 18 oz	Under 4 oz.	Culls/ No.2
1-Untreated	243.2	141.5	35.1	53.2	53.2	1.5	27.1	73.0
2- Streptomycin Sulfate	298.0	188.1	39.8	70.4	77.9	0.0	38.1	71.8
3- K-Phite	277.6	179.8	34.2	63.7	82.0	0.0	41.4	56.4
4- Actigard	270.2	149.6	33.9	56.3	59.5	1.5	35.9	83.1
5- SAver	286.6	164.2	36.5	72.3	55.5	0.0	43.6	78.8
6- KPX-B1	265.8	150.9	42.6	51.1	57.2	1.2	39.9	73.8
7- Renew	240.2	149.7	36.2	55.4	58.1	0.0	42.3	48.2
8- Actigard + SAver	261.5	146.4	35.9	54.7	55.7	1.5	38.0	75.6
9- Streptomycin Sulfate + SAver	293.2	293.2	38.9	55.3	93.5	2.6	44.3	58.6
10- KPX-B1 + Renew	299.9	202.6	45.7	60.1	96.8	4.7	33.3	59.2
Average	273.6	166.1	37.9	59.3	68.9	1.3	38.4	67.9
L.S.D. (.05)	63.6	54.0	15.8	23.8	39.7	n.s.	16.9	31.0

Acknowledgements
Sincere appreciation goes to the Texas A&M AgriLife Research Potato Breeding Program (Dr. Creighton Miller, Jeff Koym, and Doug Scheuring) for providing space and logistical support. This research was supported by grants from SCRI (2009-34381-20036) and the Texas Department of Agriculture.

References
Abad, J. A., Bandla, M., French-Monar, R. D., Liefting, L. W., and Clover, G. R. G. 2009. First report of the detection of '*Candidatus* Liberibacter' species in zebra chip disease-infected potato plants in the United States. Plant Dis. 93:108.

Boyd, V. 2011. Research bolsters foliar nutrients' role in citrus greening management. The Grower. Online. Last accessed December 14, 2012. (http://www.thegrower.com/news/citrus-greening/Research-bolsters-foliar-nutrients-role-in-citrus-greening-management-131343848.html?view=all)

Abdullah, T. L., Shokrollah, H., Sijam, K., and Abdullah, S.N.A. 2009. Control of Huanglongbing (HLB) disease with reference to its occurrence in Malaysia. Afr. J. Biotechnol. 8:4407-4015

Csinos, A. S., Pappu, H. R., McPherson, R. M., and Stephenson, M. G. 2001. Management of *Tomato spotted wilt virus* in flue-cured tobacco with acibenzolar-*S*-methyl and imidacloprid. Plant Dis. 85:292-296.

Gottwald, T.R., Graham, J.H., Irey, M.S., McCollum, T. G., and Wood, B. W. 2012. Inconsequential effect of nutritional treatments on Huanglongbing control, fruit quality, bacterial titer, and disease progress. Crop Prot. 36:73-82.

Hansen, A. K., Trumble, J. T., Stouthamer, R., and Paine, T. D. 2008. A new Huanglongbing (HLB) species, "*Candidatus* Liberibacter psyllaurous", found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). Appl. Environ. Microbiol. 74:5862-5865.

Li, W., Abad, J.A., French-Monar, R.D., Rascoe, J., Wen, A., Gudmestad, N.C., Secor, G.A., Lee, I-M., Duan, Y., and Levy, L. 2009. Multiplex real-time PCR for detection, identification and quantification of '*Candidatus* Liberibacter solanacearum' in potato plants with zebra chip. Journal of Microbiol. Methods 78:59-65.

Liefting, L. W., Weir, B. S., Pennycook, S. R., and Clover, G. R. G. 2009. '*Candidatus* Liberibacter solanacearum', a liberibacter associated with plants in the family *Solanaceae*. Int. J. Syst. Evol. Microbiol. 59:2274-2276.

Rehman, M., Melgar, J. C., Rivera-C., J. M., Idris, A. M., and Brown, J. K. 2010. First report of "Candidatus Liberibacter psyllaurous" or Ca. Liberibacter solanacearum" associated with severe foliar chlorosis, curling, and necrosis, and tuber discoloration of potato plants in Honduras. Plant Dis. 94:376

Munyaneza, J. E., Goolsby, J. A., Crosslin, J. M., and Upton, J. E. 2007. Further evidence that zebra chip potato disease in the Lower Rio Grande Valley of Texas is associated with *Bactericera cockerelli*. Subtrop. Plant Sci. 59:30-37.

Spann, T. M., Schumann, A. W., Rouse, B., and Ebel, B. 2011. Foliar nutrition for HLB. Citrus Ind. June. Pages 6-10.

Area Wide Sampling for Potato Psyllids: Comparisons of Distributions and Scouting Strategies on Potatoes, Tomatoes, and Peppers

Prager, S.M., Butler, C., and Trumble, J.T., Department of Entomology, University of California, Riverside, Riverside, CA 92521

Abstract
Effective pest management is dependent on knowledge of levels of infestation, and consequently on sampling. *Bactericera cockerelli* can use multiple host plants including various crops. However, the risk posed by this pest, and the bacterium it vectors is not equivalent among these crops. We therefore compare distributions among different plant species to determine the applicability of sampling plans to different crops. We found that while some characteristics of potato psyllid distribution are consistent, such as aggregated distributions and preferences for the top two-thirds of plants, others are not. Further, preliminary data suggests that there are limitations to the psyllids ability to use different host plants. These factors lead us to conclude that crop specific sampling plans are required.

Introduction
Effective and economically sound management decisions are dependent on knowledge of a pest's presence within fields. This knowledge, in turn, relies on sound sampling plans and knowledge of the pest insect's spatial patterns. Potato psyllids are known to use multiple host plants, including bell peppers, tomatoes and potatoes. In California in 2012, over 220,000 acres of tomatoes and 1,400 acres of bell peppers were planted (USDA-NASS). In Texas, only 203 acres of tomato were planted. However, over 3,000 acres of bell peppers were planted (USDA-NASS). Both states had over 14,000 acres of potato planted (USDA-NASS). In California, these crops may be planted in close proximity of each other. Given that *Bactericera cockerelli* is a pest on all these plants, and that they may potentially act as a source for psyllids that move onto other crops, it is important to examine these psyllids on various crops. However, the risk posed by *B. cockerelli* is not consistent among crops. For instance, there is little to no evidence of zebra chip like symptoms on bell peppers. Additionally, distribution both within plants, among plants, and among fields is likely to vary with crop. Here we examine these factors, and consider the implications on sampling for potato psyllid management.

We sampled psyllids in fields of bell pepper, tomato, and potato in California. These data were then used to examine psyllid distribution among the different crops. In addition, we present preliminary data about the influence that natal host plant plays on oviposition. This latter information is important in understanding propensity to move between crops.

Materials and Methods
Sampling
Potato psyllids were sampled on entire pepper, tomato and potato plants in both experimental and commercial fields. All psyllid life-stages were counted and the location of plants within fields in addition to the vertical location, tissue (leaf, stem, fruit etc.) and side of leaf where psyllids were located was recorded. Detailed descriptions of methods, and fields are available in Butler and Trumble (2012) and Prager et.al (in press).

Host choice experiments

To determine whether natal plant has an influence on future oviposition decisions, we conducted a series of no-choice bioassays. In bioassays, three male-female pairs of adult *B. cockerelli* from either a colony reared on tomato or a colony reared on bell pepper were caged onto either bell pepper or tomato plants. This resulted in four treatments: psyllids reared on tomato caged onto tomato, psyllids reared on pepper caged onto pepper, psyllids reared on tomato caged onto pepper, and psyllids reared on pepper caged onto tomato. Psyllids were caged onto plants for three days after which cages and psyllids were removed and the total number of eggs was counted. Each combination was replicated a minimum of 15 times.

Results and Discussion

Sampling and distributions of psyllids

In order to understand the distribution of psyllids, and to effectively sample them, a proper sampling method is required. One method that is commonly used is yellow-sticky card trapping. We compared data from yellow sticky-cards to data from counts conducted on plants on the same day in each of 2009 (Fig 1a; $F_{1, 4}$=2.46, p=0.19) and 2010 (Fig1b; $F_{1, 6}$=4.3, p=0.08) and found no statistical correlation. This indicates that sampling must be conducted directly on plants and that we cannot rely on sticky cards when sampling for management decisions.

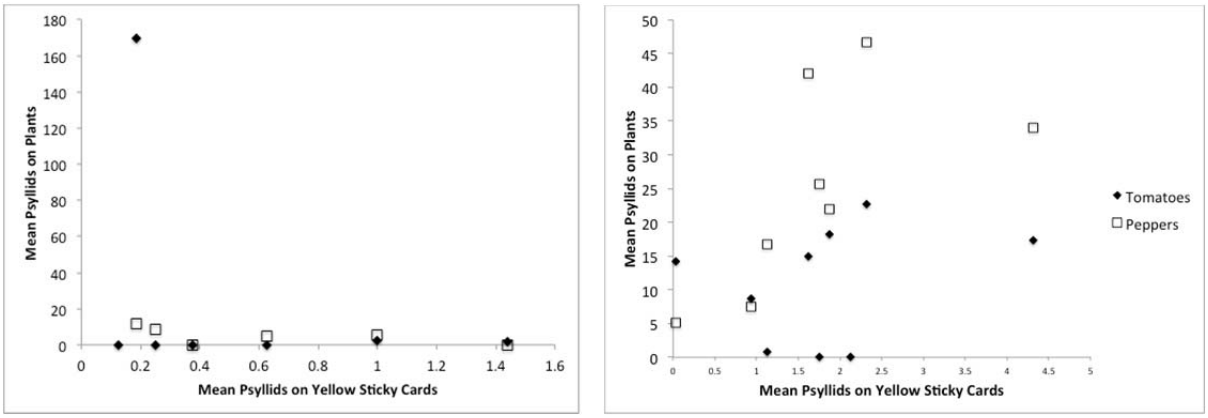


Fig 1. The relationship between potato psyllids collected on plants, and those collected on yellow sticky cards on the same day, in 2009 (left) and 2010 (right). Samples from tomato indicated by diamonds, sampled from peppers are squares.

Previous studies have indicated an "edge-effect" for potato psyllids in potato fields (Butler and Trumble, 2012). We compared those results to our sampling data for pepper and tomato fields to determine if this was a consistent trend among crops (Fig 2). Our results suggest that while an edge effect does occur in tomato, it is not present in pepper (Prager et. al, in press). Sampling in tomato and potato can thus concentrate on edges, while sampling in bell peppers will require a more comprehensive search. In previous studies, potato psyllids exhibit a clumped (aggregated) distribution within potato fields (Butler and Trumble, 2012). We found this to be a consistent trend among crops (Table 1) with aggregation being the observed on peppers and tomatoes as well.

A consistent trend is that psyllids were located predominantly in the upper two-thirds of plants (Fig 3.); a trend observed in all three crops examined. However, in peppers, when nymphs are examined, this trend changes slightly over time. Specifically, the proportion of nymphs in the top of the plant

increases, while that in the middle and bottom sections decreases (data not shown). This may reflect a choice by the psyllids, or may reflect an interaction between when eggs are initially laid and plant growth, with new growth occurring near the top of the plant. In determining where to sample for nymphs, it is also helpful to know where to look on a leaf. Butler and Trumble (2012) previously found that in potatoes the adaxial (bottom) of leaves was preferred (4.1 ± 0.50) versus the abaxial surface (1.8 ± 0.5) ($t = 3.48$, $df = 80.39$, $P = 0.0008$). This appears to be the case in tomatoes; however, when tested we found a marginally insignificant difference between the top (54.7 ± 137) and the bottom (94.3 ± 270) ($t=1.88$, $p<0.06$, $df=341.8$). We found no trend at all for leaf side preference in peppers (bottom: 10.5 ± 27; top: 14.7 ± 27) ($t=0.59$, $p=0.55$, $df=1109$). Thus one should concentrate on the bottom of leaves, but cannot completely ignore the topside. Overall, our results suggest that when sampling for psyllids, you can follow some relatively constant rules. These include examining the top and middle of plants, looking mostly on the edges of fields, and examining multiple locations within the field.

Literature suggests that *B. cockerelli* can use many host plants, some of which are planted in relative proximity of each other. Consequently, it is important to consider whether psyllids would be equally distributed among these crops. This issue has both sampling and insecticide application implications. We compared the number of psyllids sampled in adjacent fields of tomato and pepper during the same two-week periods of 2009 and 2010 and found no apparent associations (Fig. 4). One crop often was infested with psyllids when none or very little were found on the other. This suggests that potato psyllids may have a preference for some crops over others. Some preliminary results suggest that this may be the case, at least relative to natal host plant. Specifically, the data indicates that potato psyllids prefer to oviposit on the same plant species they were reared on, rather than an alternate (Fig. 5).

When all the results presented are considered, some general conclusions about sampling for management of potato psyllids can be generated. We conclude that crop specific sampling plans are necessary. These plans should have thresholds that are specific and proportional to the risk that *B. cockerelli* presents to the crop being considered. For management decisions, we suggest binomial-sampling schemes based on presence/absence. Such plans have already been developed and published for potato psyllids in potatoes (Butler and Trumble 2012), and in peppers (Prager et. al, in press). We presented a preliminary plan for tomato based around nymphs and eggs at infection levels of 27, 57 and 70% infestation. Stop lines for the tomato sampling plans, used for determining when to take management action (apply insecticides), are presented in figure 6. Finally, as detailed, we note that many elements of distribution of psyllids are similar across crops, but complete similarity cannot be assumed.

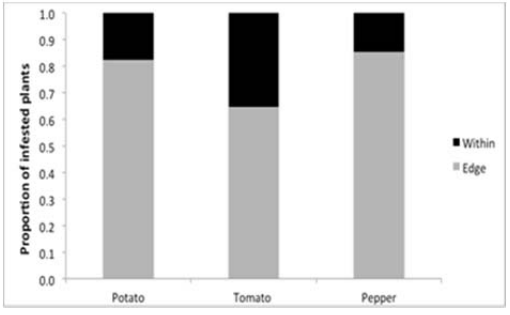


Fig 2. The number of infected plants on the edge and within fields of potato, tomato and pepper.

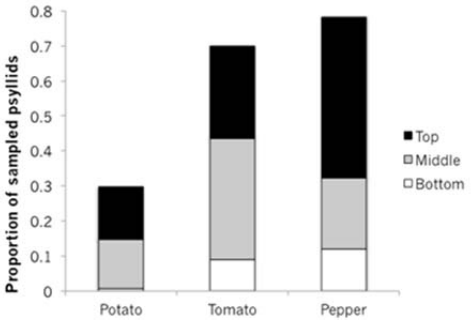


Fig 3. Proportion of psyllids collected on top, middle or bottom of plants.

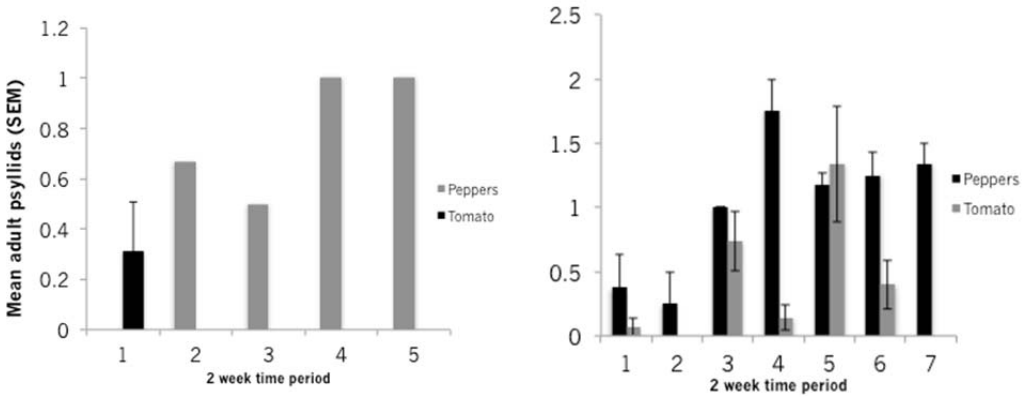


Fig 4. Mean number of psyllids sampled on pepper plants (black bars) and tomato plants (grey bars) during two-week periods in 2009 (left) and 2010 (right).

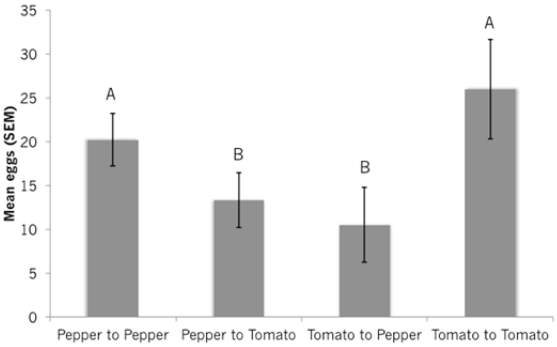


Fig 5. Mean number of eggs (standard error) oviposited when psyllids from colony reared on pepper or tomato are exposed to either tomato or pepper. Letters indicate significant differences at $p<0.05$.

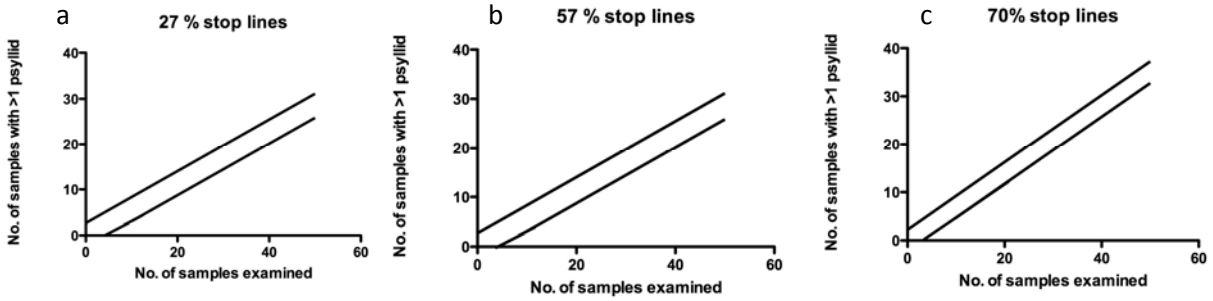


Fig 6. Stop lines for bionomial sampling plan for *B. cockerelli* in tomatoes. When the number of plants surveyed with at least one psyllid (nymph or egg) falls above the top line, take action. When the number falls below the lower line, no action is required. If between the lines, more sampling is required. Plans presented for 27% (a), 57 % (b) and 70% (c) infestation of fields.

References

Butler CD and Trumble JT, Spatial dispersion and binomial sequential sampling for the potato psyllid (Hemiptera: Triozidae) on potato. *Pest Management Science*; 142: 247-257

Prager SMP, Butler CD, Trumble JT, A sequential binomial sampling plan for potato psyllid (Hemiptera: Triozidae) on bell pepper (Capsicum annum). *Pest Management Science.* In press.

Table 1: Indices of spatial aggregation for potato psyllids in pepper, tomato and pepper fields.

Crop	*Year*	*Range of means*	*Green's index*	*Iwao's mean crowding regression*			*Taylor's power law*		
				a	*B*	r^2	*a*	*b*	r^2
Potato	*2009*	*0.08-7.2*	*1.16*	*4.83*	*2.35*	*0.79*	*0.89*	*1.46*	*0.99*
Potato	*2010*	*0.80-2.3*	*0.84*	*1.11*	*10.50*	*0.80*	*1.06*	*1.80*	*0.83*
Pepper (nymphs)	*2009 & 2010*	*2.5-50.5*	*38.27*	*0.49*	*1.57*	*0.99*	*1.9*	*1.63*	*0.89*
Tomato (nymphs & eggs)	*2009 & 2010*	*0.1-869.3*	*4.12*	*67.19*	*98*	*4.89*	*1.95*	*1.79*	*0.97*

Comparing Sampling Techniques and Challenges for Potato Psyllids Monitoring in the Columbia Basin of Oregon and Washington

Rondon[1], S.I. and A.F. Murphy [1]

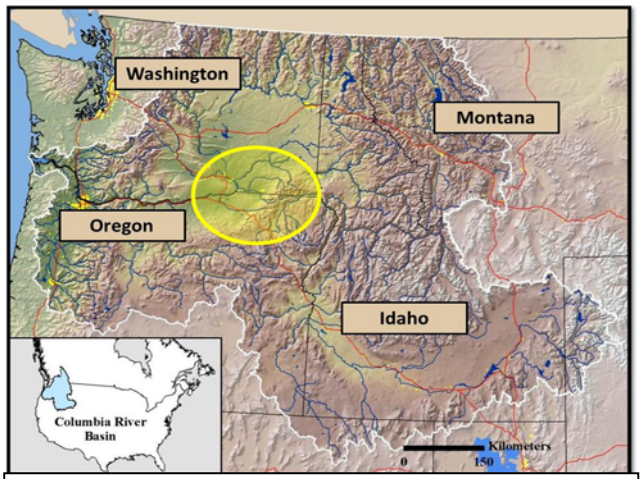
[1]Oregon State University, Hermiston Agricultural Research and Extension Center, Hermiston, OR 97838 USA

Abstract

In 2011, Zebra chip (ZC) was confirmed in the Pacific Northwest production area of Oregon and Washington; ZC was also confirmed in Idaho the same year. The research reported here was designed to determine how to best monitor the potato psyllid in the Oregon and Washington Columbia Basin region and hence successfully manage this insect-disease complex. Thus, in 2012, eight fields were sampled weekly using sticky cards, DVAC (inverted leaf blower) samples and leaf samples from June through September. At harvest, 100 tubers were collected from each field for ZC ratings using the 0-3 Texas scale. Sticky card placement, from the edge of the field to the center, was evaluated and location was found to have no significant impact on trap catches thus no edge effect was evident. DVAC and sticky card monitoring techniques were compared and DVAC sampling was slightly better than sticky cards. None of the sampling methods correlated with ZC ratings. Four different types of sticky cards were compared: 1) Pherocon AM, AlphaScents Yellow card, AlphaScents ACP card, and a standard 3 × 5 yellow card. These traps were placed 3 m apart at four different sites in a randomized order and collected weekly. The Pherocon AM card and the AlphaScents cards all performed significantly better than the standard 3 × 5 card. This paper only discusses the monitoring aspects of our comprehensive research program conducted in 2012 in Oregon and Washington. Future research must focus on improved approaches for vector monitoring and management.

Introduction

In 2011, Zebra chip (ZC) was confirmed in the Pacific Northwest production area of Oregon and Washington (Hamm et al. 2011). The same year, Idaho reported ZC damage in several commercial fields. These three states are responsible for over 50% of the U.S. potato production. Zebra chip is a serious disease caused by *Candidatus* Liberibacter solanacearum (Lso) and transmitted by the potato psyllids, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae). Because of the association of the psyllid with the bacterium, a comprehensive psyllid monitoring research program was needed in the Pacific Northwest. Previously, most of the information was coming from the southern states (e.g., Texas and California) where ZC has been a serious problem since the early 2000's (Henne et al. 2010). The research



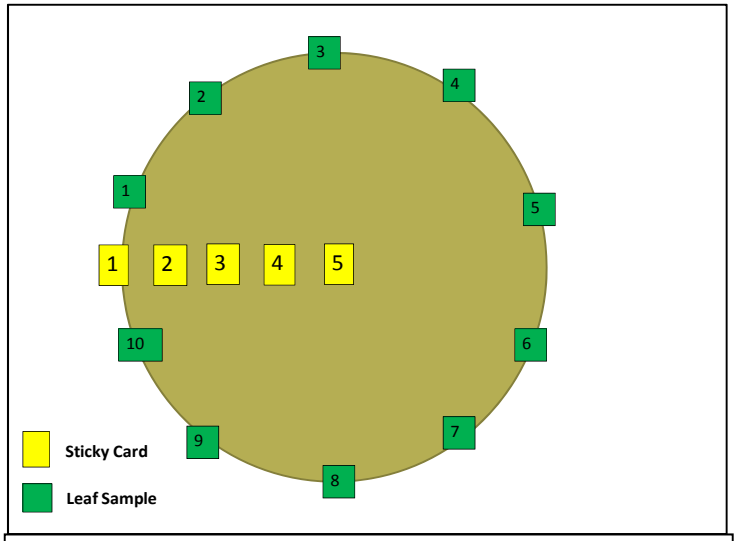
Yellow circle shows the Columbia Basin region in Oregon and Washington affected by ZC in 2011

reported here was designed to determine how to best monitor the potato psyllid in the Columbia Basin region and hence successfully manage this insect-disease complex and compare with methods used in Texas. Will the same techniques used in Texas work for the Columbia Basin? Will the unique landscape of the Columbia Basin region have any effect on potato psyllid population dynamics and distribution within fields? Will the differences between Texas and the Columbia Basin regarding crop phenology and potato psyllid arrival have an impact on potato psyllid management? Future research must focus on better estimation of ZC risk and timely application of chemicals and integrated management practices. This paper will only discuss the monitoring aspects of our comprehensive research program conducted in 2012 in Oregon and Washington.

Materials and Methods

Large scale monitoring system

Eight fields, three commercial fields in Oregon, three in Washington and two at the Hermiston Agricultural Research and Extension Center (HAREC) in Hermiston Oregon, were selected for our studies. Fields were selected based on 2011 history of ZC and psyllid populations. Following Goolsby et al. (2007) protocol, five yellow sticky cards (Cascade Ag. Services, Wenatchee, WA) were placed in each circle at even intervals from the field margin to the center; traps were collected weekly and brought to the laboratory at the HAREC for sorting and counting. Sticky cards are thought to be good indicators of migrating potato psyllids. Potato psyllid adults were removed weekly and stored for molecular assays to determine their degree of Lso infection. Leaf samples were also collected in each circle to account for nymphs and eggs. During the growing season, ten leaves from the perimeter and five leaves from around each sticky card were collected weekly. Adults also were collected using a DVAC (inverted leaf blower); sampling lasted 5 min, 1.5 – 3 m from the edge of the field (check how to use a DVAC http://oregonstate.edu/dept/hermiston/silvia-rondon). All eight circles were sampled from June to September. Each site followed standard commercial practices (i.e. fungicides, herbicides). At harvest, 20 tubers were collected from within 3 m of each sticky trap (n=100). Fifty tubers were sliced and rated within one month of harvest for ZC (stolon and bud ends) using the 0-3 Texas scale. The remaining 50 tubers were stored for subsequent rating. Weekly reports were sent via Potato Update http://oregonstate.edu/dept/hermiston/trap-reports, Potato Progress http://www.nwpotatoresearch.com/PR/Index.cfm, or directly to the participating growers. The pest management practices for each circle were recorded and correlated with insect trapping. Pest management data are not included in this report.



Sample distribution in a potato circle. Yellow sticky cards were placed from the edge (1) to the center (5) to estimate adult potato psyllid populations; ten leaf samples around the circle and five leaf samples next to each sticky card were taken to sample nymphs and eggs.

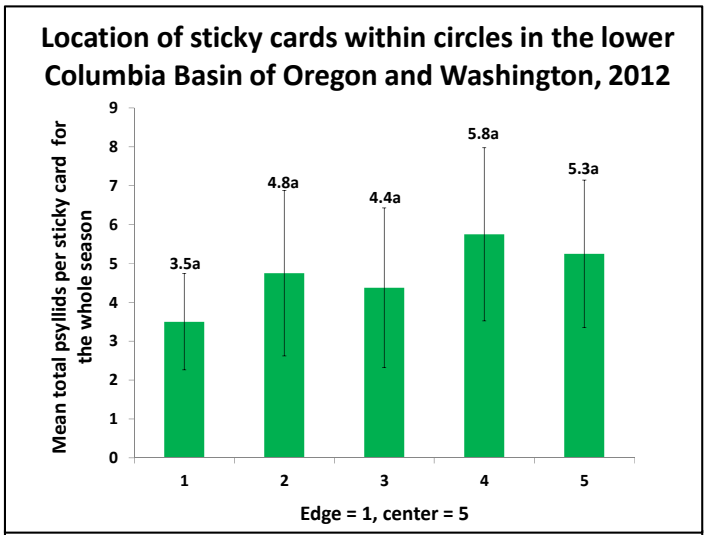
Sticky card comparisons

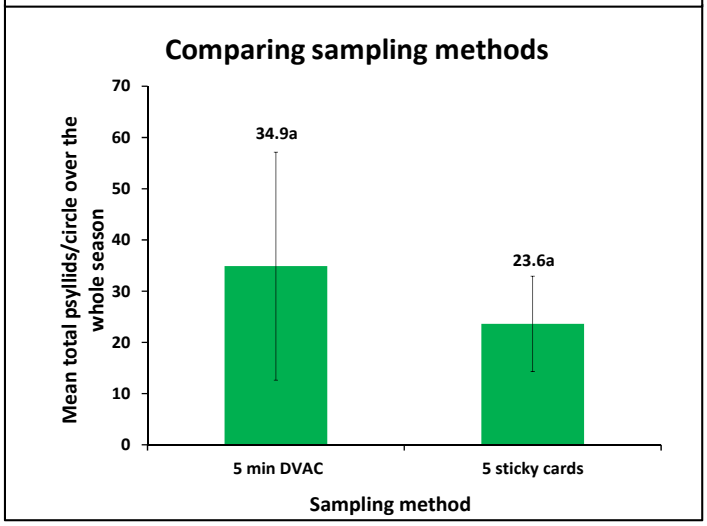
Preliminary observations in the Columbia Basin suggested that there were differences among commercial sticky cards available. Thus, a study to evaluate different types of sticky cards was conducted. Following procedures similar to Henne et al. 2010, four different sticky cards were compared: 1) Pherocon AM (Trece, Adair, OK), 2) AlphaScents Yellow Card, 3) AlphaScents ACP (AlphaScents, West Linn, OR), and 4) a 3 x 5 yellow card (Cascade Ag. Services, Wenatchee, WA). Traps were placed linearly in random order 3 m apart at four different sites. Cards were replaced weekly. The number of psyllids collected/cm^2 was used for comparison.

Results and Discussion

Large scale monitoring system

The placement of sticky cards within a field did not impact psyllid numbers (F = 2.04; df = 4, 28; P = 0.116), nor did location of leaf samples (H = 2.43; df = 5; P = 0.787). DVAC samples were comparable to sampling with sticky cards (T = -0.18; df = 14; P = 0.857) although DVACs yielded a numerically higher number of psyllids than the ones found in the sticky cards and were more efficacious in catching psyllids early (two weeks earlier). As a practical approach, growers are more interested in finding the first potato psyllids migrating to their crops, thus techniques that can help them determine when the first psyllids are arriving are the most appreciated. Potato psyllids pheromones are currently not available for growers. Current monitoring practices in the Columbia Basin include placing 1-2 sticky cards around the perimeter of a circle set in favor of prevalent winds. First psyllids were detected on trap 4 (close to the center of the pivot), thus the edge effect witnessed on southern states (i.e., Texas and California) (Butler and Trumble 2010) apparently does not apply for the Columbia Basin region based on this first year data. Therefore, monitoring and control merely around the edges may not be sufficient.





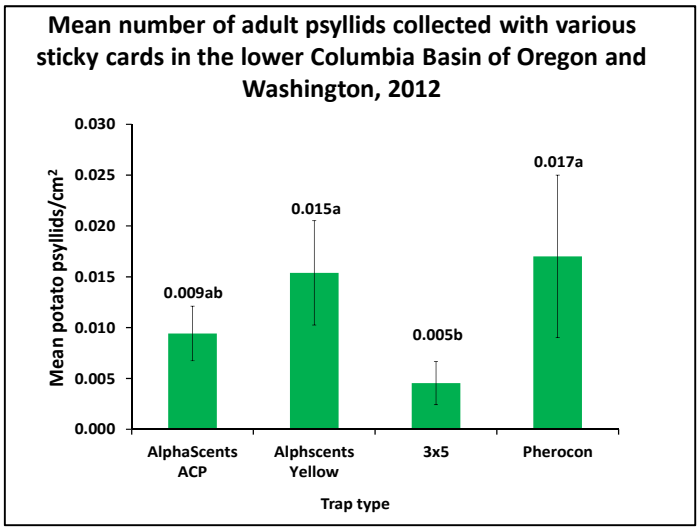
All sampling methods were compared against each other using regression analysis. While leaf samples, sticky cards and DVAC samples all correlated significantly with each other they did not correlate with ZC incidence (Table 1). This might be due to low Lso in the psyllids in the Columbia Basin in 2012 (less than 1%).

Table 1. Comparison of potato psyllid sampling methods with ZC rating using regression analysis.

Pair comparisons		(%) R^2	df	F	P
D-vac	Sticky Card	83.2	1, 6	35.62	0.001
D-vac	Leaf Sample	62.0	1, 6	12.44	0.012
Sticky Card	Leaf Sample	42.6	1, 38	29.93	< 0.001
ZC Rating	DVAC	0	1, 6	0.00	0.954
ZC Rating	Sticky Card	0	1, 38	0.28	0.600
ZC Rating	Leaf Sample	0	1, 38	0.04	0.842

Sticky cards comparisons

Pherocon AM and both AlphaScents yellow cards performed significantly better than the standard 3 × 5 yellow card (F = 6.09; df = 3, 9; P = 0.015). The 3 × 5 sticky card is currently widely used in the Columbia Basin to monitor the beet leafhopper. The beet leafhopper, *Ciculifer tenellus* Baker (Homoptera: Cicadellidae), transmits Beet Leafhopper-Transmitted Virescence Agent (BLTVA), a phytoplasma that causes purple top disease (a.k.a. Columbia purple top disease) in the region. Foliar symptoms of BLTVA are similar that ZC (i.e., leaves of potato plants infected with this phytoplasm are purple, dwarfed, crinkled, and rolled upward and inward; also aerial tubers can be present).



Potato producers in the Columbia Basin of Oregon and Washington have a significant need and interest in monitoring psyllid populations to control the spread of ZC. Our studies were designed to answer many of the most critical questions related to monitoring psyllids and ZC in Oregon and Washington. Our mission is to provide information to growers in the region in a timely manner. The progress of these studies were reported during the OSU-HAREC Potato Field Day (27 June, Hermiston); several potato psyllids workshops were offered (n=12), and numerous fieldmen and growers were constant fixtures in our Irrigated Agricultural Entomology Laboratory at the HAREC. Further research on monitoring is warranted to confirm the trends observed in 2012.

Acknowledgements

We recognize financial support from the mini SCRI grant, Oregon and Washington State Potato Commission, and the Agricultural Research Foundation. We would also like to acknowledge contributions from Aymeric Goyer, Erik Echegaray, and James Crosslin, as well as technical assistance from: Ruben Marchosky, Carol Mills, Tanner Keys, Treve Moffit, Abbye McDonough, Mary Adams, Jonathan Macias, Dale Wilkerson, and Brandi White. Thanks to Phil Hamm for reviewing this manuscript.

References

Butler, C.D. and J.T. Trumble. 2010. Distribution and phenology of the potato psyllid in southern California. *In* F. Workneh and C. M. Rush (eds.), 10th Annual Zebra Chip Reporting Session, November 7-10, 2010 2010, Dallas, TX. Zebra Chip SCRI Project.

Goolsby, J. A., J. Adamczyk, B. Bextine, D. Lin, J. E. Munyaneza, and G. Bester. 2007. Development of an IPM program for management of the potato psyllid to reduce zebra chip disorder in potatoes. Subtrop. Plant Sci. 59: 85-94.

Hamm P.B., S.I. Rondon, J.M. Crosslin, and J.E. Munyaneza. 2011. A new threat in the Columbia Basin of Oregon and Washington: Zebra Chip. *In* Proc. of the 11th Annual Zebra Chip Reporting Session. F. Workneh and C.M. Rush (editors). 7-8 Nov. San Antonio, TX. Pp 1-6.

Henne, D. C., L. Paetzold, F. Workneh, and C. M. Rush. 2010. Published. Evaluation of potato psyllid cold tolerance, overwintering survival, stickytrap sampling, and effects of Liberibacter on potato psyllid alternat host plants. *In* F. Workneh and C. M. Rush (eds.), 10th Annual Zebra Chip Reporting Session, November 7-10, 2010 2010, Dallas, TX. Zebra Chip SCRI Project.

Potato Psyllid Population Fluctuations in Natural Vegetation and in Relation to Potato Emergence Time Across Texas

Rashed, A., Paetzold, L., Archer, C., Pattison, S., Bralley, J.B., Gray, J., Workneh, F., and Rush, C.M.

Texas A&M Agrilife Research, 6500 Amarillo Blvd W., Amarillo, TX 79106

Abstract

This study was set to investigate potato psyllid movement patterns in relation to potato crop emergence in different locations across the state of Texas. In all locations potato psyllids first appeared in natural vegetation. Changes in psyllid numbers in relation to potato crop varied based on the location. Between 0.0 and 2.2% of the collected psyllids tested positive for Liberibacter, in 2012. Despite the high psyllid numbers collected, ZC incidence remained relatively low (0.05- 1.2%). This low disease incidence was not due to low transmission efficiency of the potato psyllids. Field trials also indicated that ZC severity is affected by the number of feeding infective psyllids. Based on the results of this study, monitoring should be started in natural vegetation before potato emergence. Chemical control measures in the first 3 weeks post plant emergence is recommended to reduce the initial impact of potato psyllids, as they move into the potato field. To date, chemical control has been the sole effective method of vector control in this system. This approach must be continued to maintain psyllid populations at a manageable level.

Introduction

Zebra chip disease (ZC) is now a major challenge to the potato industry across the United States, and has been impacting regional economies for more than a decade. The vector-borne bacterium, '*Candidatus* Liberibacter solanacearum' ('*Ca.* L. solanacearum'), has been identified as the etiological agent of ZC (Wen et al. 2009). '*Ca.* L. solanacearum' is transmitted by the potato psyllid *Bactericera cockerelli* (Hemiptera: Triozidae). Current chemical control treatments for the potato psyllid vector appear to be successful in limiting outbreaks (Goolsby et al. 2007). However, the high costs associated with multiple sprays within a growing season and rising concern over recent appearance of resistant potato psyllid populations (D.C. Henne, personal communication) highlight the need for studies that can lead to more targeted and integrated control approaches. Such strategies should also consider ecological interactions that may be specific to the local habitat structure. Wild plants that grow early in the season can be important hosts to potato psyllids, until the preferred potato host becomes available (Wallis 1951).

While previous surveys provide some evidence for psyllid long-range migration from southern regions (with milder winters), the significance of local vegetation in ZC epidemiology is yet to be evaluated. In addition to potato psyllids' movement, their direct interaction with host plants plays an important role in ZC epidemiology. This is because transmission efficiency of '*Ca.* L. solanacearum' could be influenced by psyllid- host interactions.

Here we present results of our potato psyllid population survey in natural vegetation in several locations in Texas. Although high populations of psyllid were detected in some locations, disease incidence remained relatively low. Therefore, we proceeded by presenting results of a greenhouse study that quantified transmission efficiency in relation to psyllid number and their titer load. We also report preliminary data of a field study that evaluated the effect of infective psyllid numbers on potato tuber disease severity.

Material and Methods

Psyllid populations were monitored in several locations across the state of Texas. These locations included Dalhart, Bushland, Springlake, Olton, Seminole, Kermit, Fort Stockton, and Pearsall.

Yellow sticky traps were placed in natural vegetation throughout the year. Additional traps were placed on the edges of the potato fields after planting and around potato emergence time. Traps were replaced every 2 to 3 weeks, and trapped potato psyllids were tested by qPCR for '*Ca.* L. solanacearum. When there were more than 10 psyllids present on a single trap, only a subset was tested.

Wild plants surrounding traps that successfully collected potato psyllids were collected. Plants were sampled at the base of the stem as well as leaf petioles, and sampled tissues were stored at -80 ºC. Plant tissue was ground in a homogenizer, after 2-min in liquid nitrogen, and DNeasy® Plant Mini Kit (QIAGEN) protocol was used for total nucleic acid extractions.

A slightly modified version of the commercial DNeasy® Blood &Tissue Kit (QIAGEN) protocol was used for potato psyllid extractions. Psyllids were placed in a screw cap tube, along with a 2.5mm stainless steel ball bearing bead, and tubes were then placed in liquid nitrogen for about 2 minutes and psyllids were ground using a Hard Tissue Grinder (VWR, Sugar Land, TX, USA). Thereafter, the standard protocol was followed. Sample DNA was eluted with 50µl of the elusion Buffer.

Comparative Ct method, also known as ΔΔCt, was performed for '*Ca.* L. solanacearum' detection/quantification, using an Applied Biosystems 7500 Real-Time PCR System (ABI) (Rashed et al., 2012).

'*Ca.* L. solanacearum'-positive psyllids used in the inoculation study and disease severity experiments were maintained in multiple bugdorm cages in the greenhouse for several generations. An inoculation success experiment was conducted in the greenhouse, and repeated three times. In each study between 18 (block 3) and 23 (blocks 1 and 2) plants were infested with either 1 or 4 infective psyllids, using leaf-cages. Insects were removed after 48hrs and quantified for their titer load. Leaf tissue under the cage area was removed for pathogen detection and quantifications (Rashed et al., 2012).

The red potato cultivar La Soda and the russet Norkotah were used to study the effect of infective psyllid numbers on disease severity. In a field study, cages were placed on planted rows prior to plant emergence. After emergence, plants in cages were thinned to 4 (one at each corner). Depending on the experimental treatment, 5, 15, or 30 psyllids were released at the base of a single plant in each cage and then removed after 1 week. Infestations were conducted 6 weeks before harvest. At harvest all potato tubers were scored for symptom severity following a 0 to 3 scale (Rashed et al., 2013). There were 5 cage-replicates per insect density and five psyllid-free cages were maintained as healthy controls.

Results and Discussion

The pattern of fluctuations in potato psyllid populations in natural vegetation and potato fields differed among different geographical locations (Fig. 1), but at all sites, potato psyllids first appeared in natural vegetation. A population fluctuation was even recorded in Kermit, where no potato fields exist, demonstrating that potato psyllid appearance did not depend on the presence of a potato crop.

In Pearsall, potato psyllid numbers declined, following a small peak in natural vegetation. This reduction was followed by a large spike in surrounding potato fields, starting about 2 to 3 weeks after emergence of the potato plants (Fig 1a, d). In Olton and Springlake, the largest peak in psyllid numbers occurred in natural vegetation. These populations soon declined but the reduction in psyllid numbers in natural vegetation was followed by a small peak in potato fields (Fig 1b, e). In Dalhart, potato psyllids increased gradually and simultaneously in both natural vegetation and potato fields (Fig 1c, f). No distinct peak was observed in this sampling location. Although, potato psyllids disappeared for several weeks after harvest they reappeared in fall and were collected even during December in northern locations with colder climate, i.e. Olton, Springlake, and Dalhart.

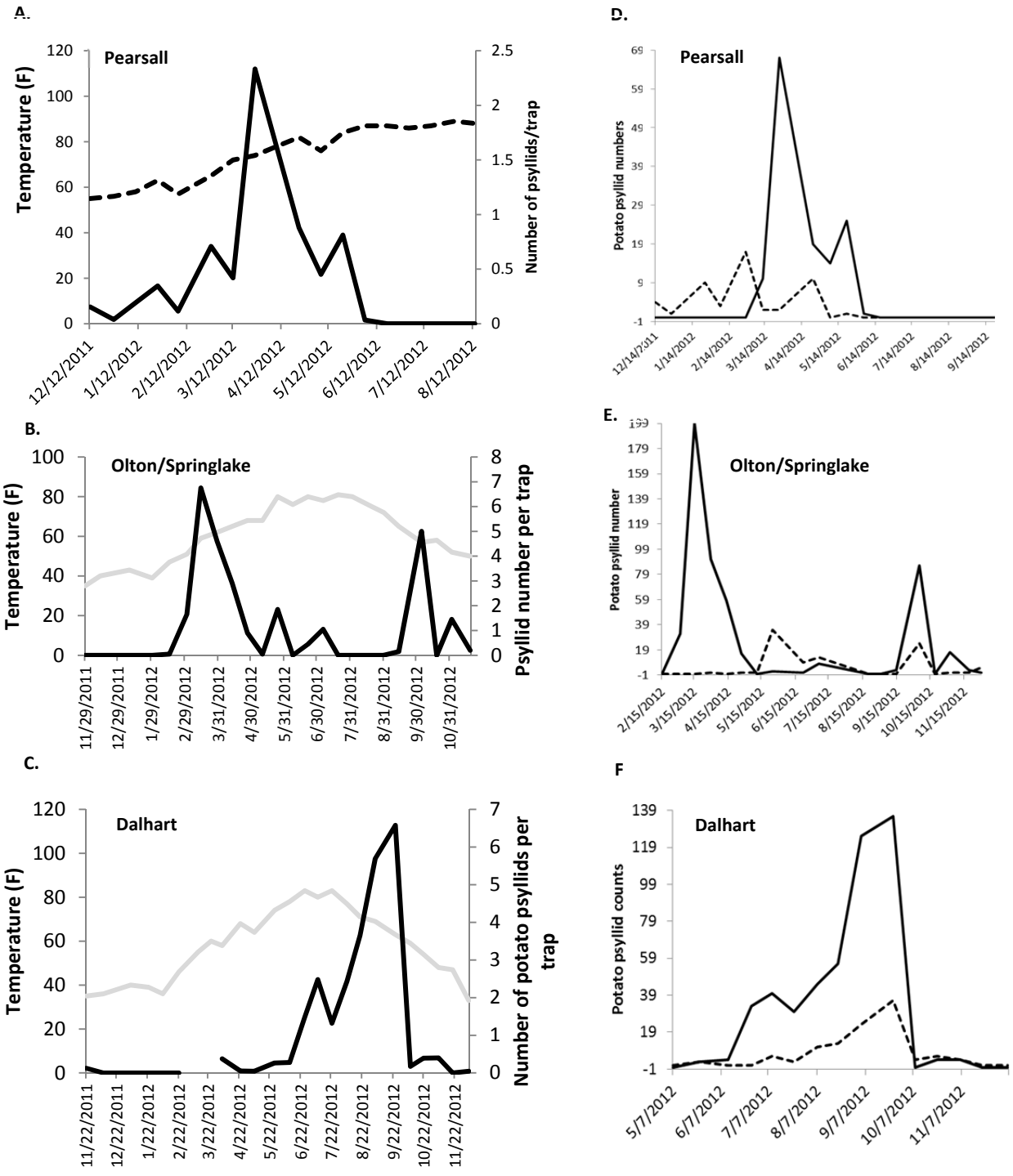


Figure 1: Fluctuations in psyllid numbers during 2011 and 2012. A and D: Pearsall, B and E: Springlake, and C and F: Dalhart. Graphs A through C represent changes in average psyllid numbers per trap (black lines) and fluctuations in environmental temperatures (gray lines). Graphs D through F illustrate changes in absolute psyllid numbers in natural vegetation (dotted lines) and potato field edges (solid line).

At all locations, peak psyllid numbers coincided with temperatures between 50 and 70 F. However, it has been reported that this species does best at temperatures around 80 F (List 1939). In this study, potato psyllids also were collected in December, when temperatures were quite cold in the northern Texas locations. This indicates that psyllids have high cold tolerance and can be active in cold temperatures. Psyllids were never collected at temperatures above 90 F indicating their sensitivity to high temperatures. Cold temperatures, at or slightly below freezing, do not appear to be detrimental to psyllid populations, but they are highly sensitive to high temperatures (List 1939).

The percentage of potato psyllids which tested positive for '*Ca.* L. solanacearum' ranged between 1.3% (in Pearsall) and 2.2% (in Olton). None of the potato psyllids collected in Dalhart, Kermit and Seminole locations tested positive for the pathogen. Despite the presence of '*Ca.* L. solanacearum'-positive psyllids, and relatively high psyllid numbers, disease incidence remained relatively low in all sampling areas, ranging between 0.05 and 1.2%. This low disease incidence was not due to inefficiency of positive psyllids in transmitting the pathogen. In our greenhouse study, inoculation success rate of potato psyllids was as high as 70%, within 48 hours, when infectious psyllids were caged individually on their potato host. Inoculation success reached as high as 100%, when there were multiple infectious psyllids feeding on the host plant. Moreover, inoculation success was independent from pathogen titer in individual psyllids. Psyllid number significantly, positively affected the quantity of '*Ca.* L. solanacearum' inoculated into plant tissue ($F_{1,77} = 7.52$; $P = 0.008$). Therefore, potato psyllids are highly efficient in transmitting '*Ca.* L. solanacearum' and variations in their pathogen titer did not influence transmission success. However, since multiple psyllids inoculated plants with a higher success rate and greater pathogen levels in relatively short inoculation access periods (see Buchman 2011), it is important to limit psyllid populations in the field. Indeed, we have shown that ZC incubation time may be affected by the number of infective psyllids that feed on the plant (Rashed et al. 2012).

To evaluate the effect of insect number on disease severity in the field, insects were released at the base of a single plant in each of the field cages which contained 4 plants. Analyzing data from the plants on which psyllids were directly released revealed no statistical difference in disease severity, indicating that having 5 or 30 psyllids on a single plant may not result in differences in disease severity. However, data from all plants within each psyllid density treatment revealed significant effect of density on ZC severity ($F_{2,107} = 4.52$; $P = 0.013$; Fig. 2). Cages which were infested with 5 infective psyllids (about 1.25 psyllids/plant) had a significantly lower symptom score compared to cages infested with 30 psyllids (Tukey HSD, $P = 0.009$) in both potato cultivars. Overall, The red cultivar La Soda had a significantly lower symptom score than Norkotah russet ($F_{1,107} = 14.44$; $P < 0.001$). Analysis of variance followed by pairwise tukey comparisons was used to analyze our preliminary symptom severity data (average tuber symptom tuber score). Plants with no symptomatic tubers were excluded from the analysis. Thus, the results of our field study indicated that disease severity under field conditions is affected by both the number of psyllids that feed on the infected plants and potato cultivar.

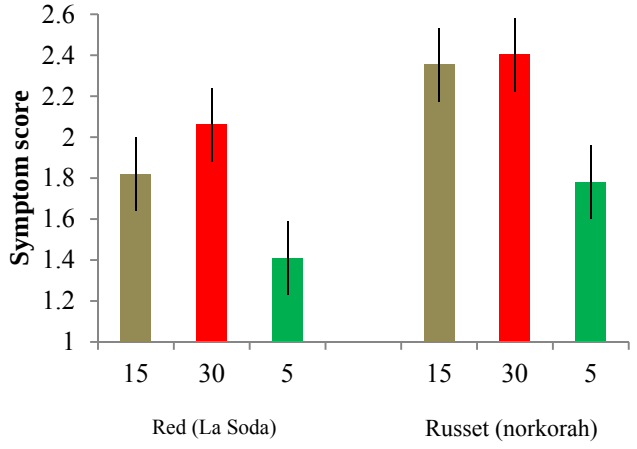


Figure 2. Tuber symptom severity of plants infested with 15 (brown), 30 (red), and 5 infective psyllids in la soda and norkotah cultivars.

Overall, the results of this study suggest that the low ZC incidence observed in 2012 was not due to low inoculation efficiency or low titer in the psyllid vectors. The results also highlighted the importance of maintaining psyllid populations at low levels. Frequent pesticide application has been successful in limiting psyllid numbers and needs to be continued. Control measures should be intense early in the season, and after potato emergence, especially if high psyllid numbers are detected in natural vegetation and field edges. Given the observed high efficiency of potato psyllids in inoculating plants with '*Ca.* L. solanacearum' in short periods of times, pesticides with immediate effect are recommended. Innovative approaches are also needed to apply chemicals more effectively.

Acknowledgements
We would like to thank our lab members for their help with different aspects of this project. We also thank Texas Department of Agriculture Zebra Chip Initiative (# ZC-1213-01) and USDA-SCRI (# 2009-511-20176) for funding this work.

References
Buchman, J. L., V. G. Sengoda, et al. 2011. Vector Transmission efficiency of Liberibacter by *Bactericera cockerelli* (Hemiptera: Triozidae) in Zebra Chip Potato Disease: Effects of psyllid life stage and inoculation access period. J. Econ. Entomol. 104: 1486-1495.

Goolsby, J. A., B. Bextine, J.E. Munyaneza, M. Setamou, J. Adamczyk, Bester, G. 2007. Seasonal abundance of sharpshooters, leafhoppers, and psyllids associated with potatoes affected by zebra chip disorder. Subtrop. Plant Sci. 59:15-23.

List G.M. 1939. The effect of temperature upon egg deposition, egg hatch, and nymphal development of *Paratrioza cockerelli* (Sulc). J. Econ. Entomol. 32: 30-36.

Rashed, A., C.M. Wallis, L. Paetzold, F. Workneh & C. M. Rush. 2013. Zebra Chip disease and potato biochemistry: Tuber physiological changes in response to '*Candidatus* Liberibacter solanacearum' infection over time. Phytopathology, in press.

Rashed, A., D. Nash, L. Paetzold, F. Workneh & C. M. Rush. 2012. Transmission efficiency of *Candidatus* Liberibacter solanacearum and potato zebra chip disease progress in relation to pathogen titer, vector numbers and feeding sites. Phytopathology, 102: 1072-1085.

Wallis, R.L. 1951. Potato psyllid selection of hos plants. J. Econ. Entomol. 44: 815-817.

Wen, A., Mallik, I., Alvarado, V. Y., Pasche, J. S., Wang, X., Li, W., Levy, L., Lin, H., Scholthof, H.B., Mirkov, T. E., Rush, C. M., and Gudmestad, N. C. 2009. Detection, distribution, and genetic variability of '*Candidatus* Liberibacter' species associated with zebra chip complex disease 1 of potato in North America. Plant Dis. 93:1102-1115.

Overwintering of the Potato Psyllid in the Northwest on *Solanum dulcamara*

Jensen, A.S.[1], Rondon, S.I.[2], Murphy, A.F.[2], and Echegaray, E.[2] [1]WA, ID, OR Potato Commissions, 4032 N Ballantyne Ln, Eagle, ID 83616; [2]Oregon State University, Hermiston Agricultural Research and Extension Center, 2121 South First Street, Hermiston, OR 97838.

Introduction

Since the first report of zebra chip (ZC) in the potato crops of the U.S. Northwest (Idaho, Oregon, and Washington), there has been much interest in the biology of potato psyllid, *Bactericera cockerelli*, in the region. The potato psyllid has been known to occur regularly in the Columbia Basin of Washington and Oregon for over 20 years, based on the personal observations of the first author, and was documented to appear in potatoes annually by Munyaneza et al. (2009). Previous to the link between ZC and potato psyllid, there had been almost no research on, or attention paid to, the psyllid in Northwest potatoes. This is because it was widespread but usually in very low numbers that did not warrant control. The advent of ZC in the Northwest raised urgent questions about the psyllid's biology outside the crop, including whether it could overwinter in the region. Therefore, studies were undertaken to identify a non-crop plant that might serve as an overwintering host for the psyllid. The first author consulted the *Flora of the Pacific Northwest* (Hitchcock and Cronquist 1973) for perennial species of Solanaceae in the region that overwinter with dormant above-ground parts. *Solanum dulcamara* L. was the species listed that fit these criteria and therefore studies of this plant commenced.

Materials and Methods

Idaho-based research: Sampling of *S. dulcamara* plants, conducted by the first author, began in various parts of the Northwest during routine travels for other purposes. Plants were sampled wherever found using a combination of leaf/stem picking and beating sheet techniques. Insects were identified using a stereomicroscope, where appropriate. Lab rearing of potato psyllids was accomplished on rooted cuttings of *S. dulcamara* and potted potato plants grown at room temperature (~65º F) under ambient lighting conditions. Samples from the field and lab colonies were preserved on several occasions in the personal collection of the first author.

Oregon-based research: Sampling of *S. dulcamara* plants started during late winter in the area surrounding Hermiston, OR. Sampling methods included leaf samples and insect-vacuum (D-vac) samples. Insects were identified using a stereomicroscope, where appropriate. Lab rearing was in a growth chamber at approximately 28°C, 50% RH, and 14:10 D:L.

Results and Discussion

On October 24, 2011, *S. dulcamara* plants growing along a stream in Stanfield, OR were found hosing immature psyllids. Cuttings from this location were returned to the first author's home in Idaho where psyllids were reared to adult and confirmed to be potato psyllid. This was apparently the first documentation of *S. dulcamara* as host of potato psyllid.

Following up on this find, two sites near Star, ID (one found in November, the other in February) and one near Caldwell, ID (found in January) were confirmed to host potato psyllids. All three sites were large patches of *S. dulcamara* growing along a ditch or canal that contained water throughout the year. The first site located near Star was monitored into the middle of December with all stages of the psyllid present. Following complete leaf drop by the plants on about December 20, only adult psyllids were found at this site until spring. The other two sites, i.e. one near Star and one near Caldwell, were

found after complete leaf drop during mid-winter. Adult psyllids were easily shaken from the plants at both sites in mid-winter. On February 1, stem cuttings were established in a vase to observe dormancy break in the plant. On February 13 cuttings had roots and shoots and were potted in potting soil for establishment of a lab colony. Three psyllids were collected from the field on February 23 and March 3 and introduced to the potted *S. dulcamara* plant. Copulation was observed on March 4 and eggs were found on the plant on March 10. The eggs hatched and these psyllids developed to adults by April 8. In the field, eggs were found on *S. dulcamara* beginning on April 16 in Caldwell, and late-instar nymphs were present by May 4, conclusively demonstrating overwintering. Psyllids collected from *S. dulcamara* were placed on a potted potato plant, and successfully established a population on this plant that persisted until it was terminated in mid-July.

In the Hermiston, OR area, *S. dulcamara* plants hosting potato psyllid were first found on March 22. Of the 38 sites sampled in March and April, only five hosted potato psyllid. On April 9 psyllid eggs were collected from *S. dulcamara* in the field and placed on cuttings of *S. dulcamara*. These psyllids developed to adults in about 15 days. By mid-May the first spring generation of psyllids had emerged in the field and was reproducing on *S. dulcamara*.

This work demonstrated for the first time that *S. dulcamara* is a good host for potato psyllid. We also showed that potato psyllid can overwinter in the adult stage in the Northwest in association with *S. dulcamara*. Questions remain, however, including whether this host plant and overwintering biology are important for the development of ZC disease in the Northwest potato crop.

Acknowledgements

This research was supported by Washington State Potato Commission, Idaho Potato Commission, and Oregon Potato Commission, and by grants to the second author from the Washington State Potato Commission, the Oregon Potato Commission, and from the Zebra Chip SCRI Minigrant Program.

References

Hitchcock, C.L. and A. Cronquist. 1973. Flora of the Pacific Northwest. University of Washington Press, Seattle. 730 pp.

Munyaneza, J. E., J. M. Crosslin, and J. L. Buchman. 2009. Seasonal occurrence and abundance of the potato psyllid, Bactericera cockerelli, in south central Washington. Am. J. Potato Res. 86: 513-518.

Assessing the Associations of Management and Environmental Factors with Regional Psyllid Abundance and Zebra Chip Intensity

F. Workneh[1], D. C. Henne[2], J. Goolsby[3], J. M. Crosslin[4], S. Whipple[5], J. D. Bradshaw[5], A. Rashed[1], L. Paetzold[1], R. Harveson[5], and C. M. Rush[1]

[1]Texas AgriLife Research, Bushalnd, TX 79012; [2]Texas AgrLife Research, Weslaco, TX 78596; [3]USDA-ARS, Edinburg, TX 78539; [4]USDA-ARS, Prosser, WA 99350; [5]University of Nebraska, Panhandle Research and Extension Center, Scottsbluff, NE 69361.

Summary

Potatoes are cultivated under a wide range of environmental conditions across the central US. Planting normally begins in December in the Lower Rio Grande Valley and ends in May in the northern regions. To understand the impact of the diverse environments, zebra chip (ZC) occurrence data were collected during the last three years (2010-2012) from a total of 26 fields across the region and related to management and environmental factors including latitudinal location, weather variables, planting dates, and pesticide applications. Location, maximum temperature, and planting dates (which were highly correlated) were found to be the most important factors in distinguishing between relatively low and high levels of ZC. Total psyllid population numbers did not contribute to the variability in ZC levels and also did not correlate with any of the variables. However, populations of psyllids testing positive for Liberibacter were significantly negatively correlated with location, planting date, and maximum temperature. During the last three years, ZC was more prevalent in the southern locations the in the northern locations and there was some degree of south-to-north declining trend. Over all variations in type and frequency of pesticide application across the region did not significantly contribute to variability in ZC occurrence.

Impact Statement

Knowledge of factors which contribute to development and spread of plant diseases is essential in development of management practices. In this study, several environmental and management factors were assessed for their effect on potato ZC occurrence from south Texas to Nebraska. The findings that field locations, planting dates, and temperature are significant factors in ZC occurrence suggest that one may need to take these factors into account when considering management options.

Introduction

Potatoes are cultivated under a wide range of environmental conditions across the central US. Planting generally begins in December in the Lower Rio Grande Valley and ending in May in the northern regions, with the respective harvest dates extending from April through September. Knowledge of the impact of the regional variations in potato culture on ZC intensity is useful in understanding of factors which affect ZC the epidemiology of the disease. The primary objective of this project was to investigate environmental and management factors associated with ZC intensity across the region. Specifically, we were interested in investigating whether variations among the fields in ZC intensity could be associated with environmental differences and management factors.

Materials and Methods

Zebra chip assessments were conducted at six locations across the region from south Texas to Nebraska, which included The Lower Rio Grande Valley, Pearsall, Olton, and Dalhart (all in Texas), Garden City

(Kansas), and Bridgeport (Nebraska) in each of the 2010, 2011, and 2012 seasons. Over the three-year period ZC intensity was assessed in 26 fields across the region. These fields were subsets of a wider regional potato psyllid detection network in which a much greater number of fields were monitored weekly using yellow sticky traps (Goolsby et al., 20121; Henne et al., 2012). Weather stations were installed at each of the 6 locations and weather variables were collected either remotely (where wireless cellular services were available) or manually. ZC assessment in each field was conducted in plots of 20 m × 30 m established systematically (approximately 160 m apart) around the field edges and in the centers of the fields (two rings of plots around the circle). The total number of plots per field ranged from 16 to 32 depending on the size of the fields. The number of symptomatic plants per plot was counted generally within two weeks of harvest and tubers from the symptomatic plants were dug, sliced from the stolon attachment end and examined for the typical internal necrosis. If a single tuber from a plant was found to be symptomatic, the plant was considered positive for ZC.

Analyses. For each field the mean number of symptomatic plants per plot was used as a measure of ZC intensity. To determine the association of various factors with ZC intensity levels, fields were classified into two classes in which those which had less than and equal to three symptomatic plants per plot were designated as one (class 1) and those with greater than three were designated as two (class 2). This classification scheme resulted in equal number of fields in each ZC intensity class. Daily weather variables, weekly counts of adult psyllids (both averaged over the growing season), planting dates (Julian), field locations (GPS), and the number and type of insecticide applications were analyzed to determine their association with the two classes of ZC intensity using discriminant function analysis and logistic regression in SAS statistical software (SAS Institute Inc., Cary, NC).

Results and Discussion

Over the three-year period the average number of symptomatic plants per plot per field ranged from near zero to over fifty (Fig. 1) and the disease was more prevalent in the southern locations than in the north.

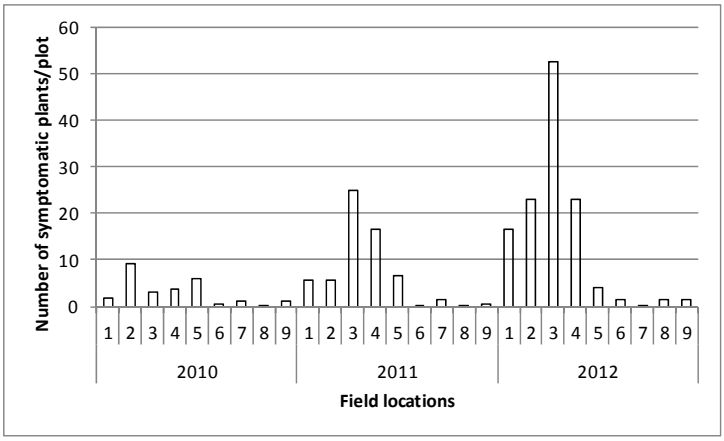


Fig. 1. Number of symptomatic plants/plot in different field locations assessed over a three-year period (2010-2012)

Means, minimum, maximum, and standard deviations of variables for each class of ZC are presented in Table 1. The selected variables correctly classified about 88.5% of the fields in to ZC class one (lees than and equal to 3 symptomatic plants per plot) or class 2 (greater than 3 symptomatic plants per plot). Of all the variables, location, planting date, and maximum temperature were the most important factors contributing to the variability in ZC prevalence across the region. The fact that these three variables were identified as important factors is not surprising because planting begins early in the southern locations (overlaps with relatively cooler temperatures than those in the northern regions) and the three variables were highly correlated [coefficients ranging from 0.67 to 0.93 ($P < 0.05$)] suggesting that anyone of the three variables could be a good candidate as predictors of ZC occurrence across the region. Location, by itself (when used alone) accounted for about 90% of the variation, followed by planting date (86%) and maximum temperature (70%) (Fig. 2 & Table 2). The fact that

location was found to be one of the important factors, and most of the high levels of ZC were from the southern region, agrees with the finding that psyllids testing positive for the pathogen were detected mostly in the southern region (Goolsby et al., 2012; Henne, et al. 2012).

Table 1. Means, maximum, minimum, and standard deviations (std.dev.) of variables for each of the zebra chip intensity class

	ZC class 1 (ZC ≤ 3)[a]				ZC class 2 (ZC > 3)[b]			
Variable	Mean	Max	Min	Std. dev.	Mean	Max	Min	Std. dev.
Avgtemp (C)	21.25	24.42	18.24	0.63	20.11	24.15	16.15	0.67
Location (Lat N)	36.55	41.63	26.44	4.53	29.21	34.09	26.38	2.97
Maxtemp (C)	29.57	32.78	24.52	2.76	27.51	33.04	24.25	2.85
Mintemp (C)	12.97	16.86	10.20	0.68	13.53	16.14	9.24	0.62
Pestap	7.08	17.00	10.20	4.52	7.46	17.00	4.00	3.45
Pldate (Julian)	99.77	140.00	1.00	44.32	31.31	88.00	3.00	33.06
Ppsyllid	0.00	0.00	0.00	0.00	1.85	8.00	0.00	2.51
Tpsyllid	27.28	244.46	1.08	65.37	25.49	150.00	0.23	47.24

[a] Average number of symptomatic plants/plot was less than or equal to three

[b] Average number of symptomatic plants /plot was greater than three

Avgtemp = average temperature; Maxtemp = maximum temperature; Mintemp = minimum temperature; Pestap = frequency of pesticide applications; Pladate = planting date; Ppsyllid = counts of psyllids positive for Liberibacter; Tpsyllid = total psyllid counts

Total psyllid population numbers did not significantly contribute to ZC variations across the region and also did not correlate with any of the selected variables. However, the number of Liberibacter-positive psyllids negatively and significantly correlated with latitudinal locations of the fields (r = -0.46; P = 0.0165), planting date (r = -0.47; P = 0.0126), and maximum temperature (r = -0.52; P = 0.0051). There was slight but significant negative correlation between latitudinal location and ZC intensity (r = - 0.499; P = 0.0094) indicating south-to-north declining trend, which also agrees with the finding that the numbers of psyllids testing positive for Liberibacter were greater in the southern regions than in the northern regions (Goolsby et al., 2012; Henne, et al., 2012).

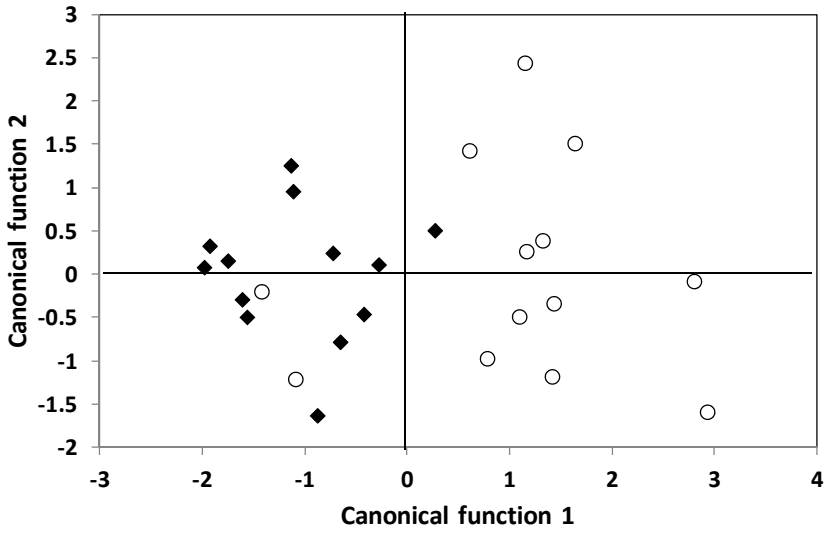


Fig. 2. Graphical representation of classification fields with ZC intensities [≤ 3 (circles) & > 3 (diamonds) symptomatic plants/plot] based on associations of selected variables

Table 2. Classification of fields based on ZC levels and % correctly classified into the class membership (≤ 3 or > 3) as affected by various variables

Actual number	≤ 3[a]	> 3[a]	% correctly classified
13	11	2	84.62
13	1	12	92.31
Total 26	12	14	88.5

[a] Average number of symptomatic plants per plot. ZC intensity class 1 is ≤ 3 and class 2 is > 3 symptomatic plants per plot.

Variations in pesticide application did not significantly contribute to the variability of ZC across the region. Cluster analysis of insecticide type and frequency of application over the three-year period provided three distinct clusters (Fig. 3). The first cluster (represented by circles) contained about 70% of the fields which were closely clustered indicating similarity in pesticide applications. This cluster had an average of 6.9 symptomatic plants per plot per field. Fields in each of the second and third clusters were not as tightly clustered as in the first indicating existence greater variability within each of the clusters. There were only three fields in the second cluster (square) and these fields had an average of 1.9

symptomatic plants per plot per field. The field in third cluster (triangle) had a little more ZC intensity than the other two with an average of 12.5 symptomatic plants per plot per field.

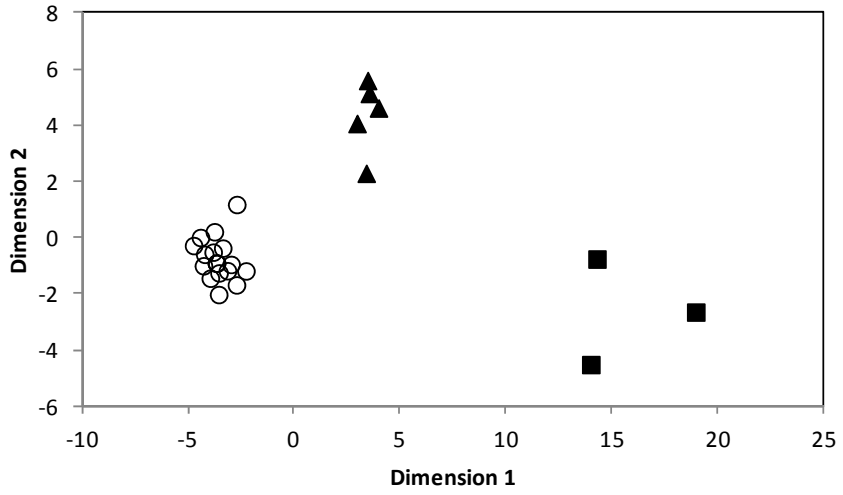


Fig. 3. Clustering of fields as impacted by pesticide type and number of applications

Acknowlegement
Thanks to our lab members for their help with different aspects of this project. Funding for this project was provided by USDA-SCRI (# 2009-511-20176).

References

Goolsby, J. A., Adamczyk, J. J., Crosslin, J. M., Toxclair, N. N., Anciso, J. R., Bester, G. G., Bradshaw, J. D., Bynum, E. D., Carpio, L. A., Henne, D. C., Joshi, A., Munyaneza, J. E., Porter, P, Sloderbeck, P. E., Supak, J. R., Rush, C. M., Willet, F. J., Zachmann, B. J., and Zens, B. A. 2012. Seasonal population dynamics of potato psyllid Hemiptera:triozidae) and its associated pathogen "*Candidatus* Liberibacter solancearum" in potatoes in the southern great plains of North America. J. Econ. Entomol. 105 :1268-1276.

Henne, D., Aniciso, J. Bradshaw, J., Whipple, S., Carpio, L,. Richmond, J., Walker, S., Porter, P., Barrett, S., Willet, J., Mathews, J., Deroo, K., Seger, P., Crosslin, J., Hamlin, L., and Goolsby, J. Overview of the 2011-2012 psyllid area-wide monitoring program. 2012. In: Proceedings of the 12th Annual Zebra Chip Reporting Session. F. Workneh, A. Rashed, and C.M. Rush editors. San Antonio, TX, Oct. 31 - Nov. 2.

Zebra Chip Update in the Lower Columbia Basin of Oregon and Washington: First year Retrospective toward Managing the Disease

Rondon, S.I.[1]

[1]Extension Entomologist Specialist, Associate Professor
Oregon State University, Hermiston Agricultural Research and Extension Center, Hermiston OR 97838 USA

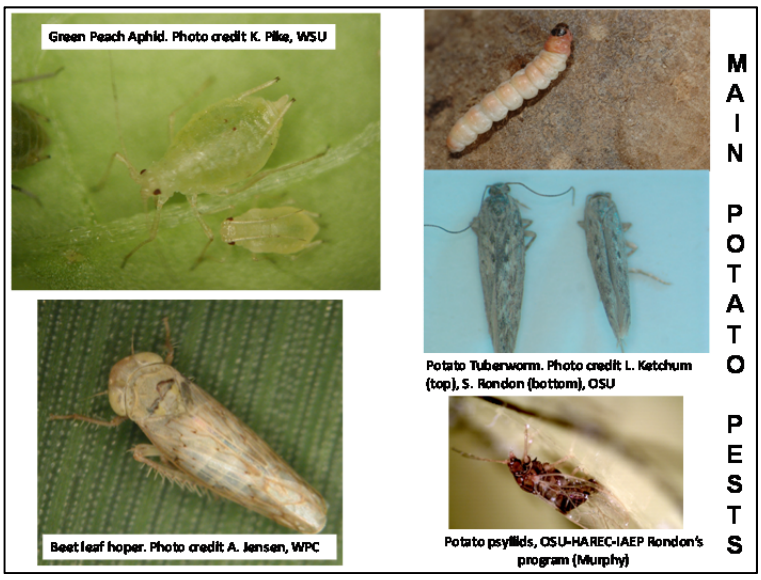
Abstract
The Columbia Basin of Oregon and Washington and Idaho were affected by zebra chip (ZC) in 2011. While potato psyllids had been consistently found in the region by early to mid-July each year, no ZC disease caused by *Candidatus* Liberibacter solanacearum (Lso) was confirmed until late 2011. Given the great importance of potato production in the region, this disease poses a new and significant threat to production. In 2011 several observations were made related to ZC in the Columbia Basin including when potato psyllids carrying the bacterium likely entered the region as well as the occurrence, incidence and spread of the disease. Also, preliminary data regarding potential control methods were studied. In 2012 my program continued studying the seasonal migration of psyllids in the Columbia Basin: evaluating the impact of pest management practices and incidence of Lso, insecticide trials for psyllid management in the Pacific Northwest, and studying the overwintering aspects of potato psyllids. The irrigated agricultural entomology program at the Hermiston Agricultural Research and Extension Center (HAREC) in Hermiston Oregon educated, communicated, and disseminated information about this pest to the industry. Considering the sudden appearance of this disease in the Columbia Basin and Idaho in 2011 and the uncertainty about the future role of ZC in the Basin, our attitude towards best management practices for controlling the insect/disease complex will certainly change as more information is obtained.

Introduction
The Pacific Northwest produces nearly two thirds of the potatoes grown in North America. The Columbia Basin of Oregon and Washington, with one of the highest yields per acre, produces over 30 percent of the total USA production. A large percentage of the potatoes produced in both states are processed into food products such as frozen French fries, dehydrated flakes, etc. A small percentage is for table stock. While potato psyllids have been consistently found in the region by early to mid-July each year, no ZC disease caused by *Candidatus* Liberibacter solanacearum (Lso) was confirmed until late 2011. This confirmation included Idaho, the main potato producer in the USA. Given the great importance of potato production in the region, this disease poses a new and significant threat to production in the region. *Candidatus* Liberibacter solanacearum, transmitted by the potato psyllid (*Bactericera cockerelli* Sülc) was unknown to the Pacific Northwest until samples were brought to the Oregon State University Plant Pathology Disease Clinic in Hermiston Oregon (Phil Hamm's program). The samples showed foliar signs of the ZC disease (e.g. purple top, leaf curling and crinkling); however, another disease known as Purple top disease, transmitted by the beet leafhopper, *Circullifer tenellus* Baker prompted samples to be tested via PCR therefore confirming ZC disease.

In 2011 several observations were made related to ZC in the Columbia Basin. In particular, inferences were made regarding when potato psyllids carrying the bacterium likely entered the region, as well as the occurrence, incidence and spread of the disease. Preliminary data regarding potential control methods were also evaluated.

When did psyllids carrying the bacterium arrive in the Columbia Basin?

Potato psyllids most likely moved into the region carrying the bacterium between 20-25th June, at least 6 weeks prior to when the insects were observed or captured in the field. Previous studies determined that the potato psyllid, at least, arrived the first week or week and a half in July. In 2011, ZC incidence was high in a few fields in the lower Columbia Basin of Oregon and Washington mainly due to the fact that no insecticides had been applied for most of the growing season (May-July) because economic thresholds had not been reached by insects commonly found in the area such as aphids (several species), beet leafhoppers (*C. tenellus*) and tuberworms *Phthorimaea operculella* Zeller) (Hamm et al. 2011, Rondon and Hamm 2012).

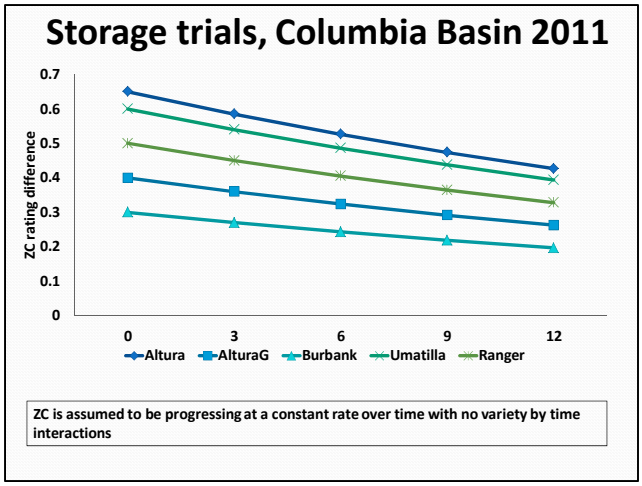


Which potato cultivars were affected?

In the Columbia Basin, potato cultivars such as Russet Norkotah, Umatilla Russet, Alturas, Russet Ranger, a red cultivar, and Pike were confirmed to have been infected by ZC (Hamm et al. 2011). Overall minor issues were reported during processing/packing (personal observations). The germplasm that is part of the Oregon State University Tri State potato breeding program planted at Hermiston became infected with a high percentage of Lso. As a consequence USDA/ARS Prosser (Dr. Chuck Brown's program) and OSU (Philip Hamm and Rondon's programs) joined forces to collect tubers from affected trials. The overall result was that approximately 25 clones showed no symptoms in about 230 clones examined. The overall incidence of symptomatic tubers was 44 % out of the 2,500 tubers that were rated. Zero ZC clones had the parent PALB03016-3. This is a selection that is derived from an attempt to incorporate late blight resistance from the Mexican wild species *S. guerreroense* L. This clone has immunity to PVY (C. Brown's unpublished data).

What happened in storage?

Potatoes from ZC infected fields were harvested between 9 September and 10 October 2011. The following varieties were included: Umatilla Russet, Ranger Russet, Russet Norkotah, Alturas and Russet Burbank. After harvest tubers were transferred to a commercial storage facility where they were kept at approximately 45°F. Tubers were removed 3 weeks after storage (WAS), 4, 6, 8, 10, 12, and 14 WAS. Each time tubers were removed from storage the stolon and bud ends were sliced and rated following the 0-3 Texas scale. Preliminary results showed that stolon end rates are higher than bud ends initially, however, as the storage season progressed; Lso became more evenly distributed (Hamm and Rondon unpublished data).



Late season control

In 2011, trials were established at the OSU-HAREC to test the efficacy of several chemicals controlling late infestations of potato psyllids. Applications were made 1 October 2011 and number of potato psyllids was taken 7 and 14 days after application. Chemicals tested included Warrior II 2.09 CS, Agrimek 0.15 EC, Endigo 2.06 ZC, Fulfill 50 WG, Movento, Oberon 2 SC, Asana XL, Vydate C 3.77 SL and Requiem 25EC. Warrior and Endigo were the best treatments controlling nymphs and eggs.

Late season application of selected pesticides, Hermiston, Oregon 2011

Treatments	# of observations	Nymphs	Eggs	Nymphs and Eggs	ZC Rate (0-3 Texas scale)
Untreated check	10	6.0±0.3 a	3.2±1.4 b	6.2±1.5	0.86
Warrior II 2.09 CS	10	0.6±0.3 b	3.2±1.7 b	3.8±1.7	0.04
Agrimek 0.15 EC	10	1.4±2.6 b	4.9±1.6 ab	6.3±2.0	0.04
Endigo 2.06 ZC	10	0.8±0.4 b	2.9±1.6 b	3.7±1.3	0.13
Fullfill 50 WG	10	1.4±0.5 b	4.9±1.3 ab	6.3±1.6	0.03
Movento 2 SC	10	1.1±0.6 b	3.8±1.7 b	4.9±2.0	0.07
Oberon 2 SC	10	1.1±0.7 b	4.0±1.4 b	5.2±1.8	0.06
Asana XL	10	2.4±1.0 ab	8.1±2.5 a	10.5±3.0	0.08
Vydate C V 3.77 SL	10	3.9±1.3 a	17±5.9 a	20.9±7.2	0.11
Requiem 25EC	10	1.4±0.5 b	5.1±2.8 ab	6.5±3.0	0.14

Application was made 1 October 2011. This data represent counts 7 days after treatment (7 October). Rondon's lab

Research in coming years

The 2012 season was intense. My program studied the seasonal migration of psyllids in the Columbia Basin, evaluated the impact of pest management practices and incidence of Lso, and evaluated insecticides for psyllid management in the Pacific Northwest. Overwintering of potato psyllids in the region was also studied (Murphy et al. 2012). We also educated, communicated, and disseminated information about this pest to the industry. In 2012, twelve potato psyllid workshops were offered; in the future, we will continue to train growers, fieldmen, etc., about how to ID potato psyllids and other potato pests. Information and relevant articles are continuously posted in potato newsletters, potato magazines,

and other journals and relevant websites (http://oregonstate.edu/dept/hermiston/silvia-rondon, http://www.nwpotatoresearch.com/PR/Index.cfm, http://zebrachipscri.tamu.edu/).

Conclusions

Considering the sudden appearance of this disease in the Columbia Basin and Idaho in 2011, and the uncertainty about the future role ZC will play in the Basin, the attitude towards best management practices for controlling the psyllid/*Liberibacter* disease complex will certainly change as more information regarding this disease and vector become available. Fortunately, risk of ZC in the Columbia Basin appears to be reduced compared to production areas in the Southeastern USA.

Acknowledgements

Rondon's summer crew and funding from the mini SCRI grant, Oregon and Washington State Potato Commissions, and Agricultural Research Foundation. Thanks to Alexzandra Murphy and Philip Hamm for comments on the manuscript.

References

Hamm P.B., S.I. Rondon, J.M. Crosslin, and J.E. Munyaneza. 2011. A new threat in the Columbia Basin of Oregon and Washington: Zebra Chip. *In* Proc. of the 11th Annual Zebra Chip Reporting Session. F. Workneh and C.M. Rush (editors). 7-8 Nov. San Antonio, TX. Pp 1-6.

Murphy A., S.I. Rondon, and A. Jensen. 2012. First report of potato psyllids, *Bactericera cockerelli*, overwintering in the Pacific Northwest. (Submitted 6/1/12; Accepted 10/22/12) (*In Print*).

Rondon S.I., and P.B. Hamm. 2012. Zebra Chip: a new potato pest in the Columbia Basin of Oregon and Washington. *In* 71st annual Pacific Northwest Insect Management Conference. 9-10 Jan. Portland, OR. Section IV. Pp 45-47.

Tomato/Potato Psyllid Phenology in a Temperate Climate

Vereijssen, J.[1], Jorgensen, N.[1], Taylor, N.[2], Read, S.[1], and Berry, N. A.[1]

[1]The New Zealand Institute for Plant & Food Research Limited Lincoln, Private Bag 4704, Christchurch Mail Centre, Christchurch 8140, New Zealand; [2]The New Zealand Institute for Plant & Food Research Limited Hawke's Bay, Private Bag 1401, Havelock North 4157, New Zealand

Abstract
To help growers in their efforts to sustainably control tomato/potato psyllid (TPP) and to understand this pest's population dynamics, seasonal monitoring in New Zealand's main potato growing areas was undertaken. Two field trials were conducted (2010–11 and 2011–12) in Lincoln (South Island, New Zealand) where TPP adults were trapped using yellow sticky traps and all life stages were counted on plants. Psyllid numbers varied greatly between the two years. Sticky traps trapped adults about 2 weeks before they were found on plants, making these traps a good monitoring option early in the season. First appearance of adults in the potato crop could roughly be forecasted by calculating Degree Days; however, peaks in summer could not be forecasted. Psyllid numbers in summer were explained by weather, where warm and dry springs and summers seem to be more beneficial to increased numbers than cold and wet ones. Rainfall also seems detrimental to population build-up. The first-guess CLIMEX model projection is a valuable step to highlight limits to understanding TPP in New Zealand. This study has provided insight into the phenology and population dynamics of TPP in Canterbury. It is now generally known when TPP may arrive in the crop, which will aid growers in controlling TPP more sustainably and also decrease insecticide resistance development.

Introduction
Since 2006, solanaceous crops grown in New Zealand have been affected by an exotic insect pest, the tomato/potato psyllid (*Bactericera cockerelli* (Sulc)), (Hemiptera, Triozidae) (TPP) (MAF Biosecurity 2009). The arrival of TPP in New Zealand and the identification of its role as a vector of the bacterial pathogen *Candidatus* Liberibacter solanacearum (Lso) have presented a considerable challenge to the New Zealand capsicum, tamarillo, tomato and potato industries. Main control of TPP is by insecticide sprays, varying from 1 to 17 in potatoes per season depending on the region. Seasonal monitoring in New Zealand's main potato growing areas was needed to understand the population dynamics of this pest and aid growers in controlling TPP more sustainably.

Here we describe data from 2 years of monitoring in a potato crop in Lincoln (Canterbury, South Island, New Zealand), the primary objective being to answer two questions: 1. Can sticky traps indicate the first appearance of TPP on potatoes in Canterbury? and 2. What is the seasonal phenology of TPP on potatoes in Canterbury?

Materials & Methods
Trial location. This study was part of a 3-year National Monitoring programme in commercial potato, tomato and tamarillo crops in New Zealand (Dohmen-Vereijssen et al. 2012). For this study we focused on two potato crops situated in Lincoln (South Island, New Zealand). The crop in the 2010–11 growing season (-43° 37' 58.24", +172° 29' 38.73") was on a 3 ha field, planted on 22 November 2010 and emerged 09 December 2010. The crop in 2011–12 (-43° 37' 54.89", +172° 29' 52.33") was on a 1.5 ha field, planted on 25 October 2011 and emerged 10 November 11.

Yellow sticky trap monitoring and plant assessments. Double-sided yellow sticky traps (BugScan®, 25 cm x 10 cm) were mounted on wire frames or stakes just above plant height. Traps were placed 5 m from the field edge and where possible at the north, south, east and west corners of a site, i.e. a total of four traps/field. Traps were collected and replaced weekly. In the lab, traps were assessed for TPP adults using a binocular microscope. Traps were out from 12 November 2010 until 3 June 2011 and from 16 November 2011 until 14 March 2012.

In the same crop, 50 randomly selected plants were assessed weekly. At first emergence, whole plants were sampled until this became too labor-intensive; then we switched to sampling whole stems. Numbers of all TPP life stages on two whole stems (all leaves attached) per plant (each stem facing other rows) were recorded. Plant assessments were conducted from 12 November 2010 until 28 January 2011 and from 16 November 2011 until 14 March 2012. Plants assessments were continued for a longer period in the second year, as the first year trapping showed some interesting dynamics after plant senescence.

All sampling was influenced by normal commercial practices (e.g. spraying) and localized environmental effects (e.g. rainfall).

Results & Discussion

Yellow sticky traps picked up TPP adults about 2 weeks earlier than plant assessments in both seasons. This makes these traps a good monitoring option early in the season. The numbers of adults on the traps and on 10 plants went up similarly until about mid- to late January (Figures 1 and 3), where after adult numbers on the plants increased faster than numbers on the sticky traps (Figure 3).

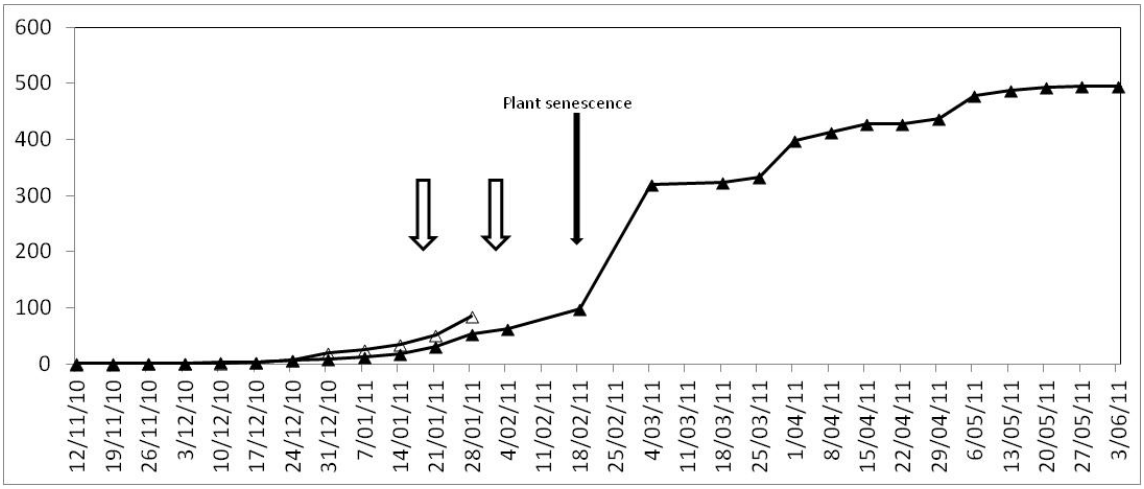


Figure 1. Cumulative number of tomato potato psyllids on yellow sticky traps (black triangles) or on potato plants (open triangles) in Lincoln in 2010–11. Arrows indicate insecticide sprays.

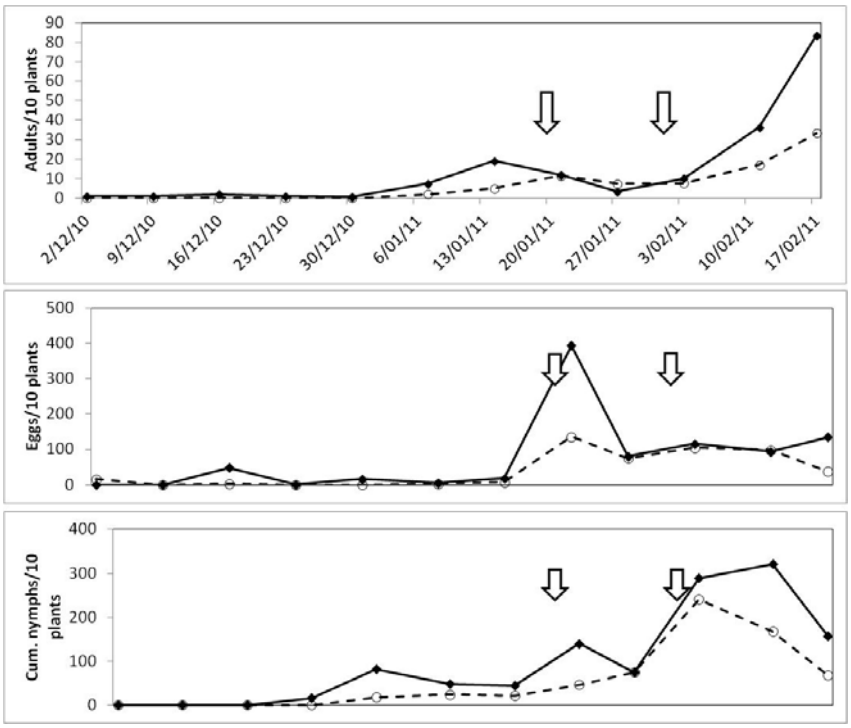


Figure 2. Number of tomato potato psyllid adults, eggs and nymphs per 10 potato plants at the edge of the crop (black diamonds) or within the crop (open circles) in Lincoln in 2010–11. Arrows indicate insecticide sprays.

In the 2010–11 season, life stages followed each other in a sequence (Figure 2), whereas in 2011–12 there was less chronology (Figure 4). This could be due to spray regimes (e.g. killing different life stages) or environmental factors (e.g. rainfall). In both years, however, survivorship of eggs and nymphs was not great. This could be caused by natural mortality, which is quite high in early instars (Yang & Liu 2009, Liu & Trumble 2006, Fung 2012), predation by beneficial insects (MacDonald et al. 2010) or insecticide sprays. Overall psyllid numbers were higher in 2010–11 than in the 2011–12 season.

Degree Days (DD), with a 7.1^{o}C base for the lower development threshold for TPP (Tran et al. 2012), from 1 June until 31 December were calculated to explain the differences between the two years. DDs were useful as a rough estimate for first appearance of TPP on sticky traps, based on the 358 DD to develop from egg into an adult (Tran et al. 2012), but not to explain psyllid peaks in summer (January–February). Running averages temperature and rainfall deviation graphs showed that warm and dry springs and summers result in higher psyllid numbers than wet and cold ones (data not shown). Rainfall seemed to be detrimental to psyllid population build up. The next step was habitat distribution modeling which would be useful to highlight limits to understanding TPP in New Zealand. This resulted in a first-guess CLIMEX model projection (D.P. Logan, unpublished) (Figure 5). Although the CLIMEX projection is very useful at the moment, it is a work in progress as not all TPP field finds or differences between regions can be explained yet.

This study has provided insight into the phenology and population dynamics of TPP in Canterbury. It is now generally known when TPP may arrive in the crop. This will aid growers in controlling TPP more sustainably; for example, by being able to omit the first insecticide sprays. Besides increasing profits, this will also decrease insecticide resistance development in TPP. The ultimate goal would be to develop

a region-specific weather-based supervised control system based on Tran et al.'s (2012) model, which would omit the sometimes unreliable and always labor-intensive psyllid monitoring.

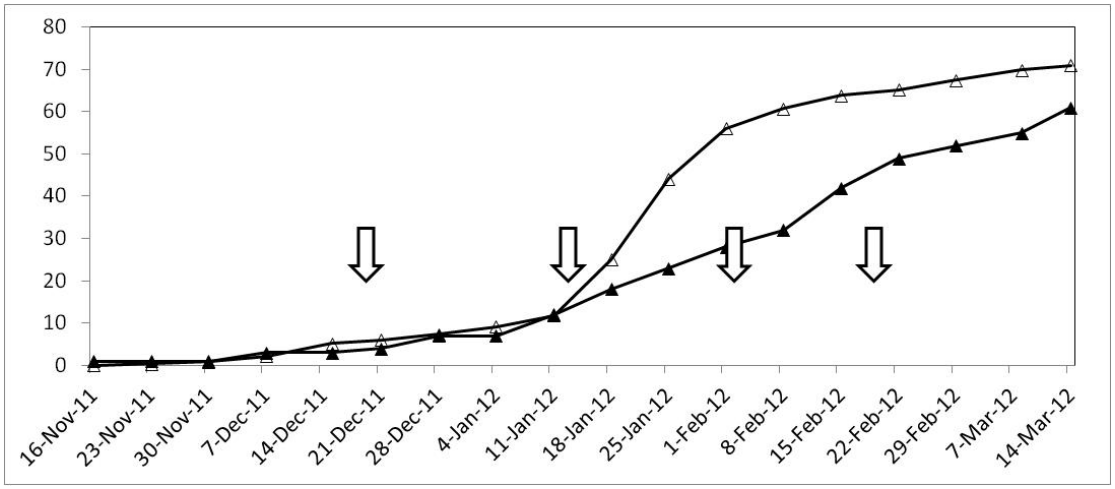


Figure 3. Cumulative number of tomato potato psyllids on yellow sticky traps (black triangles) or on potato plants (open triangles) in Lincoln in 2011–12. Arrows indicate insecticide sprays. Plants sprayed off 16 March 2012.

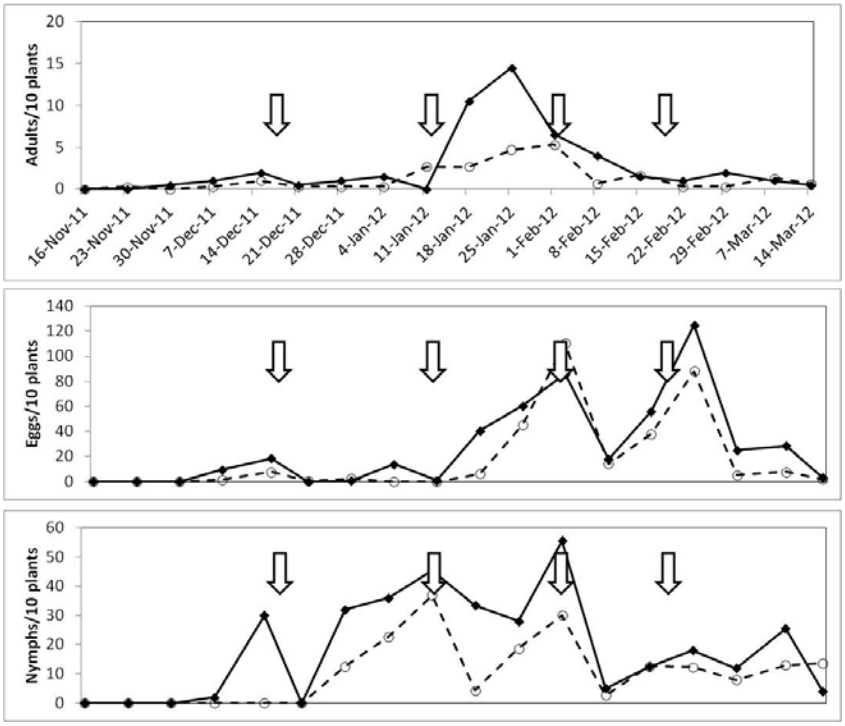


Figure 4. Number of tomato potato psyllid adults, eggs and nymphs per 10 potato plants at the edge of the crop (black diamonds) or within the crop (open circles) in Lincoln in 2011–12. Arrows indicate insecticide sprays.

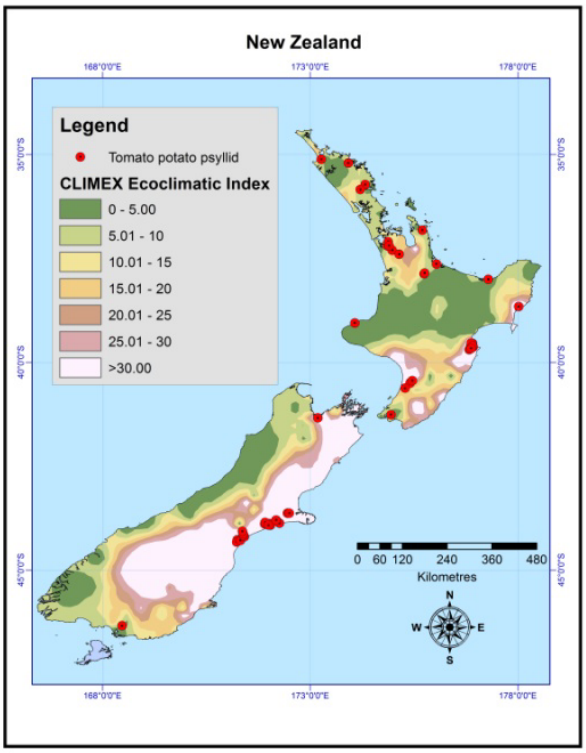


Figure 5. A first-guess tomato/potato psyllid (TPP) CLIMEX model projection for New Zealand. The Ecoclimatic Index is a numerical value for climatic suitability and relative abundance of the species. Index values >26 are considered very favorable for establishment of a permanent population at a given location. Dots indicate TPP finds in field crops (tamarillo, tomato and potato). Map courtesy of David Logan, Plant & Food Research, Te Puke, New Zealand.

Acknowledgements

The authors would like to thank the MAF Sustainable Farming Fund for providing funding for the National Monitoring project. We are also in dept to David Logan (Plant & Food Research Te Puke, New Zealand), Susan Worner and Luc Tran (Bio-Protection Research Centre, Lincoln University, New Zealand) for great discussions around psyllid phenology and sharing of ideas and data.

References

Dohmen-Vereijssen J., Taylor N., and Jorgensen N. 2012. MS 4 Psyllid National Monitoring - Year 3 activity and final report. A Plant & Food Research report prepared for Market Access Solutionz Limited. SPTS No. 7265. 9p.

Fung L. 2012. Understanding TPP. NZ Grower 67:30-31.

Liu, D., and Trumble, J.T. 2006. Ovipositional preferences, damage thresholds, and detection of the tomato-potato psyllid *Bactericera cockerelli* (Homoptera: Psyllidae) on selected tomato accessions. Bull. Entomol. Res. 96:197-204.

MacDonald F.H., Walker G.P., Larsen N.J., and Wallace A.R. 2010. Naturally occurring predators of *Bactericera cockerelli* in potatoes. New Zealand Plant Protection 63:275 (Abstract only).

MAF Biosecurity 2009. Potato/Tomato Psyllid *Bactericera cockerelli.* http://www.biosecurity.govt.nz/pests/potato-tomato-psyllid (accessed 11 December 2012).

Tran, L.T. Worner, S.P. Hale, R.J., and Teulon, D.A.J. 2012. Estimating development rate and thermal requirements of *Bactericera cockerelli* (Hemiptera: Triozidae) reared on potato and tomato by using linear and nonlinear models. Environ. Entomol. 41:1190-1198.

Yang, X.-B., and Liu, T.-X. 2009. Life history and life tables of *Bactericera cockerelli* (Homoptera: *Psyllidae*) on eggplant and bell Pepper. Environ. Entomol. 38:1661-1667.

Spatial-temporal Distribution of '*Candidatus* Liberibacter solanacearum' Haplotypes in the United States

Wen, Aimin, Johnson, Chris, and Gudmestad, Neil C., Department of Plant Pathology, North Dakota State University, Fargo, ND 58108

Abstract

A study on the temporal and spatial distribution of '*Candidatus* Liberibacter solanacearum' (Lso) haplotypes was conducted using plant samples that had either ZC or PY symptoms, and potato-psyllid samples collected from 2006 to 2012 from seven US states, Mexico, and New Zealand. A PCR assay developed by our group was used for this study. In TX, CO, NE, KS of the USA, both haplotypes of Lso were present in symptomatic potato plants and psyllids. Only haplotype A was detected in North Dakota psyllid samples collected in 2010, in Idaho and Washington ZC potato samples sampled from storage in 2011, and in Idaho ZC potato samples in 2012. Haplotype A Lso was also detected in New Zealand ZC-affected potato samples and psyllid samples collected in 2010 and 2011. Both haplotypes can be present in individual psyllids, occasionally in plants. In TX and NE, there has been a temporal shift from a mixed population to a dominate haplotype A population. A shift of haplotype was found to be associated with differences in ZC symptomology.

Introduction

Zebra chip or zebra complex (ZC) disease is an emerging disease of potato that causes significant losses to potato production. The putative causal agent has been identified as '*Candidatus* Liberibacter solanacearum' (Lso) and is vectored by the tomato/potato psyllid (*Bactericera cockerelli* Sulc) (Liefting et al. 2009). Since its first detection, ZC has spread to additional states including New Mexico, California, Nebraska, Kansas, Colorado, Wyoming, Oregon, Washington and Idaho (Gudmestad and Secor 2007, Crosslin et al. 2012a, 2012b). Studies have shown that there are two major lineages of Lso associated with ZC, namely clade 1 or haplotype A (or Lineage 2 or ST-2), and clade 2 or haplotype B (or Lineage 1 or ST-1) (Secor et al. 2009, Wen et al. 2009, Nelson et al. 2010, Lin et al. 2011, Glynn et al. 2012). Although there is currently no definitive phenotypic differences assigned to Lso haplotypes, field observations suggest haplotype B Lso produces a more severe and destructive disease symptom than haplotype A (Gudmestad, personal observation). Lso also can be detected from psyllid-yellows (PY) symptomatic potato samples (Wen et al. 2009). Geographically, New Zealand only has haplotype A Lso, whereas the southwestern states of the US have both types, and the Pacific Northwestern region only has haplotype A (Nelson et al, 2011, Wen et al, 2012). Nelson et al. (2011) recommended that haplotype should be noted for Lso reports to better understand the biological implications. However, determination of the haplotypes used to be very tedious and expensive since it involves sequencing the 16S rRNA gene or other target genes. With the availability of a rapid and simple typing PCR assay (Wen et al, 2011), Lso haplotyping is made possible on large volume samples. In this study, using this typing PCR assay, we investigated the spatial-temporal distribution of Lso haplotypes in ZC and PY-symptomatic potato samples and in psyllid samples collected in commercial potato fields from 2006 to 2012 in the USA, and Lso typing was also made on DNA samples from potato and psyllids collected in New Zealand.

Materials and Methods

Plant samples and DNA extraction. ZC and PY-symptomatic potato samples were collected from commercial fields in seven states of the USA between 2006 and 2012. DNA extraction from petioles, stolons, and tubers, if available, was performed as described previously (Wen et al. 2009). Potato tubers

displaying ZC symptoms were obtained from storage facilities in Idaho and Washington in 2011, kindly provided by Joe Rehder (ConAgra Foods--Lamb Weston, Inc., Pasco, Washington) and Dr. Nora Olsen (University of Idaho, Twin Falls Research and Extension Center, Twin Falls, ID). Total 650 archived and 593 fresh DNA samples were used in this study.

Psyllid samples and DNA extraction. Psyllid (egg, nymph and adult) samples were collected from potato production areas in California, Nebraska, North Dakota, and Texas, in 2010. DNA extraction from psyllid was conducted as previously described (Wen et al. 2009). DNA samples derived from Texas psyllid collected in 2011 and 2012, and DNA samples of Mexico psyllid raised in greenhouse in Washington, were kindly provided by Dr. Jim Crosslin (USDA-ARS, Prosser, WA). New Zealand potato and psyllid DNA samples collected in 2010 and 2011 were kindly provided by Drs. Andrew Pitman and Sam Beard (New Zealand Institute for Plant & Food Research, Canterbury Agriculture and Science Centre, Lincoln, New Zealand).

Lso typing was conducted using the SSR PCR assay developed by our group (Wen et al. 2011).

Results and Discussion

Haplotypes A and B Lso were detected in archived and fresh DNA from potato samples collected between 2006 and 2012 from Colorado, Kansas, Nebraska, and Texas (Table 1). Interestingly, only haplotype A Lso was detected in ZC-symptomatic potato samples from Idaho, Washington, Mexico and New Zealand. Likewise, only Haplotype A Lso was reported from potato samples displaying PY symptoms, which were collected from Nebraska in 2008 and 2012, and from Texas in 2011 and 2012 (Table 1). Both haplotypes A and B Lso were detected simultaneously in individual potato samples displaying ZC symptoms collected in 2010 and 2011 from Texas. Shifts in Lso haplotype detection frequency were noted in samples collected in Texas, Nebraska and Colorado from 2006 to 2012 with the detection of haplotype A Lso increasing in all three states (Figure 1). In Texas samples, collected between 2006 and 2008, the detection frequency of haplotype B Lso peaked at 67% in 2008 vs. 48% and 38%, in 2006 and 2007, respectively (Figure 1A). However, detection frequency of haplotype B Lso decreased to 6% in 2010, and remained relatively low in 2011 and 2012 with detection frequencies of 18% and 8%, respectively (Figure 1A). In Nebraska samples, haplotype B Lso detection frequency decreased from 100% in 2006 to 8% in 2009; this haplotype was not detected in 2012 (Figure 1B). In Colorado samples, detection of haplotype B Lso decreased from 100% in 2006, to 67% in 2008 and 40% in 2012 (Figure 1C). Overall, in ZC-affected potato tissues collected from the US, Mexico and New Zealand, there has been a decrease in the detection frequency of haplotype B Lso (Figure 1D).

In archived DNA samples from potato psyllids from North Dakota and New Zealand, only haplotype A Lso has been detected, while psyllids collected from California were only infected with haplotype B Lso (Table 2). Both haplotypes A and B Lso were detected in potato psyllids from Nebraska, Texas, and Mexico (Table 2). Interestingly, 13% of the psyllids from Washington in 2010 and 41% and 25% of the psyllids from Texas in 2011 and 2012, respectively, were found to be simultaneously infected with both haplotype A and B Lso (Table 2). Likewise, all of the psyllids from Mexico in 2011 were infected with both haplotypes of Lso. This study revealed the temporal and spatial distributions of the two haplotypes of Lso in potatoes displaying either ZC or PY symptoms as well as in the potato-psyllid samples collected between 2006 and 2012 in seven US states, Mexico and New Zealand. Haplotype A Lso was found in potato samples from Washington, Idaho, and Wyoming, and, for the first time, in psyllid samples from North Dakota. It was laso the first report of haplotype B Lso in the state of California. Haplotypes A and B Lso were detected in potato samples displaying PY, and ZC symptoms in Nebraska, Colorado, Kansas and Texas, whereas only haplotype A was detected in potato and psyllid samples from New Zealand, which is in agreement with previous reports (Nelson et al. 2011, Lin et al. 2012, Glynn et

al. 2012, Wen et al. 2009). Temporal shifts in Lso haplotypes were noted during the course of these studies. For example, haplotype B Lso dominated in Texas in 2008 through 2010; however haplotype A Lso has become more prevalent since 2011. It is interesting to note that concomitant with this shift in prevalence of Lso haplotype, ZC disease has become less severe and less prevalent in that state (Gudmestad, personal observation). Another important finding of this study is that two haplotypes of Lso were detected simultaneously in individual potato plants from Texas and Nebraska and potato-psyllid samples from Texas, Washington and Mexico (greenhouse raised psyllids only), which indicates that co-infection of the two Lso haplotypes exist in agricultural ecosystems. This is the first report of co-infection of the two haplotypes of Lso in potato and the potato psyllid and it raises interesting questions regarding the efficiency of transmission of each haplotype by a dual infected bacteriliferous vector, *B. cockerelli*. Furthermore, since there are three haplotypes of this insect vector (Swisher et al. 2012), studies that investigate potential Lso haplotype-*B. cockerelli* haplotype interactions are warranted. Researches of Lso have been focused on ZC-symptomatic potato plants because of the economic loss associated with the disease. Our group had previously detected Lso in PY displaying potato plants (Wen et al. 2009), also we successfully graft-transmitted PY and detected Lso in scions showing PY symptoms (Wen et al. unpublished). This study further demonstrated that Lso detected in PY-symptomatic potato plants also had two haplotypes.

At the beginning of these studies we hypothesized that the two haplotypes might be different in virulence based on observations in the field in Texas over the past two years. Based on preliminary observations in greenhouse trials (Johnson and Gudmestad, unpublished) we still believe this to be the case, however, it will be important to empirically demonstrate differences in aggressiveness between Lso haplotypes under controlled conditions and these studies are in progress. Nonetheless, the source of the two haplotypes of Lso is still in question and should also be the focus of future research. We believe the focus of that research should be on the source of Lso in overwintering psyllid populations and whether or not the overwintering plant hosts harbor different genetic populations of Lso.

Table 1. Frequency of *'Candidatus* Liberibacter solanacearum' haplotypes detected in archived DNA from potato samples obtained from seven states of the USA, New Zealand and Mexico in 2006 - 2012

Year of collection	Origin[a] and symptom[b] (number of samples)	% Lso haplotype		
		A	B	A and B
2006	CO, ZC (1)	0	100	0
	NE, ZC (2)	0	100	0
	TX, ZC (73)	52	48	0
2007	TX, ZC (34)	62	38	0
2008	CO, ZC (6)	33	67	0
	KS, PY (7)	29	71	0
	KS, ZC (17)	29	71	0
	NE, PY (4)	100	0	0
	NE, ZC (25)	47	53	0
	TX, PY (6)	50	50	0
	TX, ZC (72)	31	69	0
	MX, ZC (16)	100	0	0
2009	CA, ZC (13)	92	8	0
	NE, ZC (62)	92	8	0
2010	TX, PY (16)	93	7	0
	TX, ZC (110)	63	6	31
2011	ID, ZC (77)	100	0	0
	TX, PY (79)	100	0	0
	TX, ZC (250)	30	23	47
	WA, ZC (77)	100	0	0
	NZ, ZC (11)	100	0	0
2012	CO, ZC (10)	60	40	0
	ID, ZC (23)	100	0	0
	NE, PY (4)	100	0	0
	NE, ZC (11)	100	0	0
	TX, PY (15)	100	0	0
	TX, ZC (30)	88	12	0

[a] NZ stands for New Zealand, and MX stands for Mexico.

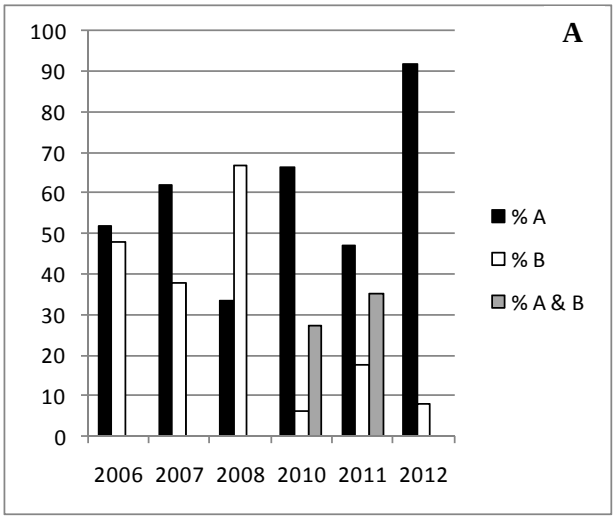
[b] ZC stands for zebra chip and PY for psyllid-yellows.

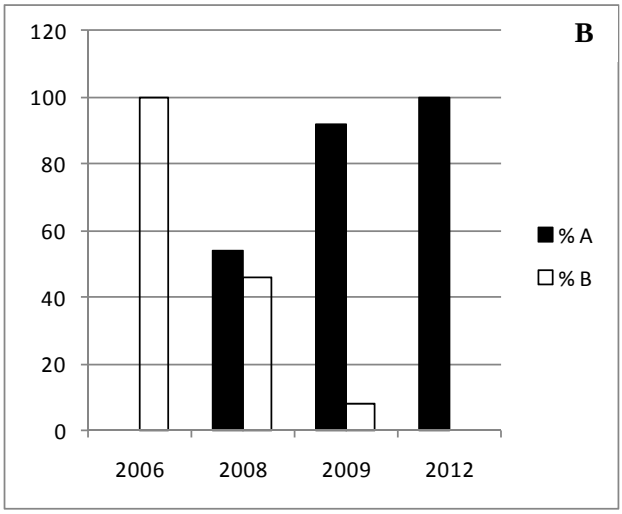
Table 2. Frequency of '*Candidatus* Liberibacter solanacearum' haplotypes detected in archived DNA from psyllid samples obtained from five states in the USA, New Zealand and Mexico in 2010 - 2012

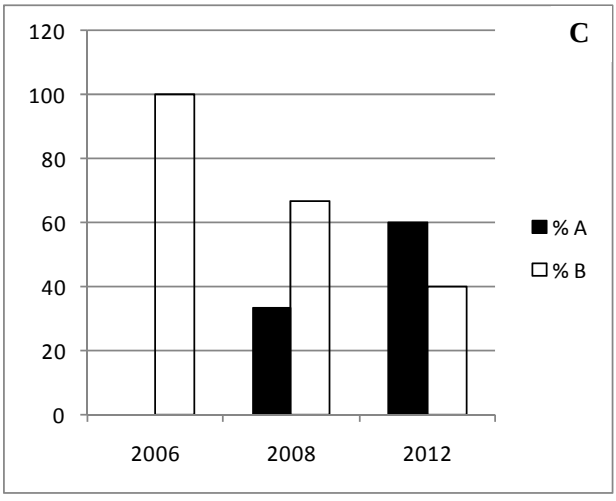
Year of collection	Origin (number of Lso positive samples)	% Lso haplotype		
		A	B	A and B
2010	CA (4)	0	100	0
	ND (37)	100	0	0
	NE (54)	98	2	0
	TX (5)	0	100	0
	WA (8)	50	37	13
2011	TX (12)	33	25	42
	NZ[a] (8)	100	0	0
	MX[b] (4)	0	0	100
2012	TX (122)	39	36	25

[a.] NZ stands for New Zealand.

[b.] Mexico psyllids were raised in growth room in Moxie, WA







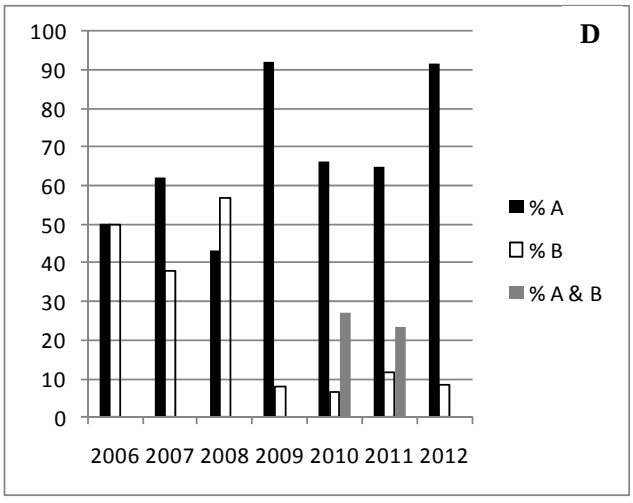


Figure. 1. '*Candidatus* Liberibacter solanacearum' haplotype detection frequency in potato samples collected in California, Colorado, Nebraska, Texas, Mexico and New Zealand from 2006 to 2012. A. Texas; B. Nebraska; C. Colorado; and D. All samples tested including those from California, USA and from Mexico and New Zealand.

References

Crosslin, J., P. Hamm, S. Rondon, J. Eggers, J.E. Munyaneza. 2012a. First report of zebra chip disease and '*Candidatus* Liberibacter solanacearum' on potatoes in Oregon and Washington State. Plant Dis. 96: 452.

Crosslin, J. M., N. Olsen, and P. Nolte. 2012b. First report of zebra chip disease and '*Candidatus* Liberibacter solanacearum' on potatoes in Idaho. Plant Dis. 96: 453.

Glynn, J.M., M.S. Islam, Y. Bai, S. Lan, A. Wen, N.C. Gudmestad, E.L. Civerolo, and H. Lin. 2012. Multilocus sequence typing of '*Candidatus* Liberibacter solanacearum' strains from North America and New Zealand. J. Plant Pathol. 94: 223-228.

Gudmestad, N. C., and G. A. Secor. 2007. Zebra chip: a new disease of potato. Nebraska Potato Eyes 19:1-4.

Liefting, L.W., B.S. Weir, S.R. Pennycook, and G.R.G. Clover. 2009. '*Candidatus* Liberibacter solanacearum' associated with plants in the family *Solanaceae*. Int. J. Syst. Evol. Microbiol. 59: 2274-2276.

Lin, H., M.S. Islam, Y. Bai, A. Wen, S. Lan, N.C. Gudmestad, and E.L. Civerolo. 2012. Genetic diversity of '*Candidatus* Liberibacter solanacearum' strains in the United States and Mexico revealed by simple sequence repeat markers. Eur. J. Plant Pathol. 132: 297–308.

Nelson, W.R., T.W. Fisher, and J.E. Munyaneza. 2011. Haplotypes of "*Candidatus* Liberibacter solanacearum" suggest long-standing separation. Eur. J. Plant Pathol. 130: 5–12.

Secor, G. A., V.V. Rivera, J.A. Abad, I.M. Lee, G.R.G. Clover, L.W. Liefting, X. Li, and S.H. De Boer. 2009. Association of '*Candidatus* Liberibacter solanacearum' with Zebra chip disease of potato established by graft and psyllid transmission, electron microscopy, and PCR. Plant Dis. 93: 574-583.

Swisher, K.D., J.E. Munyaneza, and J. M. Crosslin, 2012. High resolution melting analysis of the cytochrome oxidase I gene identifies three haplotypes of the potato psyllid in the United States. Env. Entomol. 41:1019-1028.

Wen, A., I. Mallik, V.Y. Alvarado, J.S. Pasche, X. Wang, W. Li, L. Levy, H. Lin, H.B. Scholthof, T.E. Mirkov, C.M. Rush, and N.C. Gudmestad. 2009. Detection, distribution and genetic variability of '*Candidatus* Liberibacter' species associated with Zebra complex disease of potato in North America. Plant Dis. 93: 1102-1115.

Wen, A., H. Lin, and N. C. Gudmestad. 2011. Development of PCR Assay Using SSR Primers for Detection and Genotyping of '*Candidatus* Liberibacter solanacearum'. Proceedings of the 11th Annual SCRI Zebra Chip Reporting Session (Workneh, Rashed, and Rush editors). Nov 6-9, 2010. San Antonio, Texas. pp 74-78.

Wen, A., C. Johnson, and N. C. Gudmestad. 2012. Genetic characterization of '*Candidatus* Liberibacter solanacearum' associated with zebra chip disease of potato in Washington and Idaho. Phytopathol. 102:S4.134

Plant Defenses against Psyllids and *Liberibacter*

[1]Tamborindeguy, C., [1]Huot, O., and [2]Levy, J. Texas A&M University, [1]Department of Entomology, [2]Department of Horticultural Sciences, College Station, TX 77843, USA

Abstract
Upon insect or pathogen attack, plants can activate phytohormone-mediated defense pathways resulting on the production of defensive compounds. The activation of those pathways might result in decreased insect performance and/or pathogen infection, but in some cases the attackers have evolved strategies to counteract plant defenses. Little is known about plant defenses against psyllids and/ or "*Candidatus* Liberibacter solanacearum". We examined the effects of jasmonic acid (JA) and acetylsalicylic acid (ASA) sprays on plants resistance against psyllids. We measured several psyllid life history traits such as number of eggs, nymphs and adults. We found that plant spraying with methyl jasmonate did not affect any of the psyllid life history traits measured while plant spraying with ASA resulted in decreased progeny counts. Furthermore, we detected a lower number of plants infected with "*Candidatus* Liberibacter solanacearum" when ASA was sprayed. We are currently investigating the molecular mechanisms of plant-psyllid-*Liberibacter* interaction by measuring changes in defense-related gene expression following psyllid infestation and/or "*Candidatus* Liberibacter solanacearum" infection.

Introduction
Upon insect or pathogen attack, induced plant defenses can be activated leading to the production of defensive compounds. The production of these compounds is regulated by phytohormones. The two main studied plant-defense phytohormones are jasmonic acid (JA) and salicylic acid (SA). It has been shown that, in general attack by chewing insects and necrotrophic pathogens leads to the induction of JA-mediated defenses, while the attack by sucking insects or biotrophic pathogens induces SA-mediated defenses (Walling 2000; Howe and Jander 2008). However, some attackers are capable of manipulating plant defenses by actively inhibiting the induction of particular pathways.
"*Candidatus* Liberibacter solanacearum" (Lso) is the bacterium that infects solanaceous plants and causes "zebra chip disease" in potato (Secor 2004; Munyaneza 2007; Hansen et al. 2008; Liefting et al. 2008; Munyaneza 2009). This bacterium is transmitted by the phloem-feeding insect, the potato/tomato psyllid, *Bactericera cockerelli* Šulc (Hemiptera: Triozidae) (Munyaneza 2007; Munyaneza 2007; Hansen et al. 2008). Very little is known about the defense pathways induced in solanaceous plants by these two attackers or their ability to manipulate plant defenses. The goal of the current study was to determine the effects of JA or ASA sprays (proxy for JA or SA pathway induction, respectively) on potato psyllids' life history traits.

Materials and Methods
Insect source. Psyllids were maintained in our laboratory in Texas A&M University, College Station on healthy tomato plants in separate 14'' X 14'' X 24" insect cages (BioQuip) at temperature of 23 ± 1°C and photoperiod of 16:8 h (L:D).
Plants. Tomato plants, *Solanum lycopersicum* L. cultivar Moneymaker (Victory seeds) were used for all experiments. Plants were grown in 4-inch pots with Sun Gro® Metro-Mix 900 mix and fertilized twice a week. Plants used for experiments were within 4-5 weeks of age.
Phytohormone elicitor treatments.
Jasmonic acid application: A 0.5 mM jasmonic acid solution (JA solution) was made by dissolving (±) jasmonic acid ((±)-1α,2β-3-Oxo-2-(cis-2-pentenyl) cyclopentaneacetic acid) (Sigma Aldrich) in HPLC

graded methanol at 100 g/L, and by mixing this solution with water. The 0.5 mM JA concentration is well below the toxic level for plant (Thaler et al. 2001). A control solution was similarly made but without (±) jasmonic acid. JA and control solutions were applied weekly to 11 plants each using small spray bottle. Each plant was sprayed on the top and on the sides while having one leaf covered by a wooden block to prevent any residue effect on the insects. Each plant from the control and JA treatment received a weekly spray for 3 weeks. One week after the first spray, two Lso-negative potato psyllids, a male and a female, were placed in BioQuip clip cage on to the top most fully expanded leaf of the tomato for one week. Progeny was counted weekly until the end of the experiment.

Acetylsalicylic acid application: Seven plants were caged. Each plant was sprayed weekly with either 0, 0.35 mM, 1 mM, 5 mM, 10 mM, 25 mM, or 50 mM of ASA solution as previously described. One week after the first spray, 30 potato psyllid adults from a Lso-infected colony were released in each cage. Psyllid progeny were counted weekly in each plant for three weeks. The experiment was repeated 6 times. At the end of the experiment plant tissue samples were collected and tested by PCR for Lso infection as described in (Levy et al. 2011).

Statistical analyses. To determine if JA or ASA sprays affected psyllid progeny, egg and nymph counts were analyzed using Kruskal–Wallis one-way analysis of variance test using R 2.15.2 (kruskal.test and Nemenyi-Damico-Wolfe-Dunn test from the package coin).

Results and Discussion

Jasmonic acid spray:

No significant differences in total oviposition or in nymphal counts were found after 1 or 2 weeks (Figure 1, Table 1). Similarly, no differences in development time were observed among progeny in both treatments: after 1 week only eggs and 1st instar nymphs were observed, while in the second week, all eggs had hatched and nymphs on 1st to 4th instar were observed. By week 3, all progeny had reached adulthood.

Acetylsalicylic acid spray:

Differences in eggs and nymphs were observed among plants sprayed with different ASA doses (Table 1, Figure 2). After the first week, there were significantly fewer eggs, nymphs (data not shown) and total progeny (Figure 2) in plants sprayed with 25 mM ASA than with 0 mM (control plants). After the second week, there were significantly fewer eggs, nymphs (data not shown) and total progeny (Figure 2) in plants sprayed with 10, 25 and 50 mM ASA realtive to control plants. However, high ASA concentrations resulted in premature death of the plants, therefore only lower ASA concentration will be used in future experiments. Progeny counts differences between plants sprayed with 5 mM and 0 mM were not significant, but experiments will be repeated to increase sample size since this is only a preliminary experiment. All plants sprayed with 0 mM of ASA tested positive for Lso 4 weeks after beginning of experiment, while fewer plants sprayed with ASA tested positive for Lso. This experiment will be repeated and Lso levels will be quantified using quantitative PCR (Levy et al. 2011).

These results suggest that JA spray of plants did not affect psyllid fecundity, while plant ASA spray reduced counts of psyllid progeny and Lso infection. Future experiments will evaluate psyllid and/or Lso ability to manipulate plant defenses. For instance, it has been shown that Lso can manipulate plant defenses benefiting psyllid success (Casteel et al. 2012).

Life history trait	JA spray effect	SA spray effect
Egg counts after 1 week	*H*=0.63, df=1, *P*=0.43	***H*=14.42, df=6, *P*=0.03**
Nymphal counts after 1 week	*H=0.16*, df=1, *P*=0.69	***H*=13.47, df=6, *P*=0.04**
Total progeny after 1 week	*H*=0.36, df=1, *P*=0.55	***H*=14.46, df=6, *P*=0.02**
Egg counts after 2 weeks	*H*=0.45, df=1, *P*=0.50	***H*=23.05, df=6, *P*<0.001**
Nymphal counts after 2 weeks	*H*=0.45, df=1, *P*=0.5	***H*=28.25, df=6, *P*<0.001**
Total progeny after 2 weeks	*H*=0.11, df=1, *P*=0.74	***H*=29.80, df=6, *P*<0.001**
Egg counts after 3 week	-	***H*=14.44, df=6, *P*=0.03**
Nymphal counts after 3 weeks	-	***H*=31.04, df=6, *P*<0.001**
Total progeny after 3 weeks	-	***H*=29.3, df=6, *P*<0.001**

Table 1. Significance of JA and ASA sprays potato psyllid progeny. Significant differences are in bold.

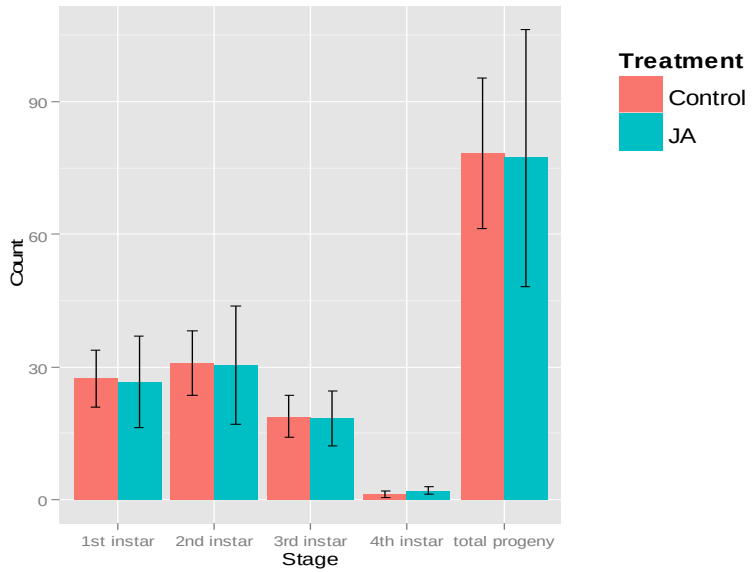


Figure 1: Effect of JA sprays on progeny counts (mean progeny counts ± SE) of potato psyllids after 14 days.

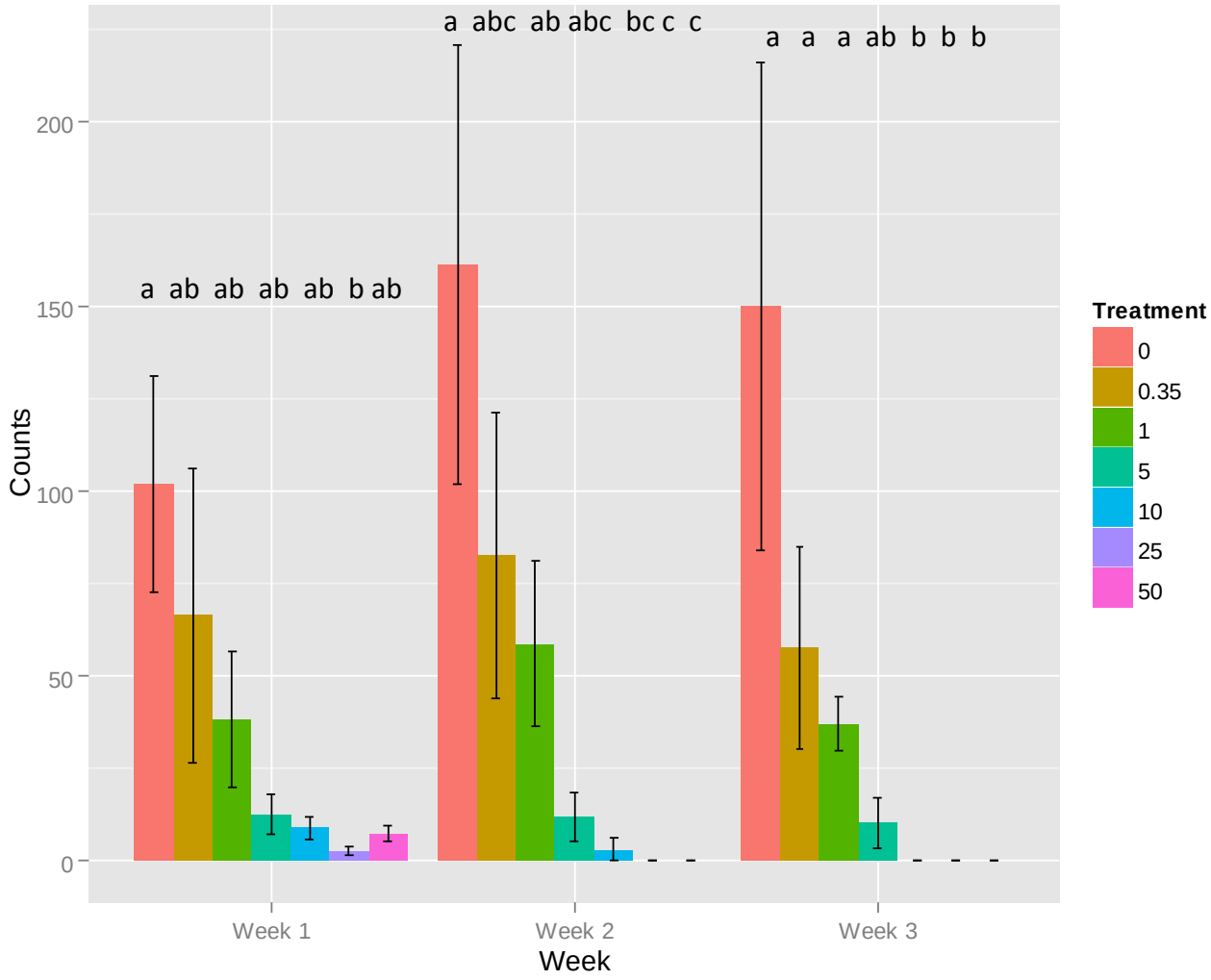


Figure 2: Effects of ASA sprays on total progeny counts (mean total progeny count ± SE) of potato psyllids. Means with different letters are significantly different within each week (Nemenyi-Damico-Wolfe-Dunn test).

References

Casteel, C. L., A. K. Hansen, et al. 2012. Manipulation of Plant Defense Responses by the Tomato Psyllid (Bactericerca cockerelli) and Its Associated Endosymbiont Candidatus Liberibacter Psyllaurous. PLoS ONE **7**(4).

Hansen, A. K., J. T. Trumble, et al. 2008. A new Huanglongbing Species, "*Candidatus* Liberibacter psyllaurous," found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). Appl. Environ. Microbiol. 74: 5862-5865.

Howe, G. A. and G. Jander. 2008. Plant immunity to insect herbivores. Annual Review of Plant Biology 59: 41-66.

Levy, J., A. Ravindran, et al. 2011. "Translocation of "Candidatus Liberibacter solanacearum", the Zebra Chip Pathogen, in Potato and Tomato. Phytopathology (In press).

Liefting, L. W., Z. C. Perez-Egusquiza, et al. 2008. A new '*Candidatus* Liberibacter' species in Solanum tuberosum in New Zealand. Plant Dis. 92: 1474.

Munyaneza, J. E., J.A. Goolsby, J.M. Crosslin, and J.E. Upton. 2007. Further evidence that zebra chip potato disease in the lower Rio Grande Valley of Texas is associated with *Bactericera cockerelli*. Subtrop. Plant Sci. 59: 30-39.

Munyaneza, J. E., J.M. Crosslin, and J.E. Upton. 2007. Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with zebra chip, a new potato disease in southwestern United States and Mexico. J. Econ. Entomol. 100: 656-663.

Munyaneza, J. E., Sengoda, V.G., Crosslin, J.M., De la Rosa-Lozano, G., and Sanchez, A. 2009. First report of '*Candidatus* Liberibacter psyllaurous' in potato tubers with zebra chip disease in Mexico. Plant Dis. 93: 552.

Secor, G. A., and Rivera-Varas, V.V. 2004. Emerging diseases of cultivated potato and their impact on Latin America Revista Latinoamericana de la Papa (Suplemento) 1: 1-8.

Thaler, J. S., M. J. Stout, et al. 2001. Jasmonate-mediated induced plant resistance affects a community of herbivores. Ecological Entomology 26: 312-324.

Walling, L. L. 2000. The myriad plant responses to herbivores. Journal of Plant Growth Regulation 19: 195-216.

Different Potato Cultivars May Vary in Zebra Chip Symptoms and Associated Tuber Physiological Changes When Infected by '*Candidatus* Liberibacter solanacearum'

Wallis, C. M., Rashed, A., Wallingford, A., and Rush, C. M. USDA-ARS-SJVASC, 9611 S. Riverbend Ave, Parlier, CA 93631, USA; and Texas A&M AgriLife Research, Amarillo, TX 79106, USA

Abstract

Zebra chip disease (ZC) is an increasingly important disease for potato production in the United States and elsewhere, as it causes undesirable browning symptoms in both fresh and fried potatoes. ZC is putatively caused by '*Candidatus* Liberibacter solanacearum' (Lso), a bacterium which is spread by the potato psyllid. Previous studies have observed that ZC symptoms are due to changes in tuber physiology, especially increased phenolic, sugar, and amino acid content. However, ZC-associated shifts in tuber physiology only have been observed in chipping cultivars of potato. Anecdotal reports have observed that some market classes of potato (such as red potatoes) may exhibit fewer ZC symptoms when infected with Lso. Thus, this study examined the progression of ZC and associated changes in tuber biochemistry that occurred when three different potato cultivars of different market classes (Red La Soda, Russet Norkotah, and the chipping variety FL1867) were infected with Lso one, two, three, or four weeks prior to harvest. At harvest, tuber symptoms, Lso titer levels, and biochemistry were assessed. Contrary to anecdotal reports, in the present study red potato tubers were observed to have greater ZC severity than russet or chipping potato tubers. Red potatoes infected for four weeks had significantly lower levels of phenolics than russet or chipping potatoes. There were no significant differences in chemical levels between cultivars when the potatoes were infected for other durations. Regardless of cultivar, tubers infected for at least three weeks had greater levels of fructose, glucose, and overall phenolics than non-infected tubers. Levels of the amino acids asparagine, aspartic acid, glutamic acid, glutamine, and methionine were greatest in tubers infected for one or two weeks. However, levels of all other amino acids were greatest in tubers infected for four weeks. ZC symptoms and Lso titer levels were positively correlated with reducing sugar, phenolic, and amino acid levels, with the exception of asparagine, aspartic acid, glutamic acid, glutamine, and methionine. These results suggest that tuber physiological alterations generally are consistent among major varieties of commercially-grown potatoes.

Introduction

Zebra chip disease (ZC) is of increasing concern for potato growers in the United States and elsewhere as it causes undesirable browning of freshly-cut and fried potato tubers leading them to become unmarketable. ZC is caused by the fastidious bacterium '*Candidatus* Liberibacter solanacearum' (Lso), which is vectored by the potato psyllid, *Bactericera cockerelli* Sulc (Hemiptera: Triozidae).

Key to the development of novel management methods and ZC disease-tolerant potato cultivars is knowledge about how ZC symptoms progress in Lso-infected potatoes. Initial work by Navarre et al. (2009) observed that some amino acids (namely tyrosine) and phenolic compounds were at greater levels in ZC-symptomatic tubers than non-infected tubers. Wallis et al. (2012) further characterized the differences between symptomatic and asymptomatic tubers, and observed that overall levels of phenolics, several amino acids, and host defense-associated proteins were positively associated with ZC symptoms. Rashed et al. (2013) observed that phenolics, reducing sugars, and several amino acids increase over the duration of Lso infections.

Greater phenolic compound levels and increased activity of polyphenol oxidases are hypothesized to result in increased browning upon exposure to air, as polyphenol oxidase changes many phenolic compounds to brown-colored products, similar to browning that occurs when apples are sliced (Mayer et al. 2006). Likewise, increased amino acid and sugar levels within tubers should increase acrylamide formation of fried tuber slices, as reducing sugars and amino acids are substrates in acrylamide-forming reactions (Friedman and Levin 2008).

Anecdotal accounts suggest that particular potato classes do not express as severe of ZC symptoms as others. For instance, fresh red potatoes appear to be more "tolerant" to ZC than russets. Because of the close relationship of tuber biochemical changes and ZC symptoms (Wallis et al. 2012), this study aimed to test changes in tuber levels of amino acids, reducing sugars, and phenolics in three different commercial cultivars of potato: Red La Soda, Russet Norkotah, and FL1867 (a chipping variety). The following hypotheses were tested: 1) ZC-symptomatic and Lso-positive tubers will possess greater levels of phenolics, amino acids, and reducing sugars regardless of cultivar; 2) more susceptible cultivars will possess greater levels of these compounds; and 3) these compounds will be positively correlated with ZC symptoms and Lso bacterial titers, suggesting potential host defense responses occurred.

Materials and Method

Seed potatoes of red La Soda, russet Norkotah, and FL1867 cultivars were planted at the Texas A&M AgriLife Research Experiment Station in Bushland, TX, in April 2012. Field cages (1x1x1-m) were established prior to plant emergence. When potato plants emerged they were thinned such that each contained four plants. There were 20 field cages per cultivar. Plant inoculations were conducted by releasing 30 Lso-positive psyllids at the base of a single plant in each of the cages. There were 4 infestation dates conducted 4, 3, 2, and 1 week(s) before harvest (July 26). In each infestation date, 4 cages per cultivar were infested with psyllids. One cage in each infestation date was maintained psyllid free to serve as uninfected control. Psyllids were allowed to feed for one week before they were removed by insecticides. At harvest, all tubers from different infestation dates were collected. Tubers were sliced at the stolon end and rated for symptom severity following a '0' (no symptoms) to '3' scale (severe). The slices were then sampled and assessed for Lso titer by qPCR by the methods of Rashed et al. (2013).

A second slice was made below the first, placed on dry ice, and shipped overnight to the USDA-ARS in Parlier, CA, for biochemical analyses. At the USDA-ARS, the slices were mashed and two aliquots of 0.10 g of material were placed into two separate 1.5 mL tubes. One aliquot was twice-extracted in 0.5 mL of methanol overnight at 4° C, for a combined total of 1 mL of methanol extract. The other aliquot was twice-extracted overnight in 0.5 mL of PBS saline buffer (pH 6.8) at 4° C, for a total of 1 mL of buffer extract.

A Shimadzu (Columbia, MD) high-performance liquid chromatograph (HPLC) system equipped with a photodiode array detector and C18 reverse-phase column separated and quantified different phenolic compounds as peaks using the methanol extracts (Wallis et al. 2012). A commercially available kit from Phenomenex (Torrance, CA) was used to assess amino acid content via gas chromatography (GC) using the buffer extracts and following manufacturer protocols (Wallis et al. 2012). The buffer extracts also were injected into a HPLC equipped with a Supelco C-611 ion-exchange column (Bellefonte, PA) and a refractive index detector to separate and quantify sugars (Rashed et al. 2012). Univariate ANOVAs with infection time (with controls as time "0") and cultivar as independent variables were used, with LSD for means separations. Associations between chemistry, ZC severity, and Lso titers were made using Spearman's correlations.

Results and Discussion

Zebra chip symptom severities were greater in red potatoes than russets and FL 1867 when infected for three or four weeks (Fig. 1). Furthermore, ZC symptoms were significantly ($P < 0.05$) positively associated with Lso titers.

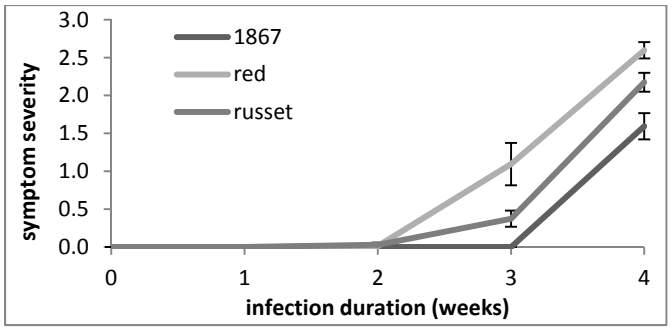


Figure 1. Mean (± SE) symptom severity rating for cultivars FL1867, La Soda (red potato), and Norkotah (russet potato).

Significant associations between ZC symptoms and Lso titer differed from findings by Wallis et al. (2012) and Rashed et al. (2013), who observed no correlation between symptoms and titers. However, Wallis et al. (2012) used field-collected potatoes with no information about when the potatoes were inoculated, and Rashed et al. (2013) extended infection to nine weeks. It appeared from Rashed et al. (2013) that symptom development and titer increases plateaued around five weeks, with no increases from five to nine weeks. This would result in no significant linear correlations being observed as a time period was included, in contrast to this study, beyond the exponential Lso growth phase and corresponding increase in observable symptoms.

Regarding tuber chemistry, several amino acids (asparagine, aspartic acid, glutamine, glutamic acid, and methionine) were at the greatest levels in tubers infected for one week before harvest, whereas the other amino acids were at greatest levels in tubers inoculated four weeks prior to harvest (Table 1). Asparagine, aspartic acid, glutamine, glutamic acid, and methionine were negative correlated with both ZC symptoms and Lso titers, whereas the other amino acids were positively associated with ZC symptoms and titers (Table 1). Because asparagine, aspartic acid, glutamine, glutamic acid, and methionine are among the amino acids with the greatest concentrations in potato tubers, this suggests changes in amino acids likely do not result in ZC fried symptom development as much as reducing sugars. Previous studies reported these amino acids were not different between Lso-infected and non-infected tubers (Rashed et al. 2013; Wallis et al. 2012), suggesting complicated responses in tubers levels of asparagine, aspartic acid, glutamine, glutamic acid, and methionine to Lso infection.

Tuber concentrations of sucrose, fructose, and glucose increased in all three potato cultivars over the four weeks of Lso-infection. Increased sucrose levels in Lso-infected tubers were not observed in prior studies, but increasing reducing sugars levels (fructose and glucose) over the duration of infection was observed by Rashed et al. (2013). All of these sugars were significantly positively associated ($P < 0.05$) with ZC symptoms and Lso titers.

		infection duration (weeks)								
		0	1	2	3	4	F (potato class or cultivar)	F (week)	symptom correlation	titer correlation
asparagine	red	25.79	37.04	34.33	31.71	12.37	*8.019*	*18.772*	*-0.434*	*-0.441*
	russet	17.58	32.54	27.66	24.09	14.99				
aspartic acid	red	6.14	7.11	6.97	4.03	1.50	*99.266*	*23.617*	*-0.619*	*-0.437*
	russet	8.23	14.31	13.84	9.48	6.14				
glutamic acid	red	0.96	1.15	1.14	0.61	0.40	*5.847*	*41.254*	*-0.709*	*-0.645*
	russet	0.59	1.20	1.15	0.35	0.36				
glutamine	red	7.72	17.70	19.72	12.68	7.32	0.054	*19.044*	-0.155	*-0.466*
	russet	3.40	20.28	18.70	8.73	15.43				
methionine	red	1.06	1.12	0.79	0.65	0.48	1.617	*11.998*	*-0.520*	*-0.443*
	russet	0.70	1.00	0.75	0.75	0.59				
histidine	red	0.59	0.76	0.76	0.94	0.82	0.005	*8.921*	*0.400*	0.155
	russet	0.52	0.68	0.71	0.84	1.10				
isoleucine	red	0.73	0.85	0.91	1.12	2.04	0.000	*38.173*	*0.654*	*0.415*
	russet	1.00	0.74	0.62	0.83	2.45				
leucine	red	0.27	0.34	0.36	1.43	2.92	2.773	*73.208*	*0.768*	*0.572*
	russet	0.93	0.41	0.39	0.82	3.92				
lysine	red	0.53	1.01	1.33	2.07	1.83	0.043	*15.748*	*0.510*	*0.291*
	russet	0.59	0.96	1.13	1.58	2.67				
phenylalanine	red	0.65	0.74	0.79	1.48	1.06	*3.957*	*10.966*	*0.557*	*0.343*
	russet	0.63	0.69	0.67	0.92	1.12				
proline	red	0.45	0.55	0.71	0.96	2.02	*8.603*	*27.535*	*0.656*	*0.416*
	russet	1.38	0.72	0.87	0.80	2.70				
serine	red	10.37	8.94	8.56	14.61	12.44	1.355	*2.523*	*0.434*	*0.188*
	russet	10.42	11.94	9.54	8.43	11.00				
tryptophan	red	0.36	0.51	0.51	0.82	0.76	0.612	*11.628*	*0.444*	*0.250*
	russet	0.35	0.49	0.49	0.62	0.83				
valine	red	3.60	3.70	3.71	4.59	5.29	3.236	*6.255*	*0.415*	*0.231*
	russet	5.35	4.27	3.25	4.43	6.69				

Table 1. Mean amino acid levels at each week, and ANOVA F-values and Spearman ρ values. Significant ($P < 0.05$) statistics are italicized. Amino acid analyses for FL1867 are currently underway.

Phenolic levels also increased in all three cultivars during the course of Lso infection (Fig. 3). Phenolic levels from red potatoes at three weeks were greater than russet and FL1867, but levels from red tubers were less than russets and FL1867 at four weeks (Fig. 3). Phenolic levels were significantly ($P < 0.05$) correlated with both ZC symptoms and Lso titers. Previous lack of associations between Lso titers and phenolic levels (Rashed et al. 2013; Wallis and Chen 2012) suggested complicated roles between phenolics and Lso, but these results observed that the presence of Lso triggered phenolic production. At and beyond four weeks of infection, phenolic compounds may have reached levels detrimental to Lso growth and survival, resulting in no significant correlations observed by Rashed et al. (2013). Further

studies are warranted to confirm that this is the case, although such studies may be dependent on successful *in vitro* culturing of Lso.

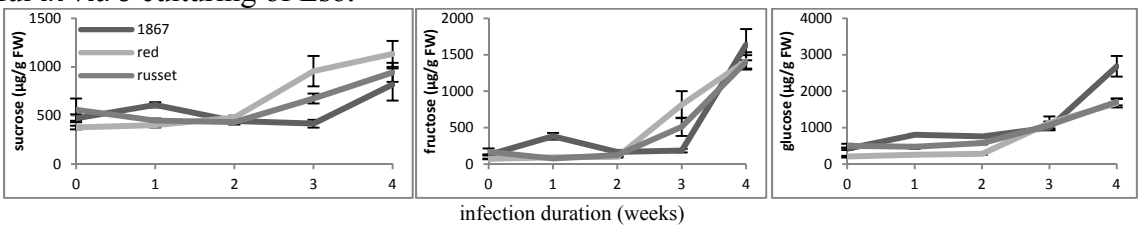


Figure 2. Mean (± SE) levels of sucrose, fructose, and glucose in FL1867, La Soda (red potato), and Norkotah (russet potato) tubers over four weeks of infection.

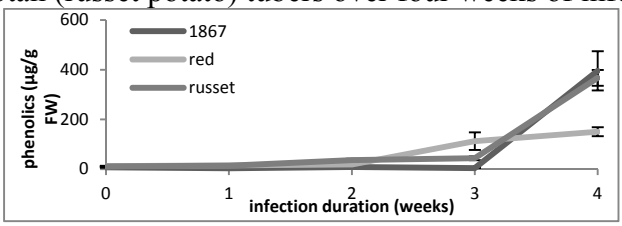


Figure 3. Mean (± SE) levels of phenolics (summed amounts of individual compounds) for FL1867, La Soda (red potato), and Norkotah (russet potato) tubers over the course of this experiment.

In conclusion, these results confirm prior conclusions that Lso infections trigger physiological changes within potato tubers that result in ZC symptom progression. However, unlike previous studies, this work observed positive correlations between Lso titers with particular amino acids, sugars, and phenolics. These findings revealed only minor differences between cultivars in terms of physiological changes and ZC symptoms in Lso infected tubers. However, additional cultivars, especially those apparently tolerant of Lso infection, warrant examination.

Acknowledgements

The authors wish to thank Li Paetzold, Griselda Aguilar, Max Henning, Nancy Goodell, Austin Fite, and Greg Phillips for their assistance in this project, and the SCRI zebra chip mini-grant program for providing the resources necessary to complete this work.

References

Friedman, M., Levin, C.E. 2008. Review of methods for the reduction of dietary content and toxicity of acrylamide. J. Ag. Food Chem. 56:6113-6140.

Liefting, L., Sutherland, P.W., Ward, L.I., Paice, K.L., Weir, B.S., Clover, G.R.G. 2009. A new '*Candidatus* Liberibacter' species associated with diseases of Solanaceous crops. Plant Dis. 93:208-214.

Mayer, A.M. 2006. Polyphenol oxidases in plants and fungi: going places? A review. Phytochemistry 67:2318-2331.

Navarre, D.A., Shakya, R., Holden, J., Crosslin, J.M. 2009. LC-MS analysis of phenolic compounds in tubers showing zebra chip symptoms. Am. J. Potato Res. 86:88-95.

Rashed, A., Wallis, Paetzold, L., Workneh, F., Rush, C.M. 2013. Zebra Chip disease and potato biochemistry: Tuber physiological changes in response to '*Candidatus* Liberibacter solanacearum' infection over time. Phytopathology, *in press.*

Wallis, C.M., Chen, J., Civerolo, E.L. 2012. Zebra chip-diseased potato tubers are characterized by increased levels of host phenolics, amino acids, and defense-related proteins. Physiol. Mol. Plant Pathol. 78:66-72.

Proteomic and Physiological Characterizations of Potato Plants in Response to Zebra Chip Disease

Nwugo, C.C. and Lin, H. Crop Diseases, Pests and Genetics Research Unit, USDA-ARS, Parlier, CA 93648

Abstract

'*Candidatus* Liberibacter solanacearum' (Lso) is a fastidious gram-negative bacterium transmitted by potato psyllids and associated with potato zebra chip (ZC) disease. Here, proteomic analysis via 2-DE and mass spectrometry was employed to compare total protein expression profiles between healthy and Lso-infected above-ground (AG) and below-ground (BG) potato tissues. Inductively Coupled Plasma (ICP) Spectroscopy was also used to elucidate the effect of Lso infection on the nutrient status of same plants. Results showed that 59 and 55 protein spots were differentially-produced in potato above-ground (AG) and below-ground (BG) tissues, respectively, in response to Lso infection. Over 60 or 80% of the differentially produced protein spots from AG or BG tissues were up-regulated in response to Lso infection and this was accompanied by an Lso-mediated increase in the concentrations of K, Mn, Fe and Cu in said tissues. While an up-regulation of several pathogen-response related and metabolism-associated proteins was observed in AG and BG tissues, there was a general down-regulation of photosynthesis-related proteins in AG tissues in addition to down-regulation of protease inhibitors in BG tissues in response to Lso infection. This study identified several defense response-related genes in host in responses to Lso infection and sheds new light on the molecular and physiological processes involved in ZC disease in potato plants.

Introduction

Zebra chip is associated with a gram-negative, phloem-limited, insect-vectored, unculturable prokaryote: '*Candidatus* Liberibacter solanacearum' (Lso) that belongs to the *Rhizobiaceae* family of α-Proteobacteria (Liefting et al. 2008). Since the first discovery in 2008 suggesting that Lso is the causal agent of ZC (Liefting et al. 2008), subsequent studies have led to a consensus agreement that Lso is etiologically associated with ZC of potatoes (Secor et al. 2009). Due to its fastidious nature, the Lso bacterium is, as of yet, unculturable and Koch's postulates have not been fulfilled. Consequently, conclusive detailed information regarding the general biology, physiology and pathogenicity of Lso is lacking. In spite of these limitations, whole genome sequence of Lso has been obtained (Lin et al. 2011) and annotation of genome information has provided insights into gene inventory account for biological functions, mode of pathogenicity, metabolic pathways and nutritional requirements for this obligated intracellular pathogenic bacterium. Effective and sustainable disease management typically relies on understanding host resistance. Unfortunately, all commercial potato cultivars are susceptible. It was thus envisaged that elucidation of molecular and physiological processes associated with host defense response to Lso infection will provided directional insights into potential improvement of host against ZC disease.

Proteomic approaches are powerful tools to study gene expression at a functional level and have been successfully used in the elucidation of molecular mechanisms involved in host defensive-response to pathogen infection (Nwugo et al. in press). The aim of this study was to employ proteomics analysis via 2-DE and mass spectrometry as well as nutritional analysis by ICP spectroscopy to elucidate the protein expression profiles and physiological responses in above-ground (AG) and below-ground (BG) potato tissues upon Lso infection.

Materials and Methods

Growth conditions and treatments

Disease free potato plants were grown in greenhouse and maintained at 24-28 ºC, 50±5% RH, and 16:8 h (Light:Dark) photoperiod. PCR confirmed Lso-positive potato psyllids were reared on potato plants in a controlled environmental for several generations at 29 ºC, 50% RH, and 16:8 (L:D) h photoperiod. Three to four weeks after planting, potato plants were inoculated with Lso by a 72 h exposure to Lso-positive adult potato psyllids at 10 psyllids per plant. Insects were eliminated by fumigation and plants were maintained as earlier described for an additional 18 days. Potato tissues (AG and BG) were separated, immediately submerged in Liquid nitrogen and stored in -80 ºC for later use. Lso-infected plants showed typical ZC symptoms and were reconfirmed by PCR.

Total protein extraction

The method used for total protein expression profile analysis was slightly modified after Nwugo and Huerta (Nwugo and Huerta 2011). Shoots and tubers from each plant were ground to a fine powder in liquid nitrogen and approximately 0.4 g of tissue was suspended in 4.5 mL of chilled solution A [5]. The mixture was incubated overnight at -80 ˚C followed by centrifugation at 4 ˚C for 20 min at 36,000 *g* (Optima L-70K Ultracentrifuge, Beckman Coulter Inc., USA). The supernatant was decanted, and the pellet was washed at least three times in solution B (Nwugo and Huerta 2011) until the supernatant was clear. The whitish pellet or crude protein extract was vacuum-dried (Vacufuge ™, Eppendorf, Germany) and protein solubilization was achieved by suspension of dried pellet in 0.5 mL of rehydration/isoelectric focusing (IEF) buffer (Nwugo and Huerta 2011) and incubating at RT 30 min. Insoluble material was removed by centrifugation and total protein quantification was performed using bicinchoninic acid (BCA) assay (Pierce, Rockford, IL, USA). Three independent extractions were performed per sample and there were three sample replicates per control or infected group.

2-DE and gel image analysis

For the first dimension electrophoresis or IEF, 11-cm long pH 4-7 ReadyStrip IPG strips (Bio-Rad, Hercules, CA, USA) were passively rehydrated overnight at RT with 200 ng of total solubilized proteins. IEF was performed in a PROTEAN IEF cell (Bio-Rad) at a current limit of 50 μA/per IpG strip at 10 ˚C as previously described (Nwugo and Huerta 2011). The second dimension electrophoresis was performed in 8-16% gradient SDS-polyacrylamide Tris-HCl gels (Criterion precast gels, Bio-Rad) in a twelve-gel cell system (Criterion Dodeca Cell, Bio-Rad). Protein spots were visualized by staining with Biosafe Coomassie and gel images were captured under identical conditions (ScanMaker 9800XL, Microtek, USA).

Gel spot detection, alignment and comparative analysis based on average intensities between healthy and Lso-infected samples were performed using the PDQuest software (version 8.0, Bio-Rad, USA). Only spots that had ≥10-fold increase over background and present in at least six of the nine gels per treatment as well as showed □1.5 fold change ($P < 0.05$) were further analyzed.

Mass spectrometry analysis and protein identification

Differentially produced protein spots were excised, trypsin digested and analyzed using MALDI-TOF-MS (QSTAR XL Hybrid Quadrupole TOF LC/MS/MS System, Applied Biosystems, USA) as previously described (Nwugo et al. in press). Prior to database queries, the Peak Erazor software (v 2.01: Lighthouse data, Odense, Denmark) was used to process peptide mass fingerprints (PMFs) generated from MALDI-TOF-MS analysis (Nwugo and Huerta 2011). MASCOT search engine (Matrix Science,

London, UK) was used to find matches of the PMF and MS/MS spectra against a custom database containing entries for potato available in the NCBI non-redundant database.

Nutrient status analysis

Macro- and micro-nutrient status of healthy and Lso-infected tissues was obtained by assaying the concentrations of Ca, K, Mg, Fe, Cu, Mn, and Zn via Inductively-Coupled Plasma Optical Emission Spectroscopy (ICP-OES) as previously described (Banuelos et al. 2007). Briefly, 0.5g of oven-dried tissues was ashed at 510 ˚C for 9hrs and digested in 1N HNO_3 for 1 h. The supernatant was filtered and the intensities of atomic emissions at 396.847nm for Ca, 766.491nm for K, 279.553nm for Mg, 238.204nm for Fe, 327.395nm for Cu, 257.610nm for Mn, and 213.857nm for Zn was measured on an ICP-OES System (Varian Vista Pro CCD Simultaneous ICP-OES, Agilent, USA).

Results and Discussion

Plants possess a variety of defense reactions at local and systemic levels in response to biotic stress. These defense responsive systems can be induced following infection by microorganisms. Recent studies have led to a dramatic increase in understanding plant-pathogen interactions involving defense signal transduction. Proteomic analysis via 2-DE revealed a high resolution of separation of total soluble proteins (Fig. 1) and identified 59 and 55 protein spots that were differentially produced in AG and BG tissues, respectively, in response to Lso infection (Table 1). Interestingly, despite a significant down-regulation in photosynthesis-related proteins, suggesting a reduction in photosynthetic activity, there was a general up-regulation of 66 and 89% of all differentially produced proteins in AG and BG tissues, respectively (Table 1), including pathogen response-related proteins (Table 1). This general up-regulation of protein production was accompanied by an increase in nutrient uptake elevating in the concentrations of K, Mn, Fe and Cu in AG and BG tissues in response to Lso infection (Fig. 2).

Table 1. Number of protein spots per functional group that were up- or down-regulated in AG or BG tissues in response to Lso infection.

Functional group	Above-ground		Below-ground	
	Up	Down	Up	Down
Photosynthesis	0	5	0	0
Redox homeostasis	0	3	10	0
Chaperones	6	0	3	0
Regulation/Protein synthesis	7	3	5	1
Pathogen response	9	1	5	2
Energy/Metabolisms	13	6	22	0
Unknown	4	2	4	3

Members of the pathogenesis related (PR) protein family differentially produced in response to Lso infection included class II chitinase, thaumatin-like protein, proteinase-inhibitor, peroxidase and ribonuclease III protein (Tables 2 and 3). The production of an array of PR proteins has been shown to increase as the result of infection, usually surrounding the site of infection but also to a lesser extent in distal tissues (Hammond-Kosack et al. 1996). The exact roles of PR proteins in ZC potato are not yet clear. In addition to PR proteins, Leucine-rich repeat-containing protein, which belongs to the R family group of plant proteins, was found to be up-regulated in AG tissues. Enhancement of *R* gene expression has been shown to be correlated with the improvement against pathogen attacks (Salmeron et al. 1996). In addition to these up-regulated proteins, there was a down-regulation of a stress-related protein, superoxide dismutase in AG tissues as well as down-regulation of protease inhibitors in BG tissues in response to Lso infection. Stress-related proteins, which include superoxide dismutase and protease inhibitors form part of the innate defense response machinery in plants against stress and pathogens, hence their down-regulation might suggest potential molecular mechanisms responsible for potato susceptibility to Lso infection. In summary, this preliminary study has analyzed nutritional status and

identified differentially expressed proteins and their putative functions of potato plants in response to Lso infection.

Table 2. Abbreviated list of differentially produced proteins in AG tissues in response to Lso infection.

Spot[a]	Δ[b]	Protein function/name[c]
		CO_2 assimilation/Photosynthesis
4	-11.19	RuBisCO large subunit
59	-3.15	Photosystem II stability factor
61	-1.90	RuBisCO activase, chloroplastic
		Redox homeostasis
70	Off	Superoxide dismutase [Fe]
85	8.11	Dehydroascorbate reductase-like protein
		Chaperones
13	4.84	Heat shock protein 70
51	4.53	Chaperonin-60alpha
		Regulation/Protein synthesis
12	3.27	GH3 auxin-responsive promoter
20	-2.30	Single-stranded DNA binding protein
83	6.88	Leucine-rich repeat-containing protein
		Pathogen response
22	9.12	Class II chitinase
26	1.65	Putative thaumatin-like protein
29	4.00	Ribonuclease III
		Energy/Metabolisms
11	2.64	Formate dehydrogenase
48	2.48	Patatin group A-2
79	2.09	Enolase

Table 3. Abbreviated list of differentially produced proteins in BG tissues in response to Lso infection.

Spot[a]	Δ[b]	Protein function/name[c]
		Redox homeostasis
4	On	Ascorbate peroxidase
31	On	Catalase
47	1.94	Thioredoxin
82	3.08	Peroxidase
		Chaperones
36	2.23	Heat shock protein 70
58	1.94	Chaperonin hsp60
		Regulation/Protein synthesis
44	On	20S proteasome alpha 6 subunit
52	-2.67	DNA replication licensing factor
		Pathogen response
26	On	Anti-bacterial protein
21	-6.36	Kunitz-type protease inhibitor
90	-10.72	Cysteine protease inhibitor 9
		Energy/Metabolisms
10	On	GTP-binding protein
11	On	NAD-dependent formate dehydrogenase
13	On	Patatin-3-Kuras 1
54	3.10	Actin1
55	3.96	Mitochondrial ATPase beta subunit
86	On	Pyruvate decarboxylase

[a] The spot numbers correspond to those given in Figure 1. [b] Protein fold change in Lso-infected tissues/uninfected tissues in both AB and BG. [c] Protein function/name was determined by http:/www.ncbi.nlm.nih.gov/BLAST/.

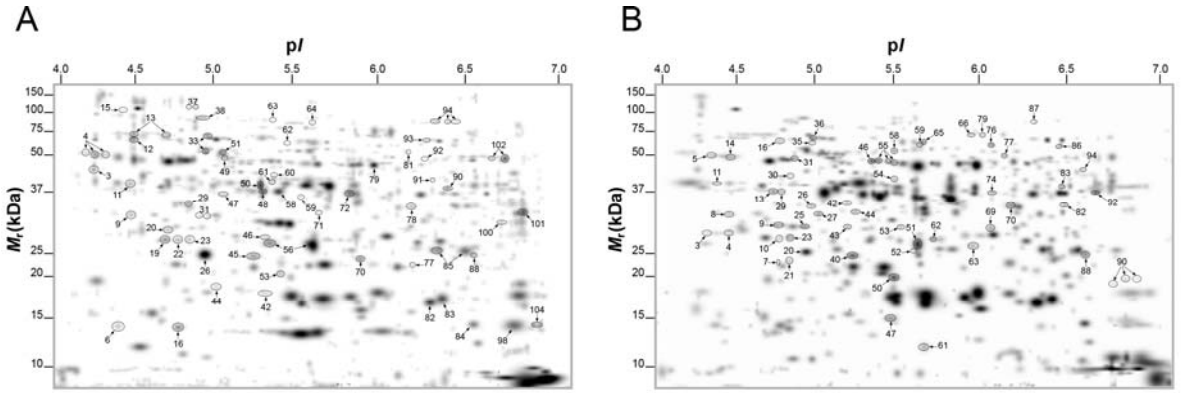


Figure 1. 2-DE gel maps of total proteins from AG (**A**) and BG (**B**) potato tissues. Labeled spots are differentially produced in response to Lso.

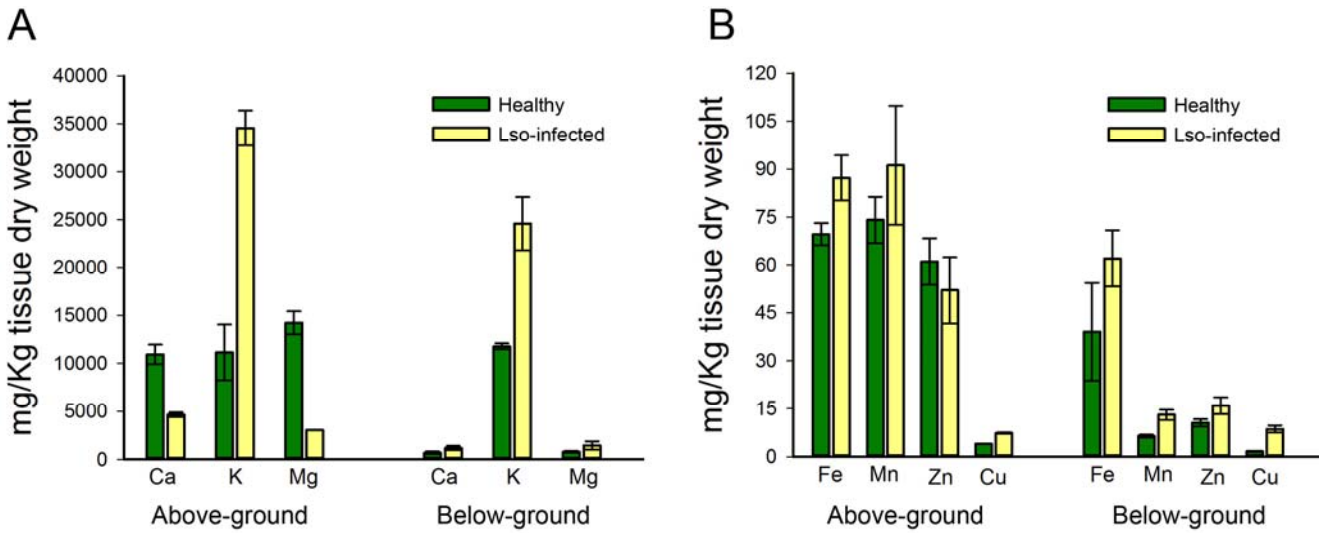


Figure 2. The concentrations of macronutrients (**A**) and micronutrients (**B**) in AG and BG potato tissues in response to Lso infection.

Acknowledgements

Funding for this research was provided by USDA-NIFA-SCRI-TAMU. We thank V. G. Sengoda and J. E. Munyaneza for providing Lso-infected potato plant materials.

References

Liefting, L.W., Perez-Egusquiza, Z.C., Clover, G.R.G., Anderson, J.A.D. 2008. A new '*Candidatus* Liberibacter' species in *Solanum tuberosum* in New Zealand. Plant Dis. 92:1474-1474.

Secor, G.A., Rivera, V.V., Abad, J.A., Lee, I.M., Clover, G.R.G., Liefting, L.W., Li, X., De Boer, S.H. 2009. Association of '*Candidatus* Liberibacter solanacearum' with Zebra Chip disease of potato established by graft and psyllid transmission, electron microscopy, and PCR. Plant Dis. 93:574-583.

Lin, H., Lou, B., Glynn, J.M., Doddapaneni, H., Civerolo, E.L., Chen, C., Duan, Y., Zhou, L., Vahling, C.M. 2011. The complete genome sequence of '*Candidatus* Liberibacter solanacearum', the bacterium associated with potato Zebra Chip disease. Plos One. e19135.doi:10.1371

Nwugo, C.C., Lin, H., Duan, Y.P., Civerolo, E.L. 2013. The effect of '*Candidatus* Liberibacter asiaticus' infection on the proteomic profiles and nutritional status of pre-symptomatic and symptomatic grapefruit (*Citrus paradisi*) leaves. BMC Plant Biology (in press).

Nwugo, C.C., Huerta, A.J. 2011. The effect of silicon on the leaf proteome of rice (*Oryza sativa L.*) plants under cadmium-stress. J. Proteome Res. 10:518-528.

Banuelos, G.S., Pasakdee, S., Benes, S.E., Ledbetter, C.A. 2007. Long-term application of biosolids on apricot production. Comm Soil Sci Plant Anal. 38:1533-1549.

Hammond-Kosack, K.E., Jones, J.D.G. 1996. Resistance gene-dependent plant defense response. Plant Cell. 8:1773-1791.

Salmeron, J.M., Oldroyd, G.E., Rommens, C.M., Scofield, S.R., Kim, H.S., Lavelle, D.T., Dahlbeck, D., Staskawicz, B.J., 1996. Tomato *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the pto kinase gene cluster. Cell. 86:123-133.

Genome Sequencing of '*Candidatus* Liberibacter solanacearum' Haplotype A and Comparison to the Haplotype B Genome CLso-ZC1

Johnson,[1]C. P., Y.P. Duan[2], A. Wen[1], and N.C. Gudmestad[1]

[1]Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA

[2]USDA ARS, Ft. Pierce, FL 34945, USA

Abstract

Ion Torrent sequencing was performed on a metagenomic DNA sample composed of two potato psyllids (*Bactericera cockerelli*) vectoring '*Candidatus* Liberibacter solanacearum' (Lso). The psyllids originated from a colony started with nymphs collected in the Lower Rio Grande River Valley, TX, in February 2012. The colony was maintained on eggplant and potato in Bugdorm 2120F enclosures in the greenhouse. Lso 16S ribosomal DNA was PCR amplified and sequenced to confirm the Lso was haplotype A. Ion Torrent sequencing generated 3.48 million reads with an average read length of 178nt. DNAStar SeqMan NGen software was used to identify Liberibacter sequences from the pool of 3.48 million reads and aligned them to the haplotype B Lso genome CLso-ZC1. The initial run pulled 108,934 sequences for an average coverage of 15.63X. Sequence homology between the two haplotypes in areas with >10X coverage was 98-99%. Gaps in the coverage appear to be regions of divergence between the two haplotypes. Two notable gaps in coverage occur at regions previously identified as phage-like regions in the CLso-ZC1 genome, supporting their proposed phage nature. The StandAloneBlast tool from NCBI is being used to correct errors in the assembly due to dissimilarities between the two haplotypes and to identify haplotype A sequences with low or little homology to haplotype B.

Introduction

Over the last 20 years, Liberibacter bacteria have been associated with some of the most damaging diseases in global agriculture. Three Liberibacter species affecting citrus, '*Candidatus* Liberibacter africanus', '*Ca.* L americanus', and particularly '*Ca.* L asiaticus' have devastated citrus production across the globe (Duan et al 2009). A Liberibacter species was discovered in Solanaceous crops in 2008 (Liefting et al 2009) and '*Ca.* L. solanacearum' (Lso) was identified as the putative cause of zebra chip disease in potato (Liefting et al 2009, Secor et al 2009). '*Ca.* L. psyllaurous' (Lps) was also identified in another solanaceous crop, tomato (Hansen et al 2008). Studies on the genetic diversity of Lso affecting potato revealed two distinct but closely related organisms most likely of the same species (Wen et al 2009, Glynn et al 2011, and Lin et al 2012). We have adopted the use of haplotype A and haplotype B (Nelson et al 2011) to describe Lso In potato. Two closely related Liberibacter species have been found in Europe infecting carrot and are designated haplotype C and D (Nelson et al 2011, Nelson et al 2012). Both haplotypes affecting potato are vectored by the potato/tomato psyllid (*Bactericera cockerelli*) and appear to have the same host range (Hansen et al 2008).

Symptoms associated with zebra chip in potato are highly variable and include foliar scorching, chlorosis, twisted stems, swollen nodes, aerial tubers, and the tuber vascular discoloration that resulted in the zebra chip name (Secor et al 2009). Along with variable plant symptoms, seasonal outbreaks of zebra chip are highly variable in severity and distribution. The geographic distribution of the two haplotypes overlaps in the Central US and Mexico, while only haplotype A is found in the Western US and New Zealand (Wen et al 2013 in press). Anecdotal evidence observed while testing field collected

materials, and from greenhouse maintenance of psyllids infected with mixtures of haplotypes, indicates that there are variations in zebra chip disease symptomatology related to Lso haplotype. The genome of haplotype B Lso has been made publicly available (Lin et al 2011) and has been a powerful tool for the study of zebra chip. However, having the genomes of both haplotypes A and B available will allow us to identify genetic elements responsible for observed variations in zebra chip symptomatology and epidemiology. Understanding how Lso genetic variability affects the interactions between bacteria, plant host, and insect vector will help us to understand how zebra chip and other Liberibacter diseases develop in plants. The objective of this study was to sequence the genome of haplotype A '*Candidatus* Liberibacter solanacearum'.

Materials and Methods

Potato foliage with potato psyllid nymphs was collected from fields in the Lower Rio Grande Valley in February 2012. Small (<.5cm) squares of plant tissue, each with an attached psyllid, were cut from the potato leaves. The cut leaf squares were placed onto healthy potato plants in Bugdorm 2120F enclosures in our greenhouse at 72-75^{0}F and 16/8 hours light/dark. Adult insects from the colonies were sampled periodically and qPCR tested for Lso with primer pair LsoF-HLBr (Li et al 2009). Lso+ insects were haplotype tested with cPCR primer pairs CJ5 and 1r/47r. Primer pair CJ5 only amplifies from haplotype A while primer pair 1r/47r only amplifies from haplotype B. One colony that contained only haplotype A Lso was kept and the other colonies were discontinued. The haplotype A colony was transferred to eggplant because eggplant appeared to survive longer than potato with Lso + psyllids.

DNA was extracted from sampled insects to determine the percentage of Lso + psyllids and the titer of Lso in those psyllids. Although most psyllids were found to contain Lso (>80%), Lso titers in sampled insects were found to be highly variable. Quantitative SYBR Green PCR with primer pair LsoF-HLBr generated ct scores ranging from 15-35, representing a million fold change in Lso titers between individual insects from the same colony. A total of 172 insects were sampled, and the two samples with the highest titers of Lso were selected for Ion Torrent sequencing. DNA library construction and Ion Torrent run were performed by Washington State University Center for Reproductive Biology: Molecular Biology Core Facility. The sequencing run was performed on a 318 chip for Ion Torrent.

The 3.48 million sequence reads generated by the Ion Torrent run were analyzed using DNAStar (Madison, WI) software. A template based assembly was performed with SeqMan NGen software suit. The haplotype B genome CLso-ZC1 accession NC_014774.1 was utilized as the assembly template. Gaps or areas with low coverage were analyzed with the StandAloneBlast tool from NCBI.

Results and Discussion

The Ion Torrent sequencing run generated 3,483,127 reads with an average read length of 178nt. SeqMan NGen was able to align 108,934 of those sequences with the CLso-ZC1 genome. Coverage of the CLso-ZC1 genome was 15.63X (figure1). The coverage map includes gaps and is therefore approximately 10% longer than the published Clso-ZC1 genome.

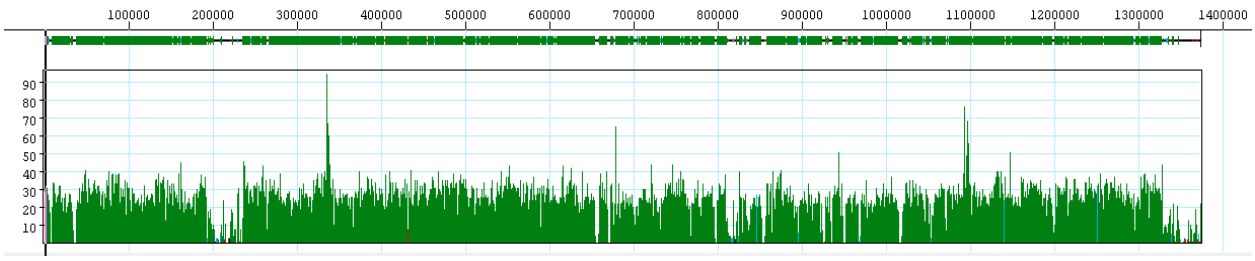


Figure 1. Coverage of CLso-ZC1 with haplotype A sequences.

The overall sequence homology between the sequenced haplotype A genome and the published haplotype B genome CLso-ZC1 was 98-99%. Two regions with phage homology appear with very low coverage (Figure 1). The regions at ~200,000 and ~1,350,000 correspond with regions previously identified as having high homology with characterized phage region found in the related '*Cadidatus* Liberibacter asiaticus' (Zhang et al 2012). The CLso-ZC1 genome also has several regions containing portions of the phage-like region that may be remnants of past phage introgressions. These other smaller areas of phage-like DNA also have very low coverage and can be seen at 35,000, 660,000, 815,000, 1,009,000, and others. We expected these regions to display high levels of variability if the phage was or is physiologically active, which is what we observed.

The genome assembled demonstrates that there is good coverage (~25X) of the approximately 130nt displayed (Figure 2). There are three haplotype A snps marked in red, one C – T at 582, one A – G at 660, and one G – A at 706. There are also twelve gaps, close to the 10% average in the genome. Mismatches that do not appear in multiple sequence files are generally read errors from individual sequencing reads and are deleted.

Figure 2 SeqMan NGen assembly. Top sequence is CLso-ZC1 template.

Sequences from CLso-ZC1 with little or no coverage were loaded into StandAloneBlast and blastn was used to search the Ion Torrent haplotype A sequence pool for homologous sequences. Selected sequence reads then were used to fill gaps or make corrections in the assembly. Many errors in the assembly were the result of small insertion/deletion mutations, multi-copy regions, or repeating sequences. A couple of large scale splicing rearrangements have also been detected but not fully characterized.

StandAloneBlast has been successfully used to pull sequences from the haplotype A pool and assemble fragments with homology to the phage-like regions with no coverage in the SeqMan NGen assembly. Homology in these areas is generally reduced from the 98-99% seen in the overall genome to ~85-90%. Long distance PCR will be used to confirm the structure and orientation of regions with low homology to CLso-ZC1.

Several sets of new PCR primers were also developed using the haplotype A sequences. Quantitative PCR primers were developed specifically to amplify regions contained in only the haplotype A or B genomes. Preliminary testing with these primers indicates that these new primers will offer many improvements over our previous primers. We will now be able to verify the haplotype of low titer infections. We will also be able to quantify relative haplotype ratios in co-infected samples. We hope that this feature will provide a powerful tool for quantifying differential haplotype titer responses to changes in host plant physiology or environmental conditions. We are planning a series of experiments where we will infect plants with both haplotypes and measure the Lso titer in psyllids and plant material in response to heat and drought stresses.

Acknowledgements
We would like to thank D. Henne and his staff for collecting and sending samples that were used to start the colonies for this project.

References
Duan YP, Zhou L, Hall DG, Li W, Doddapaneni H, Lin H, Liu L, Vahling CM, Gabriel DW, Williams KP, Dickerman A, Sun Y, Gottwald T. 2009 Complete genome sequence of citrus Huanglongbing bacterium, '*Candidatus* Liberibacter asiaticus' obtained through metagenomics. Mol. Plant-Microbe Interact. 22:1011-1020.

Glynn JM, Islam MS, Bai Y, Lan S, Wen A, Gudmestad NC, Civerolo EL, Lin H. 2012 Multilocus sequence typing of '*Candidatus* Liberibacter solanacearum' strains from North America and New Zealand. J. Plant Pathol. 94:223-228.

Hansen AK, Trumble JT, Stouthamer R, Paine TD. 2008 A new huanglongbing species '*Candidatus* Liberibacter psyllaurous,' found to infect tomato and potato, is transmitted by the psyllid Bactericera cockerelli (Sulc). Appl Environ Microb 74:5862-5865.

Li W, Abad JA, French-Monar RD, Rascoe J, Wen A, Gudmestad NC, Secor G, Lee I-M, Duan YP, Levy L. 2009 Multiplex real-time PCR for detection, identification and quantification of 'Candidatus Liberibacter solanacearum' in potato plants with zebra chip. J. Micro Methods 78:59-65.

Liefting LW, Weir BS, Pennycook SR, Clover GRG. 2009 '*Candidatus* Liberibacter solanacearum' associated with plants in the family Solanaceae. Int. J. Systematic and Evol. Micro. 59:2274-2276.

Lin H, Lou B, Glynn JM, Doddapeneni H, Civerolo EL, Chen C, Duan Y, Zhou L, Vahling CM. 2011 The complete genome sequence of '*Candidatus* Liberibacter solancearum', the bacterium associated with potato zebra chip disease. PLoS One. 6:e19135.

Lin H, Islam MS, Bai Y, Wen A, Lan S, Gudmestad NC, Civerolo EL. 2012 Genetic diversity of '*Candidatus* Liberibacter solanacearum' strains in the United States and Mexico revealed by simple sequence repeat markers. Eur J Plant Pathol. 132:297-308.

Nelson WR, Fisher TW, Munyaneza JE. 2011 Haplotypes of '*Candidatus* Liberibacter solanacearum' suggest long-standing separation. Eur J Plant Pathol. 130:5-12.

Nelson WR, Sengoda VG, Alfaro-Fernandez AO, Font MI, Crosslin JM, Munyaneza JE. 2012 A new haplotype of 'Candidatus Liberibacter solanacearum' identified in the Mediterranean region. Eur J Plant Pathol. DOI 10.1007/s10658-012-0121-3.

Secor GA, Rivera VV, Abad JA, Lee IM, Clover GRG, Liefting LW, Li X, De Boer SH. 2009 Association of '*Candidatus* Liberibacter solanacearum' with zebra chip Disease of potato established by graft and psyllid transmission, electron microscopy, and PCR. Plant Dis. 93:574-583.

Wen A, Mallik I, Alvarado VY, Pasche J, Wang X, Li W, Levy L, Lin H, Scholthof HB, Mirkov TE, Rush CM, Gudmestad NC. 2009 Incidence, distribution and genetic variability of '*Candidatus* Liberibacter' species associated with zebra chip complex disease of potato in North America. Plant Dis. 93:1102-1115.

Wen A, Johnson C, Gudmestad NC. 2013 Development of a PCR assay for the rapid detection and differentiation of '*Candidatus* Liberibacter solanacearum' haplotypes and their spatiotemporal distribution in the United States. Am J of Potato Res. In press.

Zhang SJ, Flores-Crus Z, Zhou L, Kang BH, Fleites LA, Gooch MD, Wulff NA, Davis MJ. 2011 *Ca*. Liberibacter asiaticus carries an excision plasmid prophage and a chromosomally integrated prophage that becomes lytic in plant infections. Mol. Plant-Microbe Interact. 24:458-468.

Comparison and Evaluation of '*Candidatus* Liberibacter solanacearum' Haplotyping Markers

Aimin Wen, Chris Johnson, and Neil C. Gudmestad, Department of Plant Pathology, North Dakota State University, Fargo, ND 58108

Abstract

Based on the published '*Candidatus* Liberibacter solanacearum' (Lso) genome sequence (haplotype B, LsoB) and draft haplotype A Lso (LsoA) genome sequence, forty sets of Lso typing primers were designed. Comparison and evaluation of the new primers were conducted on potato and psyllid samples infected by LsoA and LsoB. Two sets of LsoA specific primers, two sets of LsoB specific primers, and two sets of SSR primers were found to consistently type Lso infections in potato and psyllid samples. These primers were selected and subjected to more extensive testing. Haplotype specific primers can be used in conventional PCR format as well as SYBR green real-time PCR format. Real-Time PCR can quantify the number of genomes of each specific haplotype in Lso-infected potato and psyllid samples, and can calculate the ratios of each haplotype in co-infected potato and psyllid samples. This is the first report on LsoA and LsoB haplotype-specific primers.

Introduction

'*Candidatus* Liberibacter solanacearum' (Lso) was described initially associated with tomato and potato disease in New Zealand (Liefting et al. 2009). Like its closely related Huanglongbing pathogen '*Candidatus* Liberibacter asiaticus', Lso is a fastidious bacterium, which, to date, its axenic *in vitro* culturing has not been achieved. Using published primers (Liefting et al. 2009, Li et al. 2009) and newly-designed primers based on 16S-ISR-23S rRNA gene sequences numerous studies have shown that Lso is associated with Zebra chip (ZC) disease of potato in the USA, Mexico, Honduras, and Guatemala (Secor et al. 2009, Wen et al. 2009, Crosslin et al.2012a,b, Wen et al. 2012a). Single nucleotide polymorphism (SNP) in 16S rRNA and ISR-23S rRNA regions revealed that there are two Lso populations in the USA, namely clade 1 and clade 2, and one population in Mexico (Wen et al. 2009, Secor et al. 2009). Nelson et al. (2011) proposed three haplotypes (A, B and C) by compiling our rRNA sequence data, 50S ribosomal protein data deposited in the GenBank, and their own sequence data from carrot-disease samples collected in Finland. Their report showed that haplotype A (LsoA) and haplotype B (LsoB) are clade 1 and clade 2, respectively, associated with ZC disease of potato, and haplotype C (LsoC) associated with carrot disease in Finland (Munyaneza et al. 2010). With the release of Lso genome sequence (Lin et al. 2011), more in depth population studies on Lso were conducted using SSR and MLST markers (Lin et al. 2012, Glynn et al. 2012). Surprisingly, both studies confirmed the two major genetic populations, clade 1 and 2, or LsoA and LsoB. Most recently, haplotype D Lso (LsoD) was described to be associated with carrot disease in Spain (Nelson et al. 2012).

Current Lso typing techniques are based on sequencing technologies, which involve several steps including PCR, PCR product clean-up, and sequencing PCR products. These methods are costly and time-consuming and require special equipment and expertise. Using one set of SSR primers, a simple PCR assay was developed to type Lso in psyllid and plant (Wen et al. 2011) and a study on Lso haplotype temporal and spatial distribution was conducted using this PCR assay (Wen et al. 2012b). Temporal shifts in Lso haplotypes were revealed: LsoB dominated in Texas in 2008 through 2010; however LsoA has become more prevalent since 2011. It is interesting to note that concomitant with this shift in prevalence of Lso haplotype, ZC disease has become less severe and less prevalent in that state (Gudmestad, personal observation). Another important finding of this study was that LsoA and LsoB were detected simultaneously in individual potato plants and psyllids, which indicates that co-infection

of the two Lso haplotypes exists in agricultural ecosystems. It raises interesting questions regarding the efficiency of transmission of each haplotype by a dual infected bacteriliferous vector, *B. cockerelli*. Detection and quantification of single haplotype in co-infected samples are essential to conduct such research. However, due to the nature of SSR marker, single typing in co-infected samples was not successful and the ratio of the two Lso haplotypes cannot be determined. Therefore, the development of single typing markers is imperative for epidemiological studies, as well as providing unambiguous genotyping in the psyllid vector and potato plants. Using published LsoB genome sequence (Lin et al. 2011) and draft LsoA genome sequence (Johnson et al. unpublished), Lso typing primers were searched and designed, and the evaluation and comparison of the newly designed primers were conducted.

Materials and Methods

Potato and psyllid DNA samples. DNA samples were extracted from potato samples collected from commercial potato fields in the USA and from psyllid samples raised in NDSU greenhouse, using Qiagen DNA kit (Wen et al. 2009).

Primer design. Forty sets of primers were designed based on the published Lso genome sequence (Lso-B) and draft Lso-A genome sequence (Johnson et al. unpublished). Primers were synthesized by IDT (Coralville, Iowa).

Primer screening. New primer sets were screened on eight samples comprising 2 of potato-origin, 1 of psyllid-origin of each haplotype, healthy potato, and water using conventional PCR assay (Wen et al. 2009). Primer set Lso-SSR1 (Lin et al., 2012) was used as reference. A 2.5% agarose gel was used to run PCR products. Selected primers were further evaluated on 24 Lso-positive potato and psyllid samples to validate the preliminary data. Real-time PCR was performed for type specific primers on selected Lso-A, Lso-B and co-infected potato and psyllid samples using SsoAdvanced™ SYBR® Green Supermix (BioRad, Hercules, CA) following manufacturer's instructions on Stratagene Mx3005P. Relative quantification of Lso in potato and psyllid samples was obtained from real-time PCR Ct values, and ratios of two types of Lso in co-infected samples were determined from the Ct values of type specific primers.

Ratio calculation in co-infected samples. If Ct value of LsoA equals A, and Ct value of LsoB equals B, then the ratio of LsoA: LsoB in co-infected samples would be $1:2^{A-B}$.

Results and discussion

Six sets of primers were found to consistently type LsoA and LsoB in ZC-afflicted potato and Lso-positive psyllids samples (Figure 1). Two sets of LsoA primers only amplified samples infected by LsoA (Fig.1-A). An additional two sets of LsoB primers only amplified samples infected by LsoB (Fig.1-B). Conversely, two sets of SSR primers amplified both samples infected by either LsoA or LsoB with different sized amplicon (Fig.1-C). None of the healthy plant samples or water controls was amplified by the six sets of primers.

Relative quantification was obtained by the Ct values of real-time PCR (Table 1). Samples infected with LsoA had Ct values from LsoA-1 and LsoA-2 primers, but not from LsoB-1 and LsoB-2 primers, vice versa, whereas co-infected samples had Ct values from both haplotype specific primers. None of the healthy plant samples or water negative controls had Ct values. Based the Ct values, the ratios of LsoA and LsoB in co-infected samples, 8a and 9c, were calculated to be 1:256 and 1:512, respectively (Table 1).

Although there are four haplotypes of Lso, only LsoA and LsoB associated with ZC disease of potato have been reported in the USA. LsoC and LsoD associated with carrot disease are only found in Europe. This study provided LsoA and LsoB specific primers and their respective PCR assays.

Conventional PCR assays using the primers developed in this study will facilitate the Lso detection and haplotype differentiation. Also, real-time PCR assays using the new primers will provide haplotype specific quantitative data which will make it possible for determination of the ratios in co-infected samples. With the new primers, epidemiological studies and ecological studies evaluating Lso haplotypes associated with ZC can be conducted.

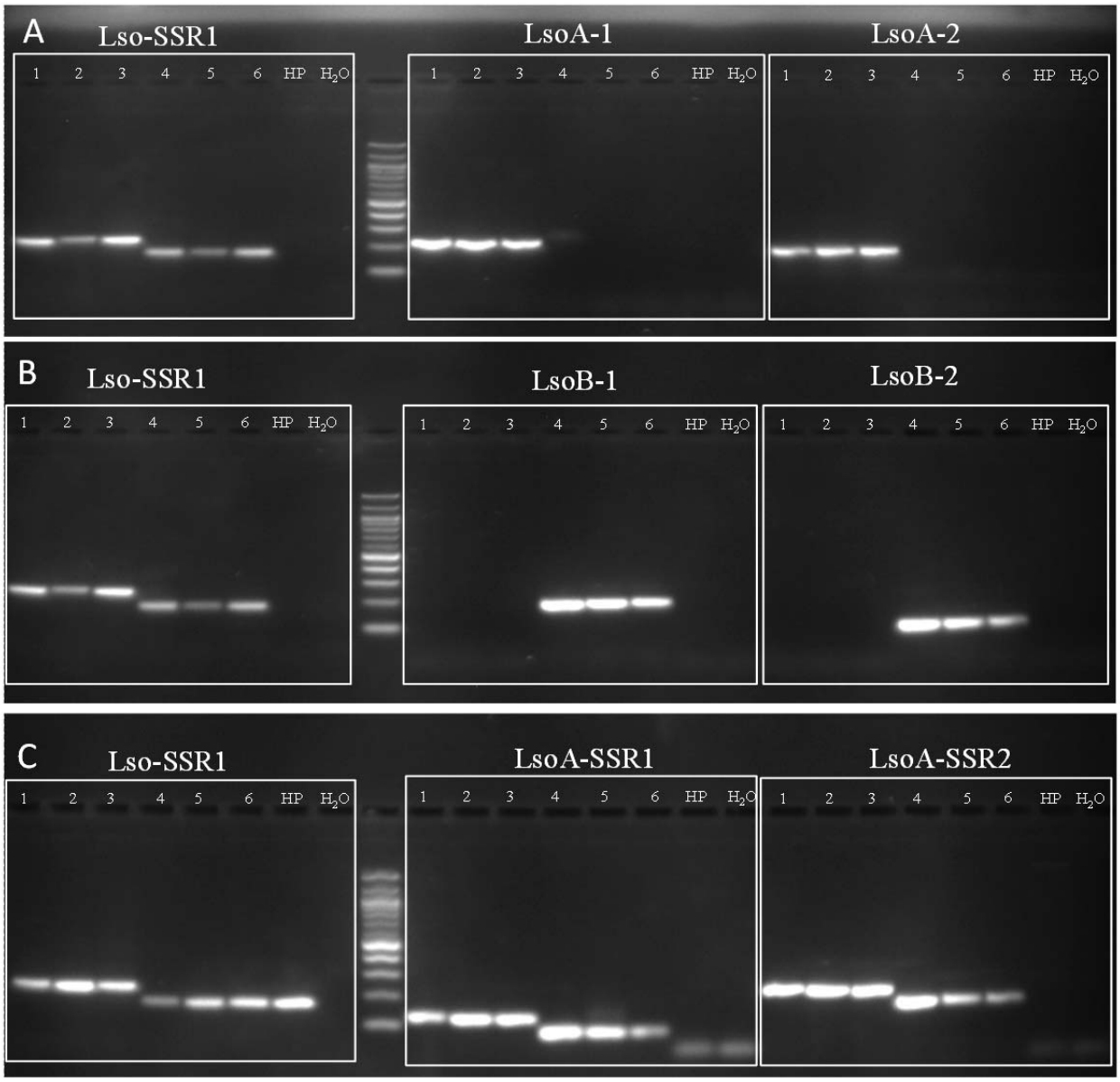


Figure 1. PCR products of Lso typing primers. Lso-SSR1 as reference. Samples: 1, 2, and 3 were LsoA-infected; 4, 5, and 6 were LsoB infected; and HP stands for healthy plant. A: LsoA specific primers; B: LsoB specific primers; C: SSR-primers.

Table 1. Ct values of real-time PCR using Lso typing primers

Primers	Ct Values of samples[a]							
	608 (A)	**Henne (A)**	**609 (B)**	**429 (B)**	**8a (mixed)**	**9c (mixed)**	**Health plant**	**Water**
LsoA-1	28.56	18.63	No Ct	No Ct	25.77	25.75	No Ct	No Ct
LsoA-2	28.95	18.89	No Ct	No Ct	26.22	26.14	No Ct	No Ct
LsoB-1	No Ct	No Ct	27.39	30.11	18.74	17.44	No Ct	No Ct
LsoB-2	No Ct	No Ct	27.40	30.04	18.57	18.11	No Ct	No Ct
Ratio A:B	NA	NA	NA	NA	**1:256**	**1:512**	NA	NA

[a] A in bracket refers to LsoA infected samples, B refers to LsoB infected samples and mixed refers to LsoA and LsoB co-infected samples.

References

Crosslin, J.M., P.B. Hamm, S.I. Rondon, J.E. Eggers, J.E. Munyaneza. 2012a. First report of zebra chip disease and '*Candidatus* Liberibacter solanacearum' on potatoes in Oregon and Washington State. Plant Dis. 96: 452.

Crosslin, J.M., N. Olsen, and P. Nolte. 2012b. First report of zebra chip disease and "*Candidatus* Liberibacter solanacearum" on potatoes in Idaho. Plant Dis. 96: 453.

Glynn, J.M., M.S. Islam, Y. Bai, S. Lan, A. Wen, N.C. Gudmestad, E.L. Civerolo, and H. Lin. 2012. Multilocus sequence typing of '*Candidatus* Liberibacter Solanacearum' strains from North America and New Zealand. J. Plant Pathol. 94: 223-228.

Li, W., J.A. Abad, R.D. French-Monar, J. Rascoe, A. Wen, N.C. Gudmestad, G.A. Secor, I.M. Lee, Y. Duan, and L. Levy. 2009. Multiplex real-time PCR for detection, identification and quantification of '*Candidatus* Liberibacter solanacearum' in potato plants with zebra chip. J. Microbiol. Methods 78: 59-65.

Liefting, L.W., B.S. Weir, S.R. Pennycook, and G.R.G. Clover. 2009. '*Candidatus* Liberibacter solanacearum' associated with plants in the family Solanaceae. Int. J. Syst. Evol. Micr. 59: 2274-2276.

Lin, H., B. Lou, J.M. Glynn, H. Doddapaneni, E. L. Civerolo, C. Chen, Y. Ping, L. Zhou, and C.M. Vahling. 2011. The complete genome sequence of `*Candidatus* Liberibacter solanacearum', the bacterium associated with potato Zebra Chip disease. PLoS One 6: e19135.

Lin, H., M.S. Islam, Y. Bai, A. Wen, S. Lan, N.C. Gudmestad, and E.L. Civerolo. 2012. Genetic diversity of '*Candidatus* Liberibacter solanacearum' strains in the United States and Mexico revealed by simple sequence repeat markers. Eur. J. Plant Pathol. 132: 297–308.

Munyaneza, J.E., T.W. Fisher, V.G. Sengoda, S.F. Garczynski, A. Nissinen, and A. Lemmetty. 2010. First report of "*Candidatus* Liberibacter solanacearum" associated with psyllid-affected carrots in Europe. Plant Dis. 94:639.

Nelson, W.R., T.W. Fisher, and J.E. Munyaneza. 2011. Haplotypes of "*Candidatus* Liberibacter solanacearum" suggest long-standing separation. Eur. J. Plant Pathol. 130: 5–12.

Nelson, W.R., V.G. Sengoda, A.O. Alfaro-Fernandez, M.I. Font, J.M. Crosslin, and J.E. Munyaneza. 2012. A new haplotype of "*Candidatus* Liberibacter solanacearum" identified in the Mediterranean region. Eur. J. Plant Pathol. DOI 10.1007/s10658-012-0121-3.

Secor, G.A., V.V. Rivera, J.A. Abad, I.M. Lee, G.R.G. Clover, L.W. Liefting, X. Li, and S.H. De Boer. 2009. Association of '*Candidatus* Liberibacter solanacearum' with Zebra chip disease of potato established by graft and psyllid transmission, electron microscopy, and PCR. Plant Dis. 93: 574-583.

Wen, A., I. Mallik, V.Y. Alvarado, J.S. Pasche, X. Wang, W. Li, L. Levy, H. Lin, H.B. Scholthof, T.E. Mirkov, C.M. Rush, and N.C. Gudmestad. 2009. Detection, distribution and genetic variability of '*Candidatus* Liberibacter' species associated with Zebra complex disease of potato in North America. Plant Dis. 93: 1102-1115.

Wen, A., H. Lin, and N.C. Gudmestad. 2011. Development of PCR Assay Using SSR Primers for Detection and Genotyping of '*Candidatus* Liberibacter solanacearum'. Proceedings of the 11th Annual SCRI Zebra Chip Reporting Session (Workneh, Rashed, and Rush editors). Nov 6-9, 2010. San Antonio, Texas. pp 74-78.

Wen, A. C. Johnson, and N.C. Gudmestad. 2012a. Genetic characterization of '*Candidatus* Liberibacter solanacearum' associated with zebra chip disease of potato in Washington and Idaho. Phytopathol. 102:S4.134

Wen, A., C. Johnson, and N.C. Gudmestad. 2012b. Development of a PCR assay for the rapid detection and differentiation of '*Candidatus* Liberibacter solanacearum' haplotypes and their spatiotemporal distribution in the United States. Am. J. Potato Res. DOI 10.1007/s12230-012-9293-9

Haplotyping Studies of the Potato Psyllid by High Resolution Melting Analysis

Swisher, K.D.[1], Munyaneza, J.E.[2], and Crosslin, J.M.[1] [1]USDA-ARS, Prosser, WA 99350; [2]USDA-ARS, Wapato, WA 98951

Abstract

To understand potato psyllid population dynamics during the 2011 potato growing season, high resolution melting analysis (HRM) was utilized to genotype psyllids collected across the central and western United States using a portion of the mitochondrial cytochrome c oxidase subunit I-like gene (CO1). These results confirmed the previously known Western and Central haplotypes, which were localized geographically to the western and central United States, respectively. Initial genotyping also identified a novel, Northwestern psyllid haplotype found geographically in the northwestern United States. The development of an HRM assay to differentiate psyllid populations was subsequently applied to archived psyllids collected between 1998 and 2010. Here, results show that the Northwestern psyllid population has existed in Washington State as early as 1998. Additionally, the Western psyllid population was identified in Washington and Oregon as early as 2008. Current studies are focusing on using the HRM analysis as a tool to haplotype the psyllids collected during the 2012 potato growing season.

Introduction

Washington, Oregon, and Idaho saw their first known outbreak of the devastating zebra chip (ZC) disease during the 2011 potato growing season. To identify the origin of the potato psyllids, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), that entered the Pacific Northwest in 2011 carrying the causal agent of ZC, *Candidatus* Liberibacter Solanacearum (Lso), HRM analyses were utilized to genotype psyllids collected across the western and central United States (Swisher et al. 2012). Using a 500-bp portion of the mitochondrial CO1 gene, three potato psyllid haplotypes were identified in the United States. The Western and Central haplotypes (previously identified as biotypes, Liu et al. 2006 and Liu and Trumble, 2007) were isolated in the western and central United States geographical regions, respectively. Additionally, a novel, Northwestern haplotype was identified and thus named because it was isolated only in Washington and Idaho (Swisher et al. 2012). Interestingly, in 2011, both the Western and Northwestern psyllid haplotypes were identified in overlapping regions in Washington, Oregon, and Idaho. However, psyllids collected from ZC-fields in Washington and Oregon, 5 – 10% of which carried Lso, were only identified as the Western psyllid population (Swisher et al. 2012). These results provide evidence that the Western psyllid population was responsible for the ZC outbreak in the northwestern United States in 2011.

To gain a better understanding of the psyllid population dynamics in the Pacific Northwest, archived potato psyllids collected between 1998 and 2010 were genotyped using HRM analysis of partial mitochondrial CO1 gene amplicons. This research identified psyllid populations present during a given year from specific locations across the United States. Additional HRM analyses are being conducted to determine the potato psyllid population dynamics within the 2012 potato growing season.

Materials and Methods

Psyllids were collected in Prosser, Washington, in 1998, 1999, 2009, and 2010 using a D-vacuum device or a sweep net. Psyllids were collected in Moxee, Washington, in 2005, 2006, 2008, 2009, and 2010, as well as Hermiston, Oregon, during 2009 and 2010 using a D-vacuum device. Additionally, psyllids were collected in Nebraska, near Garden City, Kansas, and in Texas near Edinburg, Pearsall, Dalhart,

Weslaco, and Olton, in 2009 and/or 2010, from yellow sticky traps located throughout commercial potato fields and/or research plots as part of a psyllid population study (Goolsby et al. 2012).

All psyllids were extracted using the CTAB method (Zhang et al. 1998) with slight modifications (Crosslin et al. 2006). Four primer pairs specific to the *B. cockerelli* mitochondrial CO1 were used for HRM and DNA sequencing analysis: 1) CO1 F3 (Swisher et al. 2012) and CO1 353R (5´CTAGCAATAATTGCAAATACAG3´), generating a 353-bp amplicon, 2) CO1 meltF (Chapman et al. 2010) and CO1 353R, generating a 326-bp amplicon, 3) CO1 F3 and CO1 meltR (Chapman et al. 2010), generating a 94-bp amplicon, and 4) CO1 meltF and CO1 meltR, generating a 67-bp amplicon.

The LightCycler 480 and the LightCycler 480 Gene Scanning Software (Roche Applied Science, Indianapolis, Indiana) were used for all HRM tests and subsequent analyses of results (Swisher et al. 2012). Samples were tested in 20 µl reactions: 10 µl LightCycler 480 High Resolution Melting Master mix (Roche Applied Science, Indianapolis, Indiana), 0.5 µl [20 µM] each primer, 1.6 µl [25 mM] $MgCl_2$, 5.8 µl PCR-grade H_2O, and 1.6 µl DNA. For the 353- and 326-bp CO1 amplicons, the qPCR program was as follows: Preincubation step (95^{o}C for 10 min with 4.4^{o}C/s ramp rate) and an Amplification step for 40 cycles (95^{o}C for 10 sec with 4.4^{o}C/s ramp rate, 55^{o}C for 15 sec with 2.2^{o}C/s ramp rate, 72^{o}C single acquisition for 25 sec with 4.4^{o}C/s ramp rate). For the 94- and 67-bp CO1 amplicons, the touchdown qPCR program was as follows: Preincubation step (95^{o}C for 10 min with 4.4^{o}C/s ramp rate) and an Amplification step for 35 cycles (95^{o}C for 10 sec with a 4.4^{o}C/s ramp rate, 60^{o}C with a second target of 53^{o}C at a step size of 0.5^{o}C with 1 cycle step delay for 15 sec with a 2.2^{o}C/s ramp rate, 72^{o}C single acquisition for 25 sec with a 4.4^{o}C/s ramp rate). For all samples, the melting cycle was as follows: Melting step (95^{o}C for 1 sec with a 4.4^{o}C/s ramp rate, 40^{o}C for 1 sec with a 2.2^{o}C/s ramp rate, 60^{o}C for 20 sec with a 1^{o}C/s ramp rate, and 95^{o}C with continuous acquisition at 25 acquisitions/^{o}C) and a cooling step (40^{o}C for 10 min with ramp rate of 2.2^{o}C/s).

Results and Discussion

HRM analysis using a 353-bp portion of the mitochondrial CO1 gene isolated the three known potato psyllid haplotypes: Western, Central, and Northwestern (Figure 1). Using this amplicon, the melting profile of all three haplotypes generated double-peak profiles. The Northwestern haplotype was shifted to a slightly lower temperature, the Western haplotype was shifted to a slightly higher melting temperature, and the Central haplotype partially overlapped with both the Northwestern and the Western haplotypes in the middle (Figure 1A). The signal differences of the normalized raw fluorescent data, as set to the Central haplotype, also clearly identified three distinct psyllid haplotypes (Figure 1B). A 326-bp portion of the mitochondrial CO1 gene was also used for HRM analyses to differentiate the three haplotypes (data not shown). Overall, the 353- and 326-bp amplicons were used for HRM analyses of 457 archived psyllid samples collected in the western and central United States between the year 1998 and 2010.

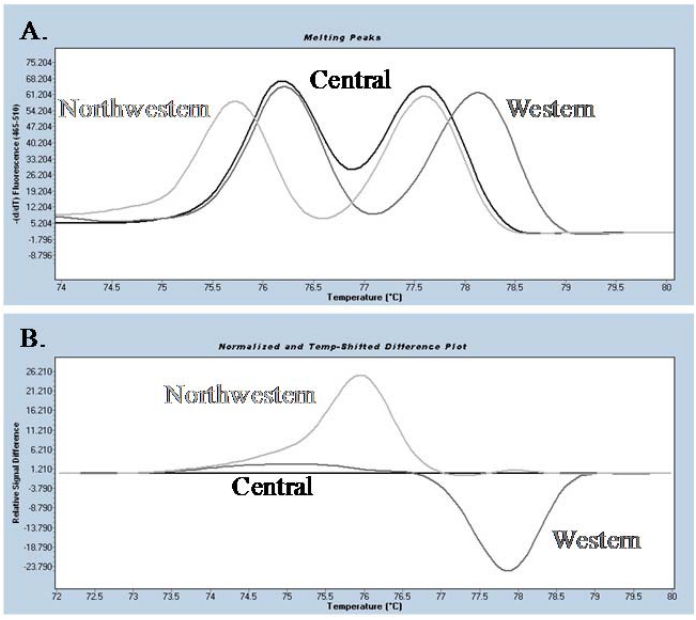


Figure 1. HRM analysis of a 353-bp portion of CO1. (A) Melting profile. (B) Difference plot.

Roughly 16% of the 457 archived psyllid samples tested did not give strong HRM results, a possible result of DNA degradation caused by the storage conditions of the archived psyllids. Therefore, two CO1 amplicons were utilized in conjunction to discern the different haplotypes. A 94-bp CO1 amplicon was used to differentiate the Central haplotype from the Western and Northwestern populations (Figure 2A). Subsequently, a 64-bp CO1 amplicon was utilized to differentiate the Western haplotype from the Central and Northwestern populations (Figure 2B). Psyllids that were not differentiated by either CO1 amplicon were determined to be the Northwestern psyllid haplotype.

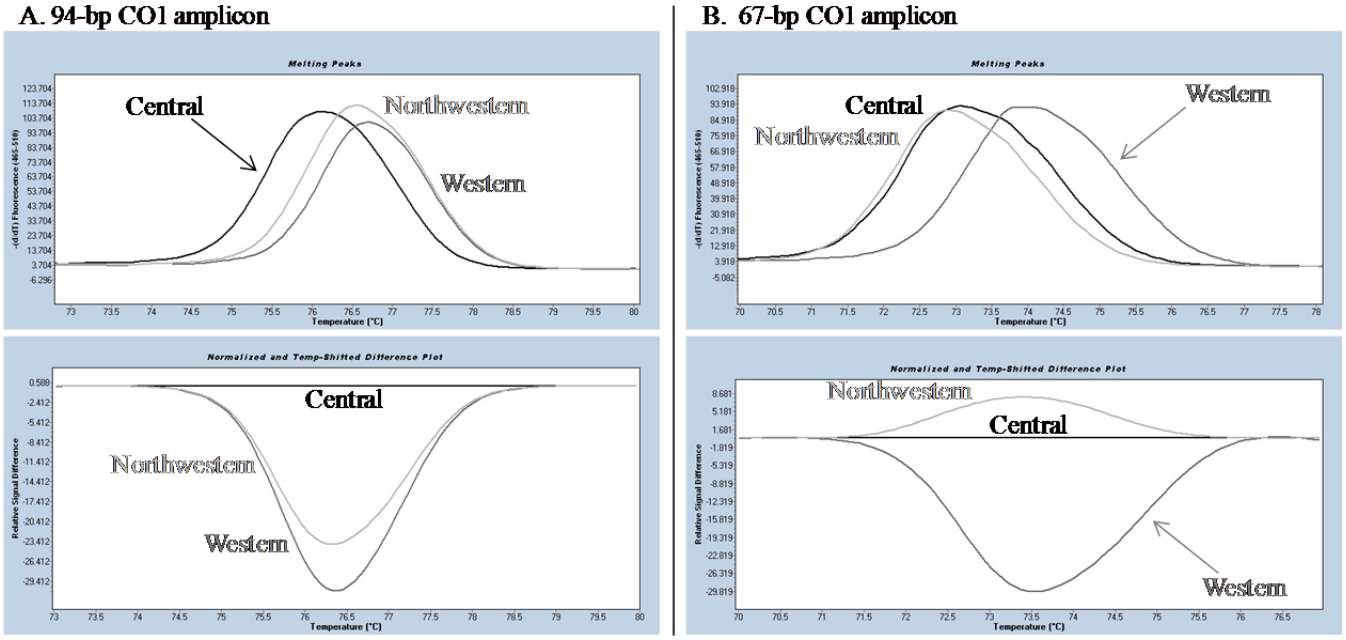


Figure 2. HRM analysis of partial CO1 amplicons. (A) Melting profile (top) and difference plot (bottom) generated using a 94-bp CO1 amplicon. (B) Melting profile (top) and difference plot (bottom) generated using a 67-bp CO1 amplicon.

HRM analysis and subsequent verification by DNA sequencing (data not shown) of the archived psyllids produced interesting results (Figure 3; Swisher et al. 2012). In Prosser, Washington, psyllids collected as early as 1998 and 1999 were identified as the Northwestern haplotype. This Northwestern population persisted in Prosser, Washington, in 2009, 2010, and 2011, with a significant presence of Western psyllids in 2009. In nearby Moxee, Washington, the Northwestern psyllid haplotype was identified in 2005 and 2006. Interestingly, the Western haplotype was identified as early as 2008, with a strong presence in 2009. Psyllids just across the Columbia River in Hermiston, Oregon, were all identified as the Western haplotype from collections made in 2009, 2010, and 2011.

The results from archived psyllids collected in the northwestern United States suggest that the Northwestern population may in fact be native to the region, with the Western population migrating into the area overtime. To date, the Northwestern psyllids have not been found outside of Washington, Oregon, and Idaho, while the Western psyllids have been found spanning from Southern California and New Mexico on up to Washington (Swisher et al. 2012; data not shown). Additional *B. cockerelli* genotyping is underway for potato psyllids collected during the winter months in the Pacific Northwest, to determine which psyllid populations can survive the cold winter temperatures, and are thus more likely to be a native population. These results also suggest that the Western psyllid population has been in Washington and Oregon prior to the 2011 season where the Western psyllids were collected in ZC-

fields and 5-10% were found to be positive for Lso. Perhaps either the changing of environmental conditions or the development of other factors caused the Western psyllid population to pick up the Lso bacterium during the 2011 season, where it had not done so before.

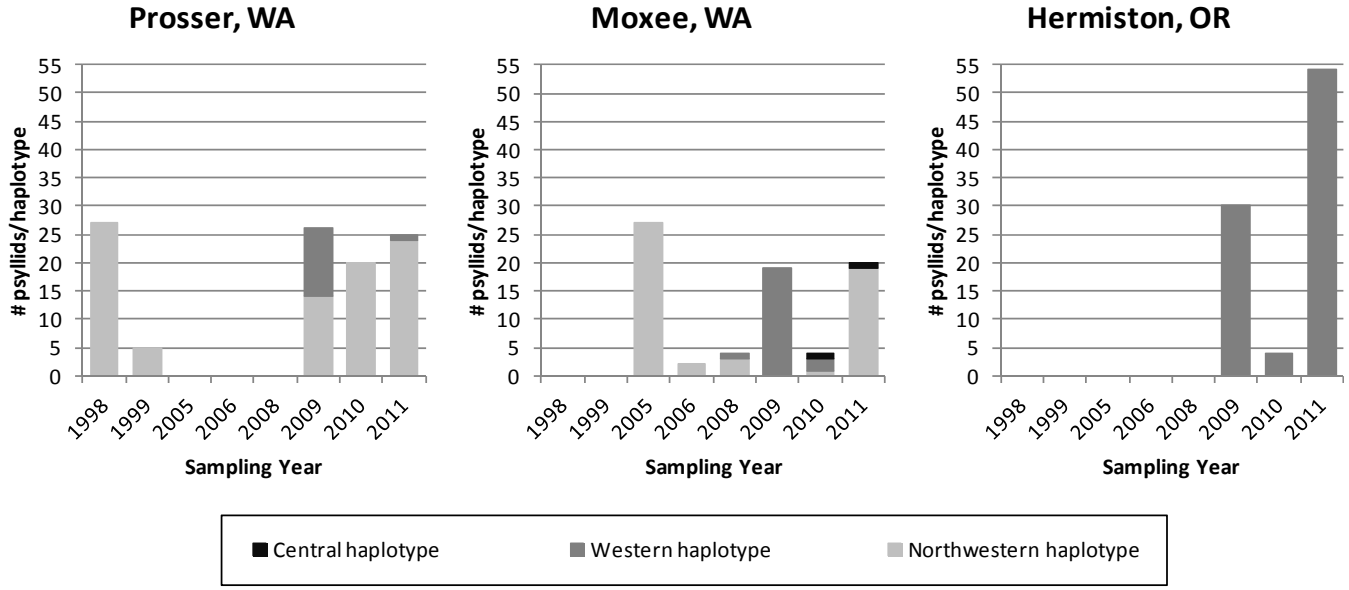


Figure 3. HRM results of all psyllids collected in the northwestern United States from 1998 - 2011. *All 2011 data has previously been published in Swisher et al. 2012.

HRM analysis of archived psyllids collected in the central United States was also conducted, although a limited number of archived samples were available (Figure 4; Swisher et al. 2012). The Central haplotype was identified in Texas as early as 2005. Psyllids collected in Nebraska, Kansas, and Texas in 2009, 2010, and 2011 were predominantly identified as the Central haplotype, although a small percentage of the Western haplotype was also identified from these areas.

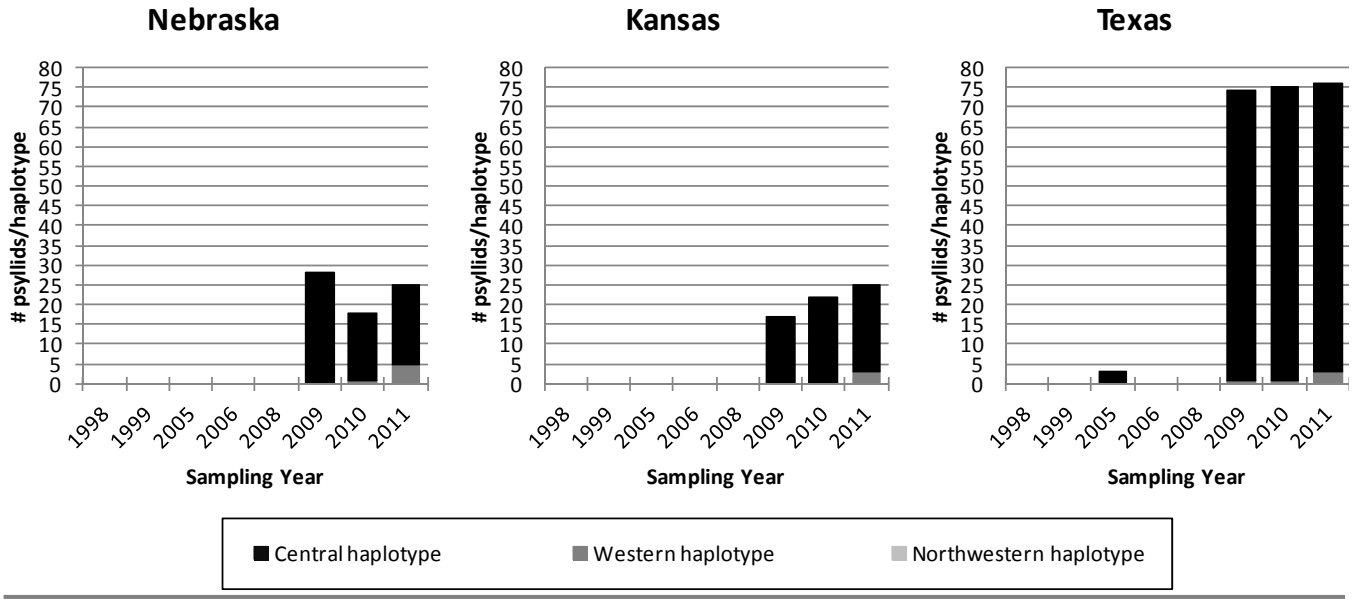


Figure 4. HRM results of all psyllids collected in Nebraska, Kansas, and Texas from 1998 - 2011. *All 2011 data has previously been published in Swisher et al. 2012.

Acknowledgements
We thank Eric Krohn, Launa Hamlin, Mary Sue Roster, Tonja Fisher, Venkatesan Sengoda, and Blaine Heilman for technical assistance. We also thank John Goolsby for providing many of the psyllids collected in the central United States. Financial support came from the USDA-SCRI Project # 2009-51181-20176 and the USDA-RAMP Project # 2009-51101-05892. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable. USDA is an equal opportunity provider and employer.

References
Chapman, R.I., L. Strube, and B. Bextine. 2010. Population genetics of the potato psyllid: Impacts on zebra chip epidemiology, pp. 64-68. Proceedings of the 10th Annual Zebra Chip Reporting Session. 7-10 November 2010. Texas AgriLife, College Station, TX.

Crosslin, J.M., G.J. Vandemark, and J.E. Munyaneza. 2006. Development of a real-time, quantitative PCR for detection of the Columbia Basin potato purple top phytoplasma in plants and beet leafhoppers. Plant Dis. 90: 663-667.

Goolsby, J.A., J. Adamczyk, J.M. Crosslin, N. Troxclair, J. Anciso, G. Bester, J. Bradshaw, E. Bynum, L. Carpio, D. Henne, A. Joshi, J.E. Munyaneza, P. Porter, P. Sloderbeck, J. Supak, C. Rush, F.J. Willett, F. Workneh, B. Zechmann, and B. Zens. 2012. Seasonal population dynamics of the potato psyllid (Hemiptera: Triozidae) and its associated pathogen "*Candidatus* Liberibacter solanacearum" in potatoes in the southern Great Plains of North America. J. Econ. Entomol. 105:1268-1276.

Liu, D., J.T. Trumble, and R. Stouthamer. 2006. Genetic differentiation between eastern populations and recent introductions of potato psyllid (*Bactericera cockerelli*) into western North America. Entomol. Exp. Appl. 118: 177-183.

Liu, D., and J.T. Trumble. 2007. Comparative fitness of invasive and native populations of the potato psyllid (*Bactericera cockerelli*). Entomol. Exper. Appl. 123: 35-42.

Swisher, K.D., J.E. Munyaneza, and J.M. Crosslin. 2012. High resolution melting analysis of the cytochrome oxidase I gene identifies three haplotypes of the potato psyllid in the United States. Environ. Entomol. 41: 1019–1028.

Zhang, Y.-P., J.K. Uyemoto, and B.C. Kirkpatrick. 1998. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. J. Virol. Methods 71: 45-50.

Relationships of Potato Psyllid Populations Based on Total Microbial Community Composition

Alex Arp and Blake Bextine. The University of Texas at Tyler, 3900 University Blvd, Tyler, TX 75799, USA

Abstract
To better understand potato psyllid migrations and range expansion the bacterial symbiont communities of individual potato psyllids were assessed using 454 pyrosequencing of the 16s rRNA gene. The psyllids used were collected from 2008-2011 from the central and western United States, New Zealand, and carrot psyllids (*Trioza apicalis)* were included to act as an outlier. 16s sequences were analyzed using the Qiime 1.5.0 software suite (qiime.org) with samples being clustered by weighted and unweighted UniFrac. Clustering analysis separated the psyllids based on their bacterial symbionts as expected with central United States and California samples, but unexpected patterns arose with Washinton, Oregon, and New Zealand samples. Disproportionately high concentrations of certain bacteria affected results in the weighted trees.

Introduction
The potato psyllid, *Bactericera cockerelli* (Sulc), is a phytophagous hemipteran in the family Triozidae. Potato psyllids occur seasonally and have a range that extends from eastern Mexico south to Nicaragua, throughout the central United States, California, the Pacific Northwest, and New Zealand. Potato psyllids feed primarily on solanaceous plants, which include crops such as: potato, tomato, peppers, and tobacco. The potato psyllid is known to transmit the bacteria *Candidatus* Liberibacter solanacaeurm, the putative causal agent of Zebra Chip disease in potato (Secor et al. 2009). Zebra Chip causes physiological changes in potato tubers rendering them unsellable to the chip industry, which results in millions of dollars in crop losses annually.

Many insects harbor symbiotic bacteria that can provide the insects with enhanced resistance to pesticides, enhanced immune response, nutrition, and reproductive advantages (Douglas 2011). Due to the lack of essential amino acids in potato psyllids' diet they rely on the bacterial symbiont *Candidatus* Carsonella rudii in their gut to provide them with these amino acids (Thao et al. 2000). Potato psyllids are also obligate carriers of *Wolbachia pipientis*, though it's relation to the psyllid and possible reproductive changes are unknown(Liu et al. 2006). Potato psyllids also commonly harbor *Rhizobium, Gordonia, Mycobacterium, Xanthomonas, Staphylococcus,* and others that can be obtained through vertical or horizontal transfer (Hail et al. 2011).

Bacterial symbionts are acquired through either vertical or horizontal transfer, thus it can be assumed that interbreeding populations of potato psyllid with similar feeding habits should have similar microbial communities. These microbial communities can provide insight into sources of outbreaks by comparing the microbial community of the possible sources with those of the new population. Regional heterogeneity within the bacterial community can also be important since these bacteria can provide resistances to pesticides and other physiological changes that could result in greater crop damages. In the study, individual potato psyllid samples from the central United States, California, the Pacific Northwest, Nicaragua, and New Zealand were subjected to 454 pyrosequencing of the 16s rRNA gene and analyzed by divergence-based diversity estimates to infer inter-population, inter-year, and emergent population relationships based on the psyllid's microbial community.

Materials and Methods

Collection and Extraction

Potato psyllids were collected from fields and laboratory colonies and were stored in 95% ethanol at -20°C until extraction. Single psyllid DNA was extracted using Qiagen DNeasy kit (Qiagen, Valencia, CA) or a psyllid optimized CTAB protocol. Extracted DNA was stored at -20°C.

454 Pyrosequencing

454 Pyrosequencing of the total 16s rRNA gene was analyzed by at the Research and Testing Laboratory in Lubbock, TX, using the bTEFAP methodology and 530 F (5'-GTG CCA GCM GCN GCG G) and 1100R (5'-GGG TTN CGN TCG TTG) generic 16s primers (Dowd et al. 2008).

Sequence Analysis

Sequences <200bp or >1000bp or with a read quality of >25 were removed prior to analysis. Chimeric sequences were removed with ChimeraSlayer (Haas et al. 2011). Sample analysis and tree building was done using the QIIME 1.5.0 package (Caporaso et al. 2010). OTU similarity was set at >97% identity. OTUs with less than 20 instances were removed to avoid inclusion of singlets or other sequencing errors. Psyllid phylogenies were created with both weighted and unweighted UniFrac with jackknife support of 10 replicates and a sample depth of 1500 sequences (Lozupone et al. 2010). Phylogenetic trees were rendered using FigTree (tree.bio.ed.ac.uk).

Results and Discussion

Weighted and unweighted phylogenetic trees show mostly similar relations between potato psyllid populations (Figure 1a-d). It can be inferred from this similarity that the abundance of bacterial reads does not influence the divergence of groups as much as an overall change in their bacterial community. Psyllids in this study were collected in multiple years at each location but the year did not influence their clustering as much as location.

There is greater regional heterogeneity in the microbial community of samples in the central United States than in samples from California (Figure 1a). The similarity of the microbes in the California samples could be the result of the closer proximity of where they were collected. The California psyllids were collected from potato, tomato, and pepper plants, but did not show any patterns relating to the plant they were collected from.

Psyllids collected in Washington and Oregon did not show direct similarity to only California, as was expected due to traditional potato psyllid migration patterns. Some Washington and Oregon potato psyllids clustered with both California and central United States samples while others were only similar to psyllids in this region (Figure 1c). The psyllids that clustered with both populations of US potato psyllids could be the result of mixing central and western populations; the other cluster may represent an isolated population.

Potato psyllids collected in New Zealand do not show a direct relation to either population in the United States (Figure 1d). This divergence from California and central United States populations is possibly the result of differences in diet and years of isolation from their founder population.

Carrot psyllids did not show relation to any groups and acted a natural outlier, except against the colony-raised New Zealand psyllids and two California psyllids on the weighted trees. The similarity of the lab

raised potato psyllids to carrot psyllids is alarming and should be investigated for transmission studies since the laboratory colony psyllids are possibly not representative of wild populations.

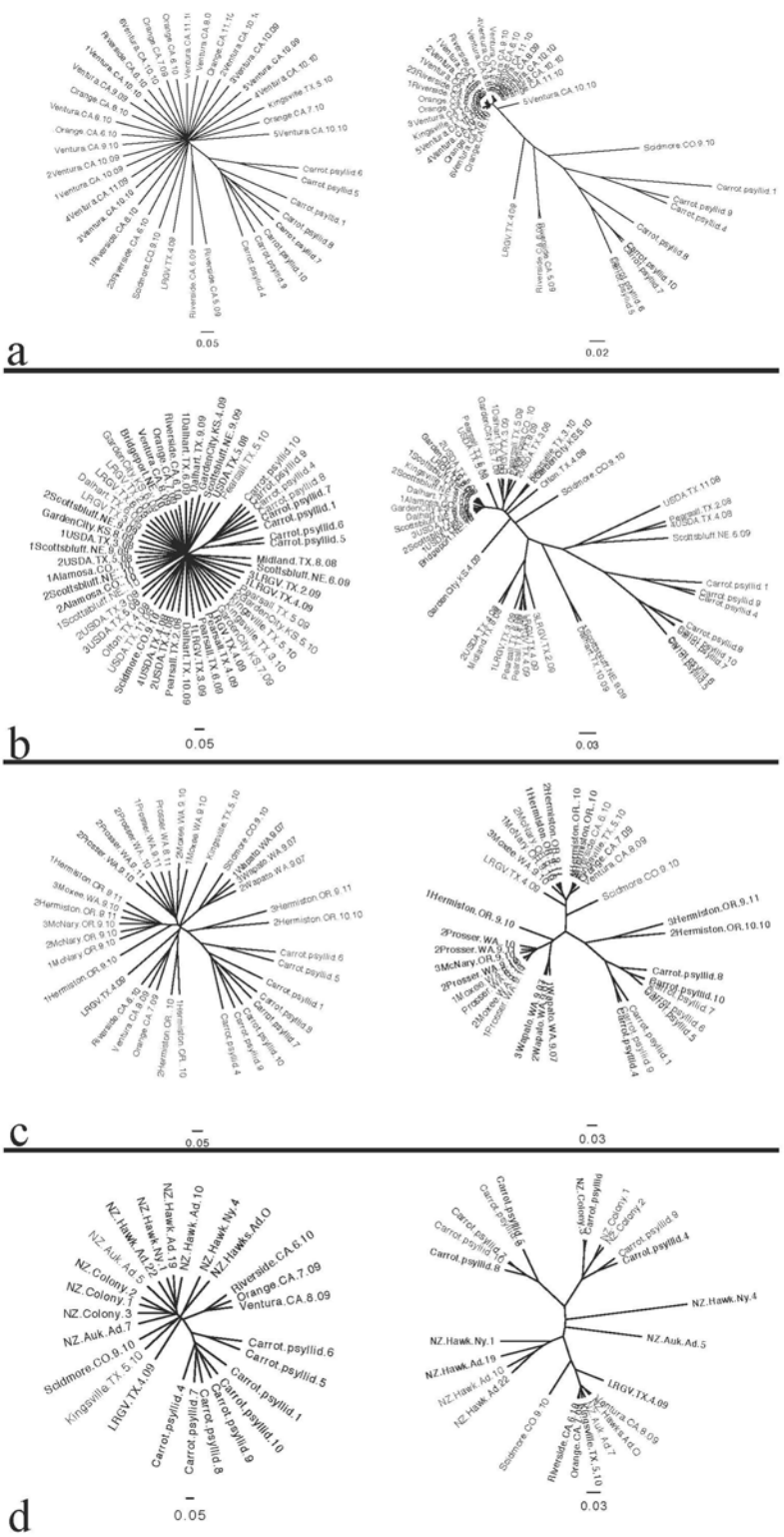


Figure 1: Unweighted (left) and weighted (right) phylogenies based on psyllid microbial communities. Carrot psyllids were included as an outlier and select central US and California psyllids were included to infer relation to possible source populations a) California b) Central US c) Washington and Oregon d) New Zealand

Acknowledgements
Funding was provided by USDA-SCRI Zebra Chip 2009-51181-20176. I would like to thank John Trumble, Casey Butler, John Goolsby, Greta Schuster, Don Henne, Joe Munyanesa, Jeff Bradshaw, and Steve Ogden for providing potato psyllids.

References
Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Meth. 7: 335–336.

Douglas, A. E. 2011. Lessons from Studying Insect Symbioses. Cell Host & Microbe. 10: 359–367.

Dowd, S. E., T. R. Callaway, R. D. Wolcott, Y. Sun, T. McKeehan, R. G. Hagevoort, and T. S. Edrington. 2008. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol. 8: 125.

Haas, B. J., D. Gevers, A. M. Earl, M. Feldgarden, D. V. Ward, G. Giannoukos, D. Ciulla, D. Tabbaa, S. K. Highlander, E. Sodergren, B. Methe, T. Z. DeSantis, The Human Microbiome Consortium, J. F. Petrosino, R. Knight, and B. W. Birren. 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Research. 21: 494–504.

Hail, D., I. Lauzìere, S. E. Dowd, and B. Bextine. 2011. Culture Independent Survey of the Microbiota of the Glassy-Winged Sharpshooter (Homalodisca vitripennis) using 454 Pyrosequencing. Environmental Entomology. 40: 23–29.

Liu, D., J. T. Trumble, and R. Stouthamer. 2006. Genetic differentiation between eastern populations and recent introductions of potato psyllid (Bactericera cockerelli) into western North America. Entomol Exp Appl. 118: 177–183.

Lozupone, C., M. E. Lladser, D. Knights, J. Stombaugh, and R. Knight. 2010. UniFrac: an effective distance metric for microbial community comparison. The ISME Journal. 5: 169–172.

Secor, G. A., V. V. Rivera, J. A. Abad, I. M. Lee, G. Clover, L. W. Liefting, X. Li, and S. H. De Boer. 2009. Association of“Candidatus Liberibacter solanacearum”with Zebra Chip Disease of Potato Established by Graft and Psyllid Transmission, Electron Microscopy, and PCR. Plant Dis. 93: 574–583.

Thao, M. L., N. A. Moran, P. Abbot, E. B. Brennan, D. H. Burckhardt, and P. Baumann. 2000. Cospeciation of psyllids and their primary prokaryotic endosymbionts. Applied and Environmental Microbiology. 66: 2898–2905.

Breeding Clones Derived from Wild Potato Species Exhibit Resistance to Potato Psyllid and Possible Resistance to Zebra Chip Disease

[1]Novy, R.G., [2]Trumble, J.T, [3]Butler, C.D, [2]Diaz-Montano, J.,[2]Gonzalez, B., [4]Manjunath, K.L., [4]Lee, R.F., and [5]Munyaneza, J. [1]USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID 83210, USA; [2]Department of Entomology, University of California, Riverside, CA 92521, USA;[3]Syngenta Crop Protection, Simi Valley, CA 93065, USA; [4]USDA-ARS, National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA 92507, USA; [5]USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951, USA.

Abstract

A haploid *tuberosum* x *Solanum berthaultii* hybrid clone and four generations of its progeny from backcrossing to cultivated potato were screened for resistance to adult potato psyllid, the insect vector of '*Candidatus* Liberibacter solanacearum' (syn. *Ca*. L. psyllaurous), which is associated with zebra chip (ZC) disease. The wild potato species *S. etuberosum* also is represented in the pedigree of the four generations of progeny. Relative to the potato variety Atlantic, significant reductions in psyllid probing (feeding) occurrences and resting duration were observed in several of the evaluated breeding clones suggesting resistance to potato psyllid; one clone also an increased duration of time off leaflet relative to Atlantic. In Liberibacter transmission assays, the percentages of Liberibacter-infected plants were significantly reduced relative to Atlantic for three clones, one of which exhibited no apparent psyllid resistance. This finding suggests possible resistance to Liberibacter, and not just the psyllid vector, may be contributing to reduced Liberibacter infection. Screening of breeding clones using Liberibacter-infective potato psyllids in controlled field cage evaluations also has identified putatively resistant/tolerant breeding clones following three years of evaluations. These breeding clones have the potato species *S. raphanifolium*, *S. chacoense*, and *S. berthaultii* in their pedigree which likely are contributing to the observed resistance/tolerance to ZC.

Introduction

Plant genetic resistance is an important component in many successful integrated pest management (IPM) programs and could also contribute to the control of ZC. However, an evaluation of nine potato varieties commonly grown in the U.S. and representing fresh-pack, chipping, and long processing market classes found none to have ZC resistance (Munyaneza et al., 2011a). The authors stated that: *Identification and development of potato varieties with resistance to or tolerance of ZC are crucial to developing effective and sustainable management strategies for this important potato disease*. Unique germplasm derived from the wild species *S. etuberosum* and *S. berthaultii* has been shown to exhibit resistance to many insect pests of potato including green peach aphid (Novy et al. 2002), Colorado potato beetle (Juan Alvarez, unpublished data), and wireworm (Alvarez et al., accepted with revisions). The myriad of resistances to insect pests, suggested that this germplasm might also express resistance to potato psyllid as well. Butler et al. (2011) reported on potato psyllid behavioral changes that suggest this unique germplasm does confer resistance to potato psyllid, and may also display resistance to Liberibacter as well; the results of which are summarized in this report.

Breeding clones 00-3115-2 and 00-3115-11 represent unique germplam with the potato species *S. raphanifolium*, *S. chacoense*, and *S. berthaultii* in their pedigree. These clones were initially requested from Dr. Shelley Jansky's program (USDA-ARS, Madison, WI) for use in the Aberdeen, Idaho potato breeding program as cold-induced sweetening resistant parents in 2007. The potato species represented in their pedigrees could provide ZC resistance not found in cultivated potato. The two breeding clones

were submitted by Drs. Novy and Jansky for screening under controlled field cage studies using Liberibacter-infective potato psyllids beginning in 2010. The results of three years of field cage evaluations are reported.

Materials and Methods

S. berthaultii/S. etuberosum breeding clones

A haploid *tuberosum* x *S. berthaultii* parent (designated 463-4) and four generations of progeny from backcrossing (BC) to cultivated potato were screened for resistance to adult potato psyllid and Liberibacter as summarized below. More detail regarding the materials and methodology is presented by Butler et al. (2011).

Psyllid Behavioral Assay: Based on the published protocols of Liu and Trumble (2004), the behaviors of psyllids for tasting, probing, walking, jumping, resting, off-leaflet duration, and cleaning were observed and data recorded over a 15 minute interval after being placed on replicated plants of submitted entries.

Liberibacter Transmission: Ten bacteriliferous psyllids were caged on plants of submitted entries for 24 hours and then removed. Plants were retained for two weeks and then were tested for the presence of Liberibacter using Taqman-based real-time PCR. The mean percentage of plants of each entry infected with Liberibacter was then calculated and compared to the control cultivar, Atlantic.

Breeding Clones 00-3115-2 and 00-3115-11

These breeding clones are full sibs of one another (same parents) with 00-3115-11 subsequently given the designation M5 (Jansky et al., 2011). Details regarding the pedigrees of these two clones and additional characteristics of M5 are described by Jansky et al. (2011). These clones were submitted for field cage screening using Liberibacter-infective potato psyllids beginning in 2010. Details of the field cage screening protocol are described by Muyaneza et al. (2010, 2011b) and in the Proceeding of the 12th Annual SCRI Zebra Chip Reporting Session (2012).

Results and Discussion

S. berthaultii/S. etuberosum breeding clones

Psyllid Behavioral Assay: Of the eleven clones evaluated, five had a significantly reduced number of probing (feeding) occurrences relative to the potato cultivar, Atlantic. Parental clone 463-4 and BC_1 progeny P2-4 had the fewest number of psyllid feeding occurrences, with two BC_2 clones (Etb 6-21-3 and Etb 6-21-5), and the potato cultivar, GemStar Russet, also having a reduced number of feeding occurrences. GemStar Russet was included in this evaluation based on previous observations of possible psyllid resistance.

Average resting duration of psyllids also was significantly reduced relative to Atlantic for four breeding clones that represented BC generations 1 (P2-3 and P2-4), 3 (A00ETB12-2), and 4 (A05379-69). Average duration off the potato leaflet was significantly increased relative to Atlantic only for parental clone, 463-4, possibly indicating a non-preference by psyllids relative to other germplasm entries.

The number and duration of the other observed psyllid behaviors did not differ significantly from Atlantic for the other eleven entries.

Liberibacter Transmission: The percentage of Liberibacter-infected plants was significantly reduced for entries P2-4, Etb 6-21-3, and A00ETB12-3 relative to Atlantic. Infection for these three clones was

30%, whereas the infection rate of Atlantic was 80%−−indicative of possible reduced transmission/ resistance to Liberibacter. Of interest, was the observation clone A00ETB12-3, with no apparent psyllid resistance, was among the three clones identified as having the lowest Liberibacter infection. This observation suggests possible resistance to the Liberibacter, and not just the psyllid vector, may be contributing to reduced Liberibacter infection. A00ETB12-2, a sibling of A00ETB12-3, had the highest infection rate of all entries at 100% infection, suggesting possible segregation for Liberibacter resistance among the two siblings.

In summary, modified potato psyllid feeding behavior suggesting resistance has been identified in unique germplasm derived from *S. etuberosum* and *S. berthaultii*. A statistically significant reduction in Liberibacter- infected plants of this germplasm relative to Atlantic was also found following Liberibacter transmission evaluations. This finding suggests possible resistance to the pathogen responsible for ZC, but further evaluations are necessary to confirm this observation.

Breeding Clones 00-3115-2 and 00-3115-11

2010: In this year, 102 entries were evaluated for ZC symptoms in tubers following challenge with Liberibacter-infective potato psyllids in field cages. Seventy-three entries and the Atlantic control plants exhibited severe ZC symptoms in cut tubers. 00-3115-2 was among twenty-nine entries with no to light ZC symptoms in cut tubers; 00-3115-11 was classified as having moderate ZC symptoms in cut tubers. Tubers of both breeding clones were subsequently processed into chips and strong ZC symptoms were observed. However, tuber size was small and immaturity may have been a confounding factor in the rating of ZC symptoms. In the final analyses, only 6 of the 29 entries with no to light ZC symptoms in cut tubers displayed little to no ZC symptoms in their chips.

2011: Thirty-four selections, including putatively resistant/tolerant entries from the 2010 screening were evaluated in field cage studies. Nine of the 34 selections displayed no or little ZC symptoms in cut tubers−−Breeding clones 00-3115-2 and 00-3115-11 were among this group displaying resistant/tolerance to ZC. Smaller tuber size among the nine entries precluded their further evaluation for ZC symptoms following processing.

2012: 00-3115-2 and 00-3115-11 were among five breeding clones previously identified in the 2010 and 2011 field cage evaluations as having putative resistance/tolerance to ZC. In 2012, they were again evaluated in four replicate field cage studies. 00-3115-2 and 00-3115-11 again showed no or little ZC symptoms in cut tubers. Following frying, chips of 00-3115-2 from plants that had been challenged with Liberibacter-infective psyllids did show mild symptoms of ZC relative to control, non-challenged plants. No ZC symptoms were observed in psyllid-challenged plants of 00-3115-11, with chip quality comparable to that of non-challenged control plants.

In summary, breeding clones 00-3115-2 and 00-3115-11 have shown putative resistance/tolerance to ZC following three years of field cage evaluations. It is not know if response to ZC is an example of actual resistance to Liberibacter, or tolerance to its presence with no associated ZC symptoms in tubers. Further research in distinguishing between resistance or tolerance to ZC is warranted.

References

Alvarez, J.M., Novy, R.G., and J.L. Whitworth. Wireworm (Coleoptera: Elateridae) resistance in unique potato germplasm derived from the wild potato species, *Solanum berthaultii* and *S. etuberosum. J Econom Entomol.* (Accepted with revisions)

Butler, C.D., Gonzalez, B., Manjunath, K.L., Lee, R.F., Novy, R.G., Miller, J.C., and Trumble, J.T. 2011. Behavioral responses of adult potato psyllid, *Bactericera cockerelli* (Hemiptera:

Triozideae), to potato germplasm and transmission of Candidatus *Liberibacter psyllaurous*. *Crop Protect* 30:1233-1238.

Jansky, S.H., Hamernik, A., and Bethke, P.C. 2011. Germplasm Release: Tetraploid Clones with Resistance to Cold-Induced Sweetening. *Am J Potato Res* 88:218-225.

Liu, D. and Trumble, J.T. 2004. Tomato psyllid behavioral responses to tomato plant lines and interactions of plant lines with insecticides. *J Econ Entomol* 97(3): 1078-1085.

Munyaneza, J.E., Buchman, J.L., Sengoda, V.G., Fisher, T.W., Bester, G., Hoopes, R., Miller, C., Novy, R., Van Hest, P. and Nordgaard, J. 2010. Potato variety screening trial for zebra chip resistance under controlled field cage conditions. Proceeding of the 10th Annual Zebra Chip Reporting Session. Workneh, F. and Rush, C.M. (eds). 200-203.

Munyaneza, J.E., Buchman, J.L., Sengoda, V.G., Fisher, T.W. and Pearson C.C. 2011a. Susceptibility of selected potato varieties to zebra chip potato disease. *Am J Potato Res* 88: 435-440.

Munyaneza, J.E., Buchman, J.L., Heilman, B.E., Sengoda, V.G., Bester, G., Hoopes, R., Miller, C., Novy, R**.** and Van Hest, P. 2011b. Update on potato variety screening trial for zebra chip resistance under controlled field cage conditions. Proceeding of the 11th Annual SCRI Zebra Chip Reporting Session. Workneh, F., Rashed, A. and Rush, C.M. (eds). 141-143. 2011.

Novy, R.G., Nasruddin, A., Ragsdale, D.W., and. Radcliffe, E.B. 2002. Genetic resistances to potato leafroll virus, potato virus Y, and green peach aphid in progeny of *Solanum etuberosum*. *Am J Potato Res* 79:9-18.

Update on the Potato Variety Screening For Zebra Chip Resistance Trial

[1]Munyaneza, J.E., [2]Buchman, J.L., [2]Bester, G., [3]Novy, R., [4]Miller, C., [5]Van Hest, P., [6]Nordgaard, J., [7]Yilma, S., and [8]Wallis, C.

[1]USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951, USA
[2]Frito-Lay Inc., Rhinelander, WI 54501, USA
[3]USDA-ARS, Aberdeen, ID 83210, USA
[4]Texas AgriLife Research, College Station, TX 77840, USA
[5]Bejo Seeds Inc., Oceano, CA 93445, USA
[6]Black Gold Farms, Grand Forks, ND 58201, USA
[7]Oregon State University, Corvallis, OR 97331, USA
[8]USDA-ARS, Parlier, CA 93648, USA

Abstract
Zebra chip (ZC), a new and economically important disease of potato has caused millions of dollars in losses to the potato industry in the Americas and New Zealand. The disease is associated with the bacterium "*Candidatus* liberibacter solanacearum" (Lso) that is transmitted to potato by the potato psyllid, *Bactericera cockerelli*. In U.S., the disease had been limited to southwestern and central states but spread to the Pacific Northwest in 2011. Currently, the only means to manage ZC is by controlling its psyllid vector with insecticide applications. Identification of ZC-resistant or tolerant varieties may offer the most efficient and useful way to manage this disease. Five promising advanced breeding lines identified in two previous screening trials were further evaluated for ZC resistance under controlled field cage conditions by exposing the plants to Lso-infective psyllids in 2012 at the USDA-ARS facility in Wapato, WA. Although plants from all of the tested plant material developed ZC foliar symptoms, none of the five evaluated lines were observed to have freshly-cut ZC symptoms, while Atlantic tubers from the same study were highly symptomatic. Upon frying, Atlantic and the line BS2 had moderate to severe ZC, whereas the lines 00-3115-2 and ZC73 had minor ZC symptoms, and 00-3115-11 and ZC74 exhibited no disease symptoms at all. In addition, PCR analyses showed that inoculated Atlantic had much greater Lso titers than the other varieties. The results suggest that these potato lines may be resistant to or tolerant of ZC and warrant further evaluation.

Introduction
Development and identification of potato varieties and/or advanced breeding lines with resistance to or tolerance of ZC are crucial to development of effective and sustainable management strategies for this emerging and economically important disease of potato. Unfortunately, it has been shown that most, if not all, of commercial potato varieties currently available in the U.S. are susceptible to ZC, underscoring the urgent need to identify and/or develop potatoes that are resistant to this important disease (Munyaneza et al. 2011). In 2010 and 2011, over 105 advanced potato breeding lines were screened for ZC by exposing the plants to Lso-infective psyllids under controlled field cage conditions at the USDA-ARS Research Farm at Moxee in WA. Nine lines developed no to light ZC symptoms in fresh or fried tubers, suggesting that some of these potato lines may be resistant or tolerant to ZC.

The objective of the present study was to further screen the promising lines identified during the 2010 and 2011 variety screening trials in a non-choice test setting and a replicated experiment. In addition, yield, sugars and other processing quality parameters were assessed. Moreover, the plant

metabolite (phenolics, amino acids, etc.) testing of ZC-infected and healthy tubers for the promising selections was conducted as a means to identify the nature of the symptom expression and possible ways to incorporate the information into breeding programs. Furthermore, 54 new advanced potato breeding lines were screened in a separate un-replicated trial (dirty screening) under field cages conditions to quickly assess susceptibility of these selections to ZC.

Materials and Methods

Similar to the 2010 and 2011 trials, potato plants were exposed to laboratory-reared Lso-infective potato psyllids and maintained under field cage conditions at the USDA-ARS Research Farm at Moxee in WA. Five advanced potato breeding lines (BS2, 00-3115-2, 00-3115-11, ZC73, and ZC74) identified during the 2011 screening trial were evaluated. Atlantic is well documented to be very susceptible to ZC and was included in the trail as a control. The screening was initiated early summer (April-May) of 2012. Potatoes were planted in small field cages (6 ft wide and 15 ft long each), each cage accommodating eight plants as described by Munyaneza et al. (2008) and Buchman et al. (2011a,b). There were six cages per breeding line. Plants in the cages were exposed to Lso-infective psyllids at bloom stage in four of the six cages for each selection as described by Buchman et al. (2011a,b). The remaining two cages did not receive psyllids and served as controls. Three psyllids per plant were released in each cage and the insects were allowed to feed for seven days before being killed with insecticide applications. After the insect removal, plants were maintained and observed for ZC symptoms. At the end of the experiment, tubers were harvested and checked for ZC symptoms to estimate disease incidence and severity. Also, plant and tuber tissues were collected and tested for Lso by PCR to confirm infection and quantify the pathogen titer. In addition, yield and reducing sugars (glucose, sucrose) were measured. Moreover, the plant metabolite (phenolics, amino acids, etc.) testing of ZC-infected and healthy tubers for each selection were conducted in Dr Wallis Lab (ARS-Parlier) as a means to identify the nature of the symptom expression and possible ways to incorporate the information into breeding programs. Furthermore, 54 new advanced potato breeding lines were screened in a separate un-replicated trial (dirty screening) under field cages conditions to quickly assess susceptibility of these selections to ZC.

Results and Discussion

Zebra chip foliar symptoms were observed on all the plants exposed to psyllids, including Atlantic positive control plants. Following harvest, no significant yield was observed between ZC-infected and control plants for the promising lines. None of the promising lines were observed to have freshly-cut ZC symptoms, but Atlantic tubers from the same study were highly symptomatic. Upon frying, Atlantic and BS2 had moderate to severe ZC, 00-3115-2 and ZC73 had minor ZC symptoms, and 00-3115-11 and ZC74 exhibited no disease symptoms at all. PCR analyses showed that inoculated Atlantic had much greater Lso titers than the other varieties. Tuber biochemistry was analyzed between plants exposed to Lso and control plants for each of the five lines. Phenolic levels were greater in Lso-infected tubers than comparative controls regardless of cultivar; however, infected Atlantic tubers had much greater phenolic content than any of the other cultivars evaluated, which directly led to observable fresh-cut symptoms. Sugar analyses revealed that levels of glucose increased in Lso-infected Atlantic and BS2 tubers, but no such increases were observed in the other three lines. Since increased levels of reducing sugars could result in increased brown-colored acrylamide formation upon frying, a lack of increased levels of glucose in Lso-infected tubers from cultivars without ZC symptoms suggests a mechanism for disease resistance. Amino acid analyses are ongoing to further examine the mechanisms of tolerance to Lso infections in the promising lines. Moreover, these five lines are currently being evaluated for potato psyllid feeding using EPG technique to assess potential source of insect resistance. Furthermore, 15 of

the 54 new advanced potato breeding lines that were screened in a separate un-replicated trial showed light to no ZC symptoms in fresh tubers, suggesting some tolerance or resistance to ZC and warrant further in-depth investigation in 2013.

Acknowledgements
We are grateful to Blaine Heilman, Francisco de la Rosa, Millie Heidt, Jacob Dixon, Jerry Gefre, and Venkat Sengoda for their invaluable technical assistance. Financial support for this work was partially provided by USDA-CSREES-SCRI (Project #2009-51181-20176).

Referencse
Buchman, J.L., Heilman, B.E. and Munyaneza, J.E. 2011a. Effects of *Bactericera cokerelli* (Hemipetra: Triozidae) density on zebra chip potato disease incidence, potato yield, and tuber processing quality. J. Econ. Entomol. 104: 1783-1792.

Buchman, J.L., V.G. Sengoda, V.G., and and Munyaneza, J.E. 2011b. Vector transmission efficiency of liberibacter by *Bactericera cokerelli* (Hemiptera: Triozidae) in zebra chip potato disease: effects of psyllid life stage and inoculation access period. J. Econ. Entomol. 104:1486-1495.

Munyaneza, J.E., Buchman, J.L., Upton, J.E., Goolsby, J.A., Crosslin, J.M., Bester, G., Miles, G.P., and Sengoda, V.G. 2008. Impact of different potato psyllid populations on zebra chip disease incidence, severity, and potato yield. Subtr. Plant Sci. 60:27-37.

Munyaneza, J.E., Buchman, J.L., Sengoda, V.G., Fisher, T.W., and Pearson, C.C. 2011. Susceptibility of selected potato varieties to zebra chip potato disease. Am. J. Pot. Res. 88:435-440.

Investigations on Putative ZC Tolerant Potato Selections

J. Lévy [1], D.C. Scheuring [1], J.W. Koym [2], D.C. Henne[3], C. Tamborindeguy [4], E. Pierson[1] and J.C.Miller, Jr. [1]

[1] Department of Horticultural Sciences, TAMU; [2] Texas A&M AgriLife Research, Lubbock TX, [3] Texas A&M AgriLife Research, Weslaco TX, [4] Department of Entomology, TAMU

Abstract

As part of the Texas A&M Potato Breeding and Variety Development Program, significant effort has gone into screening potato varieties/selections for tolerance/resistance to Zebra Chip disease (ZC). Evaluation of top performing material requires testing over multiple years and locations. Variety development involves evaluation of progeny of potentially ZC tolerant parents, and typically takes an additional year(s) from field evaluation of parent to field deployment of progeny. Furthermore, evaluation of material often requires observations in field trials and caged field trials since the insect pressure can vary dramatically from year to year. Here, we are reporting selected data for 2010, 2011 and 2012.

Introduction

Since its appearance in 1994 in Mexico, zebra chip disease has been spreading, first to Texas in 2000, and then to the western US in 2011. The use of expensive pesticides in recent years has certainly helped with control of the insect population; however, the long-term sustainable solution is the identification of potato cultivars resistant/tolerant to psyllids and/or cultivars resistant to the bacteria "*Candidatus* Liberibacter solanacearum" (Lso).

Our goal as potato breeders and molecular geneticists is to identify cultivars that are less susceptible to ZC. That is, cultivars able to grow and develop normally in the presence of psyllids and the pathogen Lso. As part of the Texas Potato Breeding and Variety Development Program, we have screened over 600 cultivars/selections representing all market classes, including red, white, chip, fry and russet.

It is critical to understand that resistant potato cultivars offer the optimum long-term sustainable solution to disease and insect problems. In addition, the identification of resistant/tolerant cultivars provides extremely valuable tools for pathologists, entomologists, physiologists, and others investigating disease – vector - host interactions. Therefore, resistant/tolerant cultivars enable the scientific community to understand the developmental mechanism of a new disease. Understanding of these mechanisms enables breeders or chemical companies to specifically target their efforts to counteract ZC.

The objectives of this work were: 1) to confirm and further characterize putative ZC tolerant cultivars, develop field protocols and facilitate the identification of tolerant cultivars/selections ("Fast track breeding" Miller et al., these proceedings), and 2) to further understand the mechanisms of resistance / tolerance to ZC.

After tolerant/resistant cultivars are identified, further breeding will likely be required, as cultivars identified must also meet industry standards for chipping and fresh market characteristics such as shape, color, yield, etc.

Environmental effect on ZC disease

Cultivar improvement for this disease is very complex, due to involvement of two major factors - an insect vector and a bacterium. Moreover, the environment has an effect on the three partners: plant, insect, and bacterium. Very little is known about how the environment affects these relationships. Obviously, temperature and humidity are major factors, but we also need to consider the effect of plant age at the time the insect is present, plant health, and the age and growth stage of the insect population.

Materials and Methods

Material potentially tolerant to ZC was grown and evaluated during two seasons and two locations. The advantage of this methodology is that these locations have different environments which is important in properly evaluating selections/cultivars.

The plant material was grown from January to May at the Texas A&M AgriLife Research and Extension Center at Weslaco and the Jack Wallace farm near Edinburg. This is the first location that ZC was detected in the US. Moreover, natural populations of psyllids are present year round in variable numbers. From May to August, the trials were also conducted near Springlake on the Bruce Barrett farm. The major differences between the locations are elevation and temperature.

Plant material

The plant material used this study were identified in previous field evaluations (Miller et al., proceedings 2011), and were selected based on results from the 2010 and 2011 confirmation cage studies. They were: NY138, ATX85404-8W, ATX99194-3Ru, BTX1544-1Y, BTX1749-1W/Y, NDTX050070-1R, NDTX059828-2W, TX03196-1W, TX05249-11W, TX05249-3W, TX1673-1W, TX1674-1W/Y, and Atlantic as a control.

Experimental environment

The tests were conducted in both Weslaco and Springlake. In Weslaco, a trial was conducted in the field where plants were exposed to the natural population of psyllids (Fig 1). In both locations, cage trials were also conducted (Fig2) which allowed for control of insect numbers and infection level.

Tuber and plant traits measured

Traits measured included:

1- Number of tubers per plant
2- Tuber weight per plant
3- Chip (one slice from each tuber)
4- Evaluation of fresh cut (discoloration)
5- Frying (College Station, CSS Farms in Dalhart and the Jack Wallace Farm near Edinburg).
6- Score for ZC (0 to 5 scale: 0 no symptoms – 5 heavy striping)
7- Sample either all tubers from a single plant or a single tuber for both DNA followed by qPCR analysis (Levy et al. 2011) and phenolics analysis (Folin test)

Results and Discussion

Observational analysis

The survival rate of plants in the field was different from that in cages. Plants in cages were more affected by early death, probably due to psyllid infestation or Lso infection. Atlantic was usually the

most susceptible to ZC, with at least one tuber exhibiting ZC symptoms. Yield data showed an effect of psyllids or ZC on tuber production - lower yields were observed when plants were ZC infected as compared to uninfected, and this was especially noticeable in Atlantic.

There are different measures that can quantify the effect of ZC, but in this report the focus was on the incidence of ZC. We report the percentage of plants with ZC symptoms based on the produced tubers. If a plant produced at least one tuber with ZC symptoms, it was considered ZC positive. Each trial was analyzed independently (Table 1).

All cultivars and selections included in our studies thus far and using our strategy of comparing both caged and uncaged field studies, show ZC symptoms. For example, in the Weslaco 2012 cage study we observed a high percentage of ZC incidence in all the selections. In conclusion, none of our selections as of November 2012 was resistant to ZC. Nevertheless, some varieties performed extremely well in a given year, perhaps due to the environment in that year. For example NDTX059828-2W ranked first for ZC incidence in the cage study in Weslaco 2011 (Table 1, 11% of plants were ZC positive) and performed well in the 2012 Weslaco field study. Similarly, BTX1544-1Y and TX03196-1W presented only a limited number of plants infected with ZC in those experiments (Table 1).

The qPCR tests conducted on this material showed that several of the plants not exhibiting tubers with ZC symptoms were positive by qPCR, indicating the presence of the bacteria in the tuber samples. This means that Lso was able to move and replicate in these plants. Although we cannot be sure of the time these plants were infected, presumably those in the cage were infected seven weeks before harvest, which should have been enough time for development of symptoms in the tubers.

We also measured other parameters such as Lso levels and total phenolics in the same tuber samples (Levy et al., publication in preparation).

Our results show that:

- As expected, a correlation between the percentage of tubers with ZC and Lso titer was found in all trials.
- Similarly, a correlation between the percentage of tubers with ZC and total phenolics in the tuber was found: the higher the percent ZC in tubers from a single plant, the higher the quantity of phenols in those tubers.
- There were differences in Lso quantification by qPCR among these selections, which indicates they were infected with Lso. Symptom development was not correlated with the quantity of Lso.
- No single selection produced the level of tolerance across all years and all sites that would make it commercially viable. Albeit, some selections produced remarkable results under certain conditions.

We are interested in understanding the interaction between environment and those selections that produced such noteworthy results.

In conclusion, it is not surprising to find variable data in term of ZC incidence knowing it is expected that potato cultivars perform differently in different locations, in different times of the year, in different years and possibly dealing with different psyllid populations and/or different quantities of Lso. It cannot be excluded that differences in seed quality, including viruses, might have an impact on the susceptibility of the plants to insects and diseases.

In the future we will continue to work with the most promising chip selections from all US public breeding programs. These will be evaluated for yield and quality characteristics including ZC.

- The outstanding entries based on these trials will be subjected to controlled caged confirmation screening for ZC tolerance/resistance.
- Crosses between the most promising selections have been initiated to stack genes from our material and that of other programs.
- Laboratory characterization is ongoing.

An important outcome of this work is the integration of our laboratory research toward applications for field production. Material and data collected from the field experimentation will guide our future laboratory studies. In summary, the following points are highlighted:
Field study: enables us to better understand or gain experience with screening for resistant cultivars/selections, identification of tolerant cultivars/selections, real-world constraints (weather, insect population, etc.), contact with industry and producers, adapt our molecular tools for use in the field.
Laboratory studies: Better characterization of some tolerant cultivars and translocation study (Levy et al. 2011), adapt growth control conditions (light and temperature), develop more molecular tools compatible with field diagnostics (Levy et al., 2011; Ravindran et al., 2011; Ravindran et al., 2012), qPCR, LAMP, and FAST DNA protocol.

Acknowledgements
Financial support for this work was partially provided, by the Texas Department of Agriculture/Texas A&M AgriLife, USDA-CSREES-SCRI (Project#2009-51181-20176), and USDA/NIFA Special Research Grants Program - Potato Research (Agreement #2009-34141-20129). In kind support was generously provided by Bruce Barrett, Springlake Potato Sales, Milt Carter, CSS Farms, and Jack Wallace, Jack Wallace Farms.

Figure 1. Diagram of randomized field planting

Row1	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl
Row 2	1	Atl	3	4	5	Atl	7	8	9	6	11	12	13	Atl	2		Atl	Atl
Row 3	13	7	Atl	5	3	10	9	Atl	1	4	6	Atl	6	8	12		Atl	Atl
Row 4	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl	Atl

Figure 2. Example of field cage with design: 16 plants randomly distributed in each cage in units of four, including the check cultivar, Atlantic

2	5	3	AT	2	4	6	AT
4	AT	1	6	1	AT	3	5

Table 1. % ZC incidence in seven selections and the check cultivar Atlantic in Weslaco, 2011 and 2012

	Weslaco Cage 2011	**Weslaco Cage 2012**	**Weslaco Field 2012**
Atlantic	86%	100%	50%
BTX1544-1Y	nd	**25%**	**14%**
BTX1749-1W/Y	100%	83%	75%
NDTX059828-2W	**11%**	100%	**25%**
NY138	71%	50%	40%
TX03196-1W	100%	**40%**	**0%**
TX05249-11W	70%	100%	25%
TX05249-3W	nd	100%	29%

References

Miller, J.C., Jr., Koym, J.W, Scheuring, D.C., and Miller, J.P. 2011 Southwestern Regional Potato Variety Trial Report 2010. Texas AgriLife Research, College Station and Lubbock. 22p.

Miller, J.C., Jr., Scheuring, D.C., Koym, J., Henne, D., Jiffon, J, Levy, J.C., Tamborindeguy, C. and E.A. Pierson. 2011. Further studies on host-plant tolerance to ZC. pp134-136. In: A. Workneh, A. Rashed, and C.M. Rush (eds.), Proceedings of the 11th Annual SCRI Zebra Chip Reporting Session, San Antonio, TX, November 6-9, 2011.

Levy, J., Ravindran, A., Gross, D., Tamborindeguy, C. and Pierson, E. 2011. Translocation of "*Candidatus* Liberibacter solanacearum", the Zebra Chip pathogen, in potato and tomato. Phytopathology (In press).

Ravindran, A., Levy, J., Pierson, E. and Gross, D.C. 2011. Development of primers for pmproved PCR detection of the potato Zebra Chip pathogen, '*Candidatus* Liberibacter solanacearum'. Plant Dis. 95, 1542-1546.

Ravindran, A., Levy, J., Pierson, E. and Gross, D.C. 2012. Development of a loop-mediated isothermal amplification procedure as a sensitive and rapid method for detection of '*Candidatus* Liberibacter solanacearum' in potatoes and psyllids. Phytopathology, 102: 899-907.

Investigations of Potato Psyllid Repellents

Prager, S.M., Kund, G., and Trumble, J.T., Department of Entomology, University of California, Riverside, Riverside, CA

Abstract
Potato psyllid management is highly dependent on the application of "traditional" insecticides. Materials that are repellent to psyllids have potential for supplementing of insecticides, and limiting feeding resulting in reduced pathogen transmission. We examined two products, a nutrient supplement and a sprayable wax matrix for potential repellent properties. The nutrient solution did not influence infection with *Candidatus* Liberibacter solanacearum, but did elicit some behavioral responses. The wax matrix was not repellent, but did show ovicidal properties.

Introduction
The tomato-potato psyllid *Bactericera cockerelli* vectors *Candidatus* Liberibacter psyllaurous (solanacearum) (CLP), the causal agent of zebra chip (ZC) disease. Currently, management of *B. cockerelli* is based around multiple insecticide applications. Additionally, transmission of CLP has been shown to occur with only a few hours of feeding. These factors, combined with developing resistance to many commonly applied insecticides, has led to an increased need for alternative management strategies. These strategies can include products that are repellent or otherwise alter suitability of plants to feeding, oviposition, or settling by the psyllid. Similarly, products that reduce bacterial growth might also be helpful in preventing ZC symptoms despite feeding and consequent bacterial transmission.

We investigated two materials of potential value to potato psyllid management. The first was a foliar nutrient supplement (Tech-Flo®, Nutricop-20) that is commonly used to increase copper levels in deficient soils. This material was investigated for three purposes. First, we tested the hypothesis that increased levels of *in situ* copper would lead to decreased rates of CLP infection. Copper has been shown to have some antibacterial properties, and the manufacturers of the product had unpublished studies suggesting efficacy in other crops. Second, Nansen (unpublished) has observed that growing potato plants with supplemental phosphorous fertilizer resulted in altered *B. cockerellli* host choice. We tested the hypothesis that a similar effect would be observed with the addition of copper. Third, *B. cockerelli* may respond to visual or tactile stimuli and the copper solution appears to alter both when applied to leaves.

In the second project, we examined a wax matrix (SPLAT, ISCA Technologies) that can be impregnated with essential oils. Choice tests conducted in Y-tube olfactometers have previously demonstrated that *B. cockerelli* is repelled by the scents of some essential oils (Diaz-Montano and Trumble, 2012). In particular, peppermint and clove oils were found to be significantly repellent even at the lowest dose of 1 µl. Additionally, the wax matrix is quite tacky and may function as a tactile repellent or ovicide. We tested the wax matrix both for repellency when impregnated with peppermint oil and also for potential ovicidal properties.

Materials and Methods
Nutricop-20
All bioassays were conducted on tomato plants grown from seed in the greenhouse. Beginning at two weeks, plants were treated with either a 5% or 10% dilution of the Nutricop-20 solution, or an

equivalent volume of water for the control. Solutions were applied using a hand-sprayer and applied till run-off. Treatments were repeated biweekly for 6 weeks.

Hypotheses about Nutricop-20's effect on infection, and to a lesser extent on behavior, are dependent on the Nutricop-20 resulting in increased copper levels in tissue. Therefore, the amount of copper in whole plants treated with Nutricop-20 was examined. Plants were treated as above, and at six weeks were cleaned to remove any residual product from leaf surface. Once cleaned, levels of copper concentration were determined using ICP-MS.

To determine the effect of Nutricop-20 solution on CLP infection, plants were experimentally infected. Infection was achieved by caging three male-female pairs of psyllids from a known infected colony onto plants using organza "sack cages" for three days. Following the three days, psyllids and cages were removed and the plants maintained in the greenhouse for an additional 7 days. Following the 7-day period, caged leaves were excised and CLP presence was tested via qPCR.

To test the hypothesis that treatment with Nutricop-20 will influence potato psyllid behavior, we conducted behavioral assays. Assays were conducted on greenhouse grown tomato plants treated as above, with the exception of being transferred to 10-inch pots at approximately four weeks. Individual, unsexed, adult psyllids were placed into glass arenas and observed for 15 minutes. During observation periods, behavioral responses were recorded using Noldus Observer® software. Behaviors recorded include: time off leaf, resting, jumping, probing, feeding, and walking.

SPLAT Sprayable Wax Matrix

We tested whether the wax-matrix impregnated with peppermint oil would be repellent to *B. cockerelli* oviposition using choice bioassays. In an assay, 50 unsexed, adult psyllids were placed into a cage containing one tomato plant treated with sprayable peppermint wax matrix and one plant treated with water. During assays, the location of psyllids was noted daily and eggs on each plant were counted after 5 days.

To test potential ovicidal properties of the wax matrix, we compared emergence of eggs sprayed with wax matrix and water. First, we caged three male-female pairs of pysillids onto untreated tomato plants for three days. This resulted in eggs on caged leaves, which were counted prior to treatment. Once egg numbers were determined, plants were hand sprayed with water or the wax matrix. Nymphs were counted daily to determine emergence, which was calculated as the maximum number of nymphs on any day divided by the number of eggs counted prior to treatment.

Results and Discussion

Nutricop-20

Treatment with Nutricop-20 solution resulted in greater concentrations of copper than untreated plants, and levels in the 5% solution were greater than those in the 10% solution (Fig 1). There was no significant difference in the number of CLP infected plants between treatments (5%: X^2=2.5, p=0.14; 10%: X^2=0.57, p=0.4410%) (Fig. 2). These trends suggest that while foliar treatment did increase the concentrations of copper within plants, this increased copper did not lead to changes in bacterial transmission or growth. It is possible that specific analysis of titer levels, rather than categorizing as positive/negative for CLP might reveal some differences. However, such differences are of little

practical relevance in managing the psyllids since ultimately the plants were infected and are likely to display symptoms.

Treatment with Nutricop-20 solution influenced potato psyllid behavior. Specifically, psyllids spent more time resting and walking on untreated leaves, and more time off-leaf on treated leaves (Fig 3). These results suggest some potential for this product as a repellent. Time spent off-leaf likely indicates that the psyllids found the treated leaves unsuitable and thus were attempting to leave in search of new leaves. Although, more time off-leaf could also imply tactile discomfort resulting in the psyllid settling onto portions of the arena instead of on the leaf. The increased walking and resting behaviors that involve contact with the leaf surface also support the latter interpretation. Unfortunately, as conducted these assays do not allow us to distinguish between effects from the topical presence of the copper solution on leaves and plant physiological responses from the change in the nutrient profile. Further experiments are in order to distinguish between these possibilities.

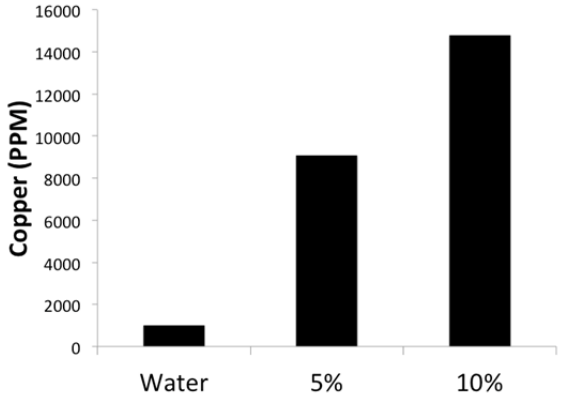


Fig 1. Mean levels of copper in tomato plants treated with 5% or 10% dilutions of Nutricop-20 solution and water controls.

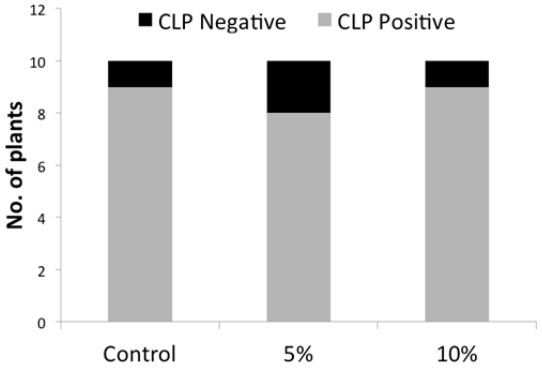


Fig 2. The number of CLP infected (grey) and uninfected (black) plants when treated with water, 5% or 10 % dilutions of Nutricop-20.

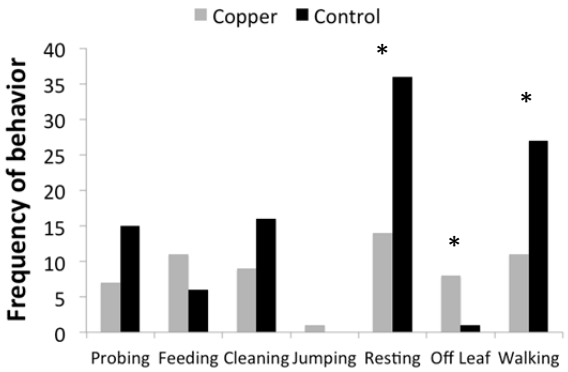


Fig 3. Frequency of behaviors on treated and untreated plants. Significant differences at $p<0.05$ indicated by asterisks.

SPLAT Sprayable Wax Matrix

There was no significant difference in the number of eggs laid on plants treated with the sprayable wax matrix and the water control ($T=0.5$, $p<0.62$, $df=18$) (Fig. 4), indicating no repellency with respect to oviposition. This is an interesting result, as it contradicts those results from olfactometry suggesting psyllids are repelled by peppermint scent. It remains to be determined if the psyllids spend less time on the treated plants. The possibility exists the psyllids spend less time on treated plants, but oviposit at equal rates. Similarly, psyllids may be repelled from feeding on plants, but not from ovipositing. Finally, we will need to examine other scents to determine if this is a result specific to peppermint oil, or one that is representative of a greater overall trend. Contrary to the repellency assays, we did observe ovicidal properties as significantly fewer eggs sprayed with SPLAT emerged than those sprayed only with water ($T=3.6$, $P<0.001$, $df=18$) (Fig. 5.). Consequently, further studies may be in order to determine the feasibility of its use as a foliar ovicidal product. However, this property is likely more beneficial in conjunction with application as a repellent versus use solely for reducing egg numbers.

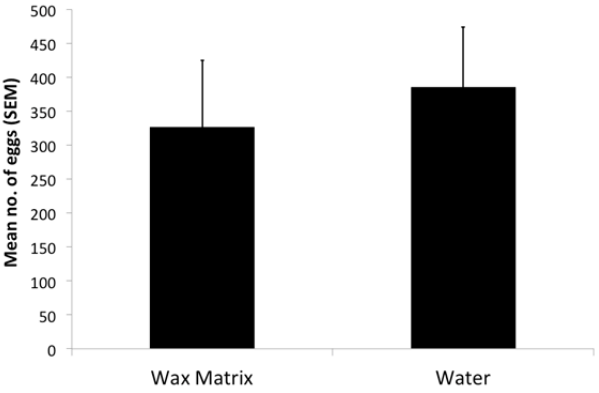


Fig 4. The number of eggs in whole plant choice bioassays of plants treated with either water or sprayable peppermint wax matrix.

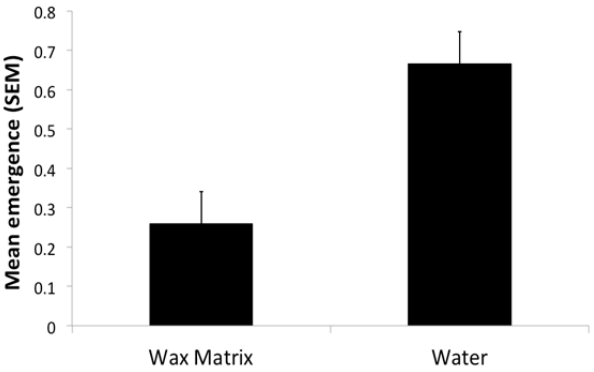


Fig 5. Mean proportion of eggs emerging following treatment with either water or SPLAT matrix.

Acknowledgements
We thank B. Carson, B. Vindiola, S. Gilbert, and N. Drew for assistance in conducting bioassays and K. Gilbert for maintenance of plants and psyllid colonies. ISCA Technologies provided SPLAT wax matrix. Tech-Flo® Nutricop-20 was provided by Nutrient Technologies. This research was supported by grants from SCRI (2009-34381-20036) and the Texas Department of Agriculture.

References
Diaz-Montano, J. and Trumble, J.T. 2012. Behavioral Responses of the Potato Psyllid (Hemiptera: Triozidae) to Volatiles from Dimethyl Disulfide and Plant Essential Oils. Journal Insect Behavior.

Potato Psyllid Insecticide Evaluation Trials in the Lower Rio Grande Valley of Texas

D.C. Henne. Texas A&M AgriLife Research, Weslaco, TX 78596, USA

Abstract
Management of zebra chip disease currently relies on sensible management of the potato psyllid, and frequent testing of insecticides is necessary to provide growers with updated information on product efficacy. Insecticide evaluation trials were performed in Weslaco, TX to assess current and prospective products for potato psyllid management. Several promising products (e.g. Sivanto, Torac) are, or will soon become, available to assist commercial potato growers with management decisions. It was also shown that potato psyllids are either weakly affected by certain products, or populations significantly increased in response to insecticide applications. More attention needs to be directed at identifying products that will repel and/or inhibit feeding by potato psyllids.

Introduction
A crucial part of managing zebra chip (ZC) disease is effective management of the insect vector of '*Candidatus* Liberibacter solanacearum', the pathogen responsible for causing potato plant mortality and tuber defects. Ongoing efforts are being made by researchers in a number of geographic regions of the United States to evaluate different pesticides for their efficacy to reduce not only potato psyllid (*Bactericera cockerelli*) populations, but incidence of ZC as well. Here, insecticide evaluation trials performed in the Lower Rio Grande Valley of south Texas during the winter of 2012 are summarized.

Materials and Methods
Potato plots (cv 'Atlantic') were established at the Texas A&M AgriLife experiment station at Weslaco, TX. Potatoes were planted 12" apart in plots 2 rows (30") x 25' during January 2012. At 3 weeks post emergence N32@150 lbs/acre was applied, and again @ bloom. Insecticide rates were applied according to manufacturer's instructions. A backpack sprayer was used for applying insecticides by placing a boom consisting of 3 nozzles/40" bed @ 75 psi at a height of 38 cm over the plants. At each evaluation date, a sample of 3 compound leaves was haphazardly selected from each of 10 plants at various locations within each plot. Counts of eggs and nymphs were made using a stereomicroscope. At harvest, a carrot digger was used for unearthing and mixing tubers in each plot. A minimum of 20 tubers/plot were randomly selected, bagged, weighed, and scored for ZC defects. All treatments were arranged according to a randomized complete block design with a minimum of 4 replicates/treatment. *Laboratory bioassays:* Contact and leaf dip bioassays were performed to determine adult psyllid mortality under conditions of exposing insects to treated leaves. Products that were tested included AgriMek (abamectin), Sevin (Carbaryl), Torac (Tolfenpyrad), Transform (Sulfoxaflor), Neem oil, Baythroid XL (Cyfluthrin), and Requiem (*Chenopodium* extract) and water (control). Insects were exposed to a previously treated potato leaf that was either wet or dry. Each treatment was replicated four times, with 10 adults/replicate. Counts of living, dead or moribund insects were made at 1, 4, 8 and 24 hours after treatment/exposure. *Statistical analyses:* As psyllid egg and nymph counts are highly overdispersed, the numerical values required a log x+1 transformation before ANOVA (or t-test) was performed. As there were no time x treatment effects, data were pooled over time (excluding pretreatment counts) prior to analysis. A Tukey HSD test was performed to identify treatment differences. Percent ZC and percent mortality data were arcsine-transformed prior to a nonparametric analysis (Kruskall-Wallis or Mann-Whitney). Data outliers were identified using Grubbs test and removed prior to analysis.

Results and discussion (refer to figures at end of report)

A) *Trial 1:* Treatments 30 ga/acre in 1 gal mix. 1= Requiem EC @ 1 qt /acre (32ml/gal), 2= Agri-Mek 0.15 EC @ 8oz/acre (8ml/gal), 3= Requiem EC @ 1 qt /acre (32ml/gal) alternated with Agri-Mek 0.15 EC @ 8oz/ac (8ml/gal), 4= Oberon 8oz /ac, 8ml/gal, 5= Radiant 1 SC 8 oz/ac, 8ml/gal, 6= Untreated control. Note: Spray applications done 2/21, 3/6 and 3/20. Efficacy evaluations done 2/28, 3/13, 3/27, and 4/3. Harvest and scoring done 5/7. No significant differences in numbers of eggs were found among treatments (P=0.97). However, the Requiem alternated with AgriMek treatment had significantly higher nymph counts than the control (P=0.04), suggesting that this combination of pesticides may be detrimental to potato psyllid natural enemies. Mean tuber weights and % ZC were not significantly different (P>0.05) among treatments.

B) *Trial 2a:* 1 Untreated control, 2 Sivanto@14 oz/acre, 3 Sivanto@10.5 oz/acre+10.5 ml Dyne-Amic (DA) 0.25%, 4 Sivanto @ 12 oz/acre+12 ml DA 0.25%, 5 Sivanto@14 oz/acre+14 ml DA 0.25%, 6 Assail 30SG@3 oz/acre+2.8 gr DA 0.25%, 7 Agrimek@10 oz/acre+10 ml DA 0.25%, 8 Radiant@6 oz/acre+6 ml DA 0.25%. Note: Spray applications done 2/9 and 3/5. Efficacy evaluations done 2/10, 2/13, 2/16, 2/23, 3/6, 3/9, 3/13, and 3/20. Harvest and scoring done 5/7. Only the Radiant treatment had significantly lower # eggs than the control (p=0.01). Nymph counts in the Sivanto @ 14oz/acre treatment only was significantly lower (p=0.04) than the control. Mean tuber weights and % ZC were not significantly different (P>0.05) among treatments.

C) *Trial 2b:* 1 Untreated control1, 2 Sivanto @ 7 oz/acre + 7 ml Dyne-Amic 0.25%, 3 Sivanto @ 8.7 oz/acre + 8.7 ml Dyne-Amic 0.25%, 4 Sivanto @ 10.5 oz/acre + 10.5 ml Dyne-Amic 0.25%, 5 Sivanto @ 12 oz/acre + 12 ml Dyne-Amic 0.25%, 6 Sivanto @ 14 oz/acre + 14 ml Dyne-Amic 0.25%, 7 Leverage @ 2.8 oz/acre + 2.8 ml Dyne-Amic 0.25%, 8 Radiant @ 6 oz/acre + 6 ml Dyne-Amic 0.25%, 9 Untreated control2, 10 Movento @ 5 oz/acre + 5 ml Dyne-Amic 0.25%, 11 Agri-Mek @ 8 oz/acre, Note: Spray applications were done 3/13. Follow up efficacy evaluations were done 3/14, 3/16, 3/20, 3/27. Harvest and scoring were done 5/7. The controls had significantly (P<0.0001) higher psyllid egg and nymph counts than all other treatments. Tuber yields in the Sivanto @ 10.5oz/acre treatment were significantly higher (P=0.01) than the Sivanto @12oz/acre and AgriMek treatments. Differences in percent ZC were not significantly different (P>0.05) among treatments.

D) *Trial 3:* 1 MBI-203 DF1@1 lb/acre+15 gr Kinetic 0.25%, 2 MBI-203 DF1@2 lb/acre+30 gr Kinetic 0.25%, 3 MBI-203 DF1@4 lb/acre+60 gr Kinetic 0.25%, 4 Untreated control1, 5 MBI-206@1 ga/acre+126 ml Kinetic 0.25%, 6 MBI-206@2 ga/acre+252 ml Kinetic 0.25%, 7 MBI-203 DF1@2 lb/acre+30 gr Kinetic 0.25%, then Movento@350 ml /ha+5.3 ml Kinetic 0.25%, then MBI-203 DF1@2 lb/acre+30 gr Kinetic 0.25%, 8 MBI-205@1qt/acre+32 ml Kinetic 0.25%,9 MBI-205@2 qt/acre+64 ML Kinetic 0.25%, 10 Untreated control2. Note: Spray applications done 3/9, 3/23 and 4/7. Follow up efficacy evaluations done 3/22, 4/6, and 4/19. Harvest and scoring done 5/7. The controls had significantly (P<0.0001) higher egg counts than all other treatments, except MBI203@1lb/acre. Nymph counts in the MBI203 @ 4lb/acre and MBI205@ 2 qt/acre were significantly lower (p=0.0002) than the controls. Mean tuber weights and % ZC were not significantly different (p>0.05) among treatments.

E) *Trial 4:* 1 Untreated control, 2 Onager @ 24 oz/acre, Note: Spray application was done 3/15. Follow up efficacy evaluations were done 3/22, 3/29, and 4/5. Harvest and scoring were done 5/7. The control had significantly higher egg and nymph counts than the Onager treatment (eggs p=0.02, nymphs p=0.01). Mean tuber weights and % ZC were not significantly different (p>0.05).

F) *Trial 5:* 1 Movento @ 5oz/acre+Dyne-Amic 0. 25%, 2 apps starting @ wk. 6 (2/16 and 2/23), then Agri-Mek @ 8oz/acre+Dyne-Amic 0.25%, 2 apps starting @ wk. 8 (3/1 and 3/8), then Oberon @ 5oz/acre+Dyne-Amic 0.25%, 2 apps starting @ wk. 10 (3/15 and 3/22). 2 Torac @ 24oz/acre+Dyne-Amic 0.25%, 1 app @ wk. 6 (2/16), then Movento @ 5oz/acre+Dyne-Amic 0. 25%, 2 apps starting @ wk. 7 (2/23 and 3/1), then Torac @ 24oz/acre+Dyne-Amic 0.25%, 1 app @ wk. 9 (3/8), then Oberon @ 5oz/acre+Dyne-Amic 0.25%, 2 apps starting @ wk. 10 (3/15 and 3/22). 3 Torac 24oz/acre+Dyne-Amic 0.25% 1 app at wk. 6 (2/16) then Movento @ 5oz/acre+Dyne-Amic 0. 25%, 2 apps starting @ wk. 7 (2/23 and 3/1), then Torac @ 24oz/acre+Dyne-Amic 0.25%, 1 app @ wk. 9 (3/8). 4 Torac @ 24oz/acre+Dyne-Amic 0.25%. Apply weekly, weeks 6-10 (2/16, 2/23, 3/1, 3/8, and 3/15). 5 Untreated control. Note: All treatments (except control) had Admire Pro at planting (@7 oz/acre). The egg counts were marginally nonsignificant (p=0.05). However, egg counts were lower than the control in treatments 2 and 4. Treatments 2-4 had significantly lower (p=0.006) nymphs and significantly higher (p=0.007) tuber weights than the control. No differences in %ZC were detected (P>0.05).

G) *Trial 6:* 1. Vydate C-LV 34 fl oz/ac 2 applications 14 days apart during flowering, 2. Sevin @ 33 fl oz/ac applied post-flowering, 3. Lannate@2pts/ac applied post-flowering, 4. Asana@5.8 fl oz/ac 14 days apart during flowering, 5. Baythroid@2.8 fl oz/ac applied post-flowering, 6. Untreated control. Sevin treatment had significantly higher (p=0.0009) egg counts than the vydate treatment and the untreated control. The Asana treatment had significantly higher (p=0.006) nymph counts than the untreated control. Mean tuber weights and %ZC were not significantly different (p>0.05) among treatments.

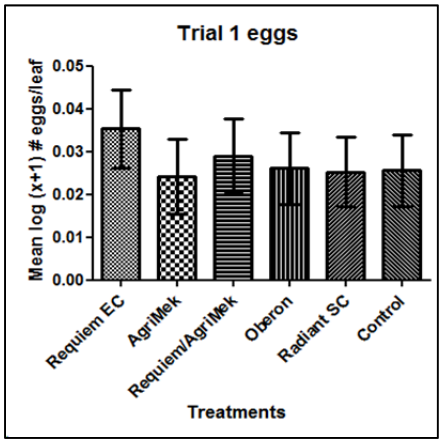
H) *Laboratory bioassays: a) Dry Contact*: Overall, Baythroid and Agrimek yielded the greatest mortality after 24 hours. As expected, Baythroid caused rapid knockdown and had significantly greater mortality than Sevin and the untreated control (p=0.003). *b) Wet Contact*: Baythroid, Agrimek and Torac yielded the greatest mortality after 24 hours. As expected, Baythroid caused rapid knockdown. Overall, only Torac caused significantly greater mortality than Sevin and Neem Oil (p=0.001).

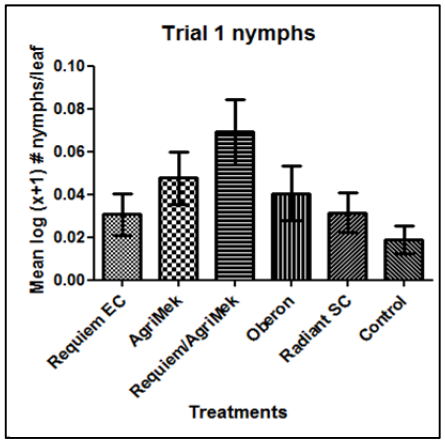
General Discussion

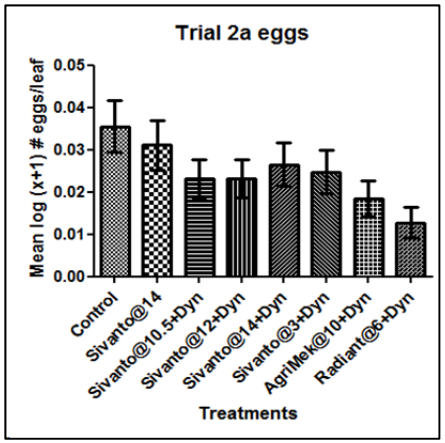
In general, potato psyllid populations throughout the trial locations were not uniform, and some field trials had inadequate psyllid pressure to enable satisfactory evaluation of treatment effects. Also, counts were only made for psyllid eggs (as a proxy for adult activity), and nymphs. The case is made here to perform more insecticide efficacy trials for potato psyllid adult control by testing under field cages and/or laboratory assays using leaf-dip, contact, and dose-response bioassays, on a known number of insects released into the cages. Furthermore, **there is a need for products that will not only repel adult potato psyllids, but also prevent feeding**. Many products now available will effectively kill and reduce psyllid populations, but their ability to repel psyllids and prevent transmission of Liberibacter is poorly understood. More focus should be directed at controlling the adults that are migrating into potato fields, as these are likely causing much of the early season ZC damage.

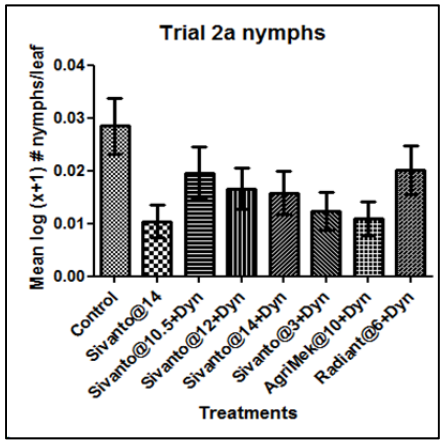
Acknowledgements

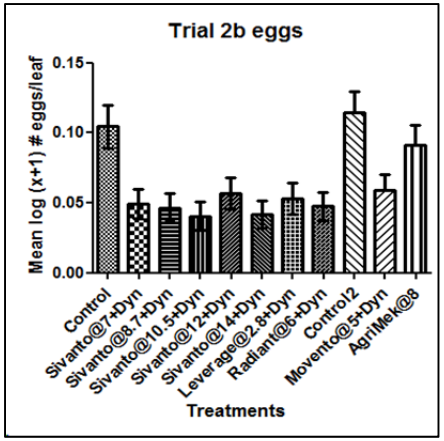
Thanks to J.A. Martinez, A. Pena, E. Huerta, and A. Yanez for assistance with performing these laboratory and field trials. This research was supported by grants from SCRI (2009-34381-20036) and the Texas Department of Agriculture. Additional funding was provided by AgraQuest, Bayer CropScience, Dow AgroSciences, Marrone Bio Innovations, Nichino America, and the Washington State Potato Commission. Thanks to J.W. Farms for providing seed material for planting.

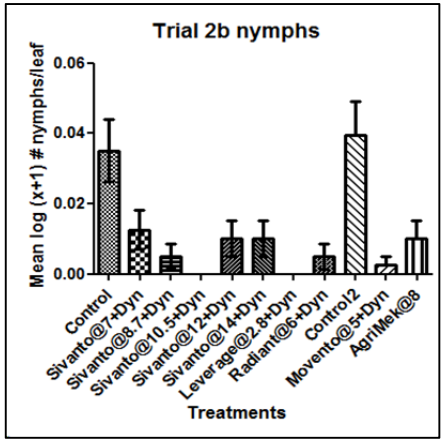

Trial 1 eggs
Mean log (x+1) # eggs/leaf
0.05
0.04
0.03
0.02
0.01
0.00
Requiem EC
AgriMek
Requiem/AgriMek
Oberon
Radiant SC
Control
Treatments

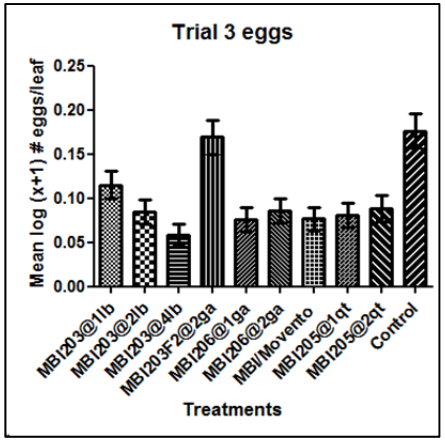

Trial 1 nymphs
Mean log (x+1) # nymphs/leaf
0.10
0.08
0.06
0.04
0.02
0.00
Requiem EC
AgriMek
Requiem/AgriMek
Oberon
Radiant SC
Control
Treatments

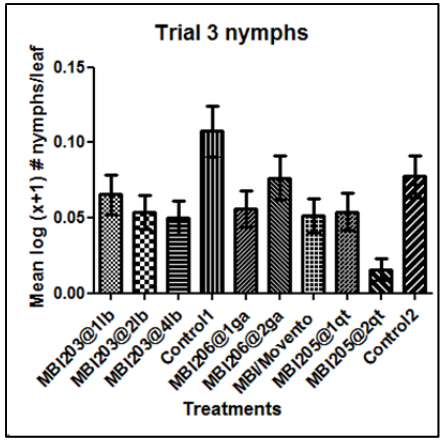

Trial 2a eggs
Mean log (x+1) # eggs/leaf
0.05
0.04
0.03
0.02
0.01
0.00
Control
Sivanto@14
Sivanto@10.5+Dyn
Sivanto@12+Dyn
Sivanto@14+Dyn
Sivanto@3+Dyn
AgriMek@10+Dyn
Radiant@6+Dyn
Treatments

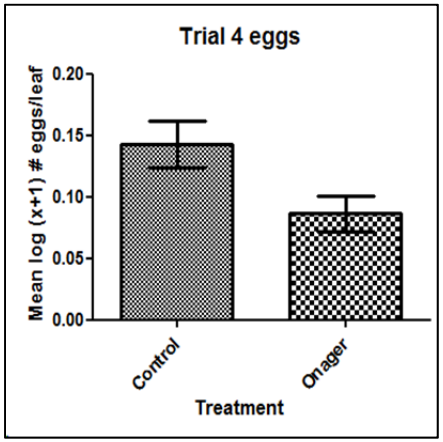

Trial 2a nymphs
Mean log (x+1) # nymphs/leaf
0.04
0.03
0.02
0.01
0.00
Control
Sivanto@14
Sivanto@10.5+Dyn
Sivanto@12+Dyn
Sivanto@14+Dyn
Sivanto@3+Dyn
AgriMek@10+Dyn
Radiant@6+Dyn
Treatments

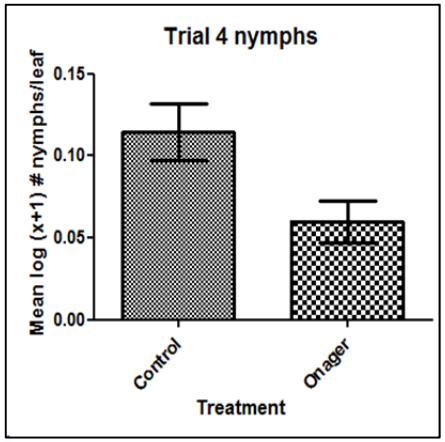

Trial 2b eggs
Mean log (x+1) # eggs/leaf
0.15
0.10
0.05
0.00
Control
Sivanto@7+Dyn
Sivanto@8.7+Dyn
Sivanto@10.5+Dyn
Sivanto@12+Dyn
Sivanto@14+Dyn
Leverage@2.8+Dyn
Radiant@6+Dyn
Control2
Movento@5+Dyn
AgriMek@8
Treatments

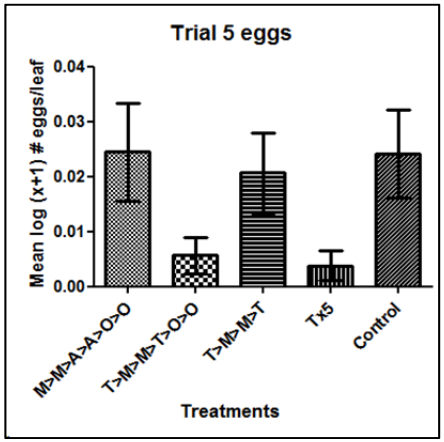

Trial 2b nymphs
Mean log (x+1) # nymphs/leaf
0.06
0.04
0.02
0.00
Control
Sivanto@7+Dyn
Sivanto@8.7+Dyn
Sivanto@10.5+Dyn
Sivanto@12+Dyn
Sivanto@14+Dyn
Leverage@2.8+Dyn
Radiant@6+Dyn
Control2
Movento@5+Dyn
AgriMek@8
Treatments

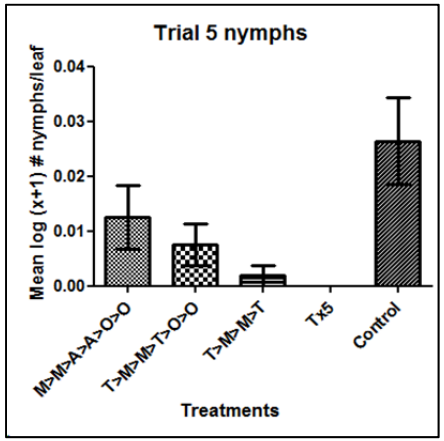

Trial 3 eggs
Mean log (x+1) # eggs/leaf
0.25
0.20
0.15
0.10
0.05
0.00
MB1203@1lb
MB1203@2lb
MB1203@4lb
MB1203F2@2ga
MB1206@1ga
MB1206@2ga
MBI/Movento
MB1205@1qt
MB1205@2qt
Control
Treatments

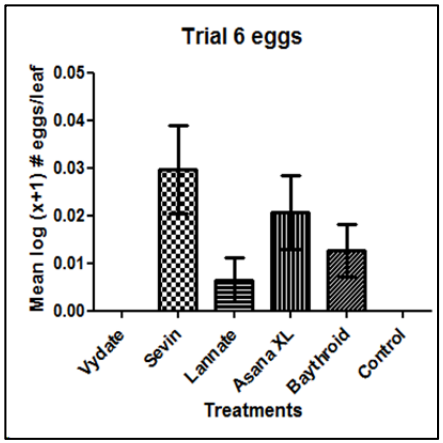

Trial 3 nymphs
Mean log (x+1) # nymphs/leaf
0.15
0.10
0.05
0.00
MB1203@1lb
MB1203@2lb
MB1203@4lb
Control1
MB1206@1ga
MB1206@2ga
MBI/Movento
MB1205@1qt
MB1205@2qt
Control2
Treatments

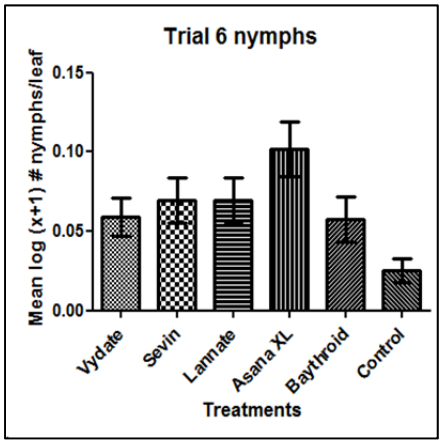

Trial 4 eggs
Mean log (x+1) # eggs/leaf
0.20
0.15
0.10
0.05
0.00
Control
Onager
Treatment

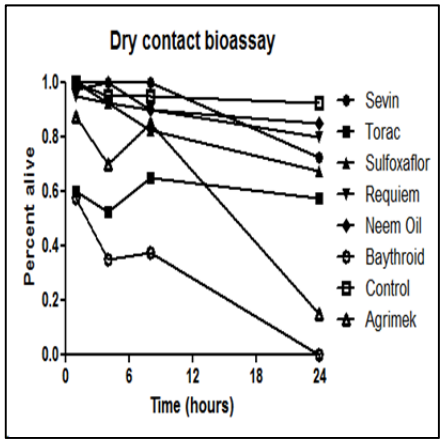

Trial 4 nymphs
Mean log (x+1) # nymphs/leaf
0.15
0.10
0.05
0.00
Control
Onager
Treatment

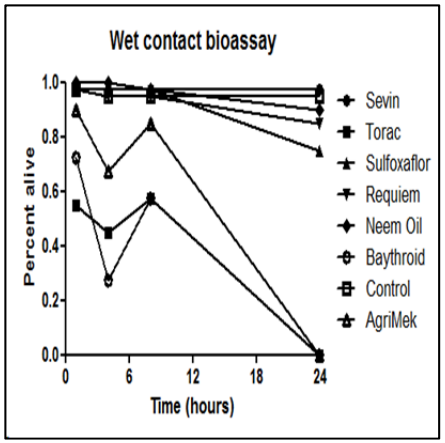

Trial 5 eggs
Mean log (x+1) # eggs/leaf
0.04
0.03
0.02
0.01
0.00
M>M>A>A>O>O
T>M>M>T>O>O
T>M>M>T
Tx5
Control
Treatments


Trial 5 nymphs
Mean log (x+1) # nymphs/leaf
0.04
0.03
0.02
0.01
0.00
M>M>A>A>O>O
T>M>M>T>O>O
T>M>M>T
Tx5
Control
Treatments


Trial 6 eggs
Mean log (x+1) # eggs/leaf
0.05
0.04
0.03
0.02
0.01
0.00
Vydate
Sevin
Lannate
Asana XL
Baythroid
Control
Treatments


Trial 6 nymphs
Mean log (x+1) # nymphs/leaf
0.15
0.10
0.05
0.00
Vydate
Sevin
Lannate
Asana XL
Baythroid
Control
Treatments


Dry contact bioassay
Percent alive
1.0
0.8
0.6
0.4
0.2
0.0
0
6
12
18
24
Time (hours)
Sevin
Torac
Sulfoxaflor
Requiem
Neem Oil
Baythroid
Control
Agrimek


Wet contact bioassay
Percent alive
1.0
0.8
0.6
0.4
0.2
0.0
0
6
12
18
24
Time (hours)
Sevin
Torac
Sulfoxaflor
Requiem
Neem Oil
Baythroid
Control
AgriMek

Evaluating the Efficacy of Insecticides and Insecticide Regimes against Potato Psyllid to Control Zebra Chip

M. Lewis[1], J. Michels[1], J. Bible[1], E. Jones[1], R. Lange[1], D. Footle[1], J. Brazille[1], S. Crites[1], and B. Carroll[1]
[1]Texas A&M AgriLife Research, Bushland, TX 79012

Abstract
Using artificially infested field cages, a preliminary planting time trial and a primary insecticide trial was conducted to control the potato psyllid, the vector of *Candidatus* Liberibacter solanacearum (Lso), in an effort to eliminate zebra chip (ZC) in commercial fields. In the planting time treatment trial, phorate (Thimet 20G) outperformed thiamethoxam (Platinum 75 SG) by numerically increasing mortality and reducing oviposition, prompting us to perform a primary planting time treatment trial during the 2013 growing season. The primary insecticide trial was affected by an *Erwinia* infection, which rendered the yield (not shown) inconclusive, since the *Erwinia* and ZC interaction is unknown. ZC incidence is presented assuming *Erwinia* does not produce false positives in the fry test (see methods) and was unacceptably high across all treatments.

Introduction
Zebra chip (ZC) is an important disease of potatoes caused by the phloem dwelling bacterium '*Candidatus* Liberibacter solanacearum'. The disease causes dark brown stripping in the tuber when fried, rendering them unmarketable. Often, entire fields are rejected if disease reaches 15% ZC incidence. Almost all control measures are aimed at controlling its vector, the potato psyllid, *Bactericera cockerelli* (Sulc), using a vigorous insecticide regime, often spraying weekly during the growing season. Since rotating chemicals is essential in reducing insecticide resistance, potato growers are always looking for novel insecticides as well as novel insecticide regimes. Here, we examine the possibility of alternative planting time treatments in a preliminary trial as well as examine current and new insecticides and insecticide regimes in a caged field study.

Materials and Methods
Insect colony. Lso infected potato psyllids used in this experiment were reared on potato (cv. 'FL1867') under greenhouse conditions using potato psyllids collected from the Lower Rio Grande Valley, TX and from the Texas Panhandle (mixed). To verify infection of Lso, a sample of potato psyllids from the colony were periodically subjected to RT-qPCR by the lab of Dr. Charlie Rush, Texas A&M AgriLife Research, Bushland, TX 79012. Infection status was verified to be Lso positive with one week of each infestation date.

Preliminary planting time treatment trial. Nine plots, 4 rows wide by 20 feet in length, were established before planting. In three plots, Platinum 75 SG at 0.15oz/1000 row feet was applied, Thimet 20-G at 9.9oz/1000 row feet in three plots and the remaining three plots were left untreated. Experimental design was complete block. At weeks 6, 7 and 8 after planting, five sleeve cages per treatment, measuring 6 in x 12, was fitted to a single compound leaf from the middle-to-upper portion of five randomly selected plants (excluding plants that had already been used in the study). Five potato psyllids were inserted into the sleeves and sleeves were closed. After two (week 6) or four (weeks 7 and 8) days, the compound leaf was removed with the cage intact. Number of alive and dead potato psyllids and number of eggs were counted and recorded.

Table 1. Single Insecticide Tests Spray and Infestation Schedule

Material	Rate/acre	15-Apr	22-Jun	26-Jun	3-Jul	5-Jul	10-Jul	18-Jul	20-Jul	31-Jul
Torac[1]	24 oz	P	I		S		S	S	I	S
UTC - Early		P	I						I	
Requiem EC	1 qt	P		I	S			S	I	S
Requiem EC	2 qt	P		I	S			S	I	S
Agri-Mek	8 fl oz	P		I	S			S	I	S
Sivanto*	10.5 fl oz	P		I	S		S		I	
Sivanto*	12 fl oz	P		I	S		S		I	
Sivanto*	14 fl oz	P		I	S		S		I	
Radiant 1 SC* (a) 6oz	6 fl oz	P		I	S		S		I	
UTC - Mid		P		I					I	
Sulfoxaflor*	1.5 fl oz	P			S	I		S	I	
Sulfoxaflor*	2.0 fl oz	P			S	I		S	I	
Sulfoxaflor*	2.25 fl oz	P			S	I		S	I	
Radiant 1 SC* (b) 6oz	6 fl oz	P			S	I		S	I	
Radiant 1 SC*	8 fl oz	P			S	I		S	I	
Movento*	5 fl oz	P			S	I		S	I	
Blackhawk*	3.5 fl oz	P			S	I		S	I	
UTC - Late		P				I			I	

*Used Dyne-Amic at 0.32 oz/ gal of mixed spray solution as a surfactant. P = planting. I = infested with 4 psyllids per plot. S = sprayed. UTC = untreated control. Material name and Rate/acre used as x-axis in graphs.

Single insecticide and insecticide regime tests. One-hundred plots, 4 rows wide by 20 ft in length, were established and assigned treatments, each replicated four times in a randomized complete block design. The cages, 5 feet in width and 7 feet in length, were placed over the center two rows of the established plots. Spray applications were made using a 4-nozzle boom and CO2 backpack sprayer and were timed according to Tables 1 and 2. Application rates are recorded in Table 3.

At the conclusion of the trial, potato psyllid nymphs were sampled using an established leaf washing method (Martini, 2012): ten leaves were sampled from each cage, washed with hot water to extract the nymphs, then filtered through organza cloth, and counted under a stereomicroscope.

Potatoes in the cages were hand dug, placed into bags, and stored at temperatures between 25°C and 30°C, until tuber evaluation was complete. Tubers were sorted into two grades based on size and weight, acceptable and non-acceptable. Potatoes were subjected to a fry test where one thin slice was taken from the stolon end of each potato and fried in vegetable oil at 190°C. After frying, each chip was visually inspected for discoloration and brown stripping, the hallmark symptom of ZC.

Table 2. Insecticide Regime Tests Spray and Infestation Schedule

Identifier	15-Apr	26-Jun	3-Jul	10-Jul	18-Jul	20-Jul	24-Jul	31-Jul
Requiem 2 qt /Agrimek	(none)	I	Requiem EC		Agri-Mek	I		Requiem EC
Standard Sequence	Platinum 75 SG	I	Fulfill	Fulfill	Movento	I	Movento	Agri-Mek
Torac 1st	Platinum 75 SG	I	Torac	Torac	Movento	I	Movento	Agri-Mek
Torac 5th	Platinum 75 SG	I	Fulfill	Fulfill	Movento	I	Movento	Torac
Maxim Seq. 1	Maxim 4FS seed treatment & Platinum 75 SG	I	Fulfill	Fulfill	Movento	I	Movento	Beleaf
Maxim Seq. 2	Maxim 4FS seed treatment & Platinum 75 SG	I	Agri-Mek	Agri-Mek	Movento	I	Movento	Fulfill
Cruiser Maxx	Cruiser Maxx seed treatment	I	Agri-Mek	Agri-Mek	Movento	I	Movento	Fulfill
UTC - Mid	(none)	I				I		

See Table 3 for rates. I = infested with four potato psyllids per plot. UTC = untreated control. "Identifier" column used in graphs as X-axis.

Table 3. Insecticide Regime

Product	Rate
Agri-Mek 0.15 EC	8 oz/A
Beleaf*	2.4 oz/A
Fulfill*	2.75 oz/A
Movento*	5 fl oz/A
Requiem EC*	1 qt/A
Torac*	24 oz/A
Platinum 75 SG	0.15 oz/1K ft
Maxim 4FS seed treatment	.08 oz/cwt
Cruiser Maxx seed	0.3 fl oz/cwt

*Used Dyne-Amic at 0.32 oz/ gal mixed spray solution as a surfactant

Results and Discussion

Preliminary planting time treatment trial. Results are summarized in Fig. 1. The preliminary planting time treatment trial showed promising results, especially considering that Thimet could be a possible alternative to Platinum since Platinum's widespread and heavy use could lead to thiamethoxam resistance in the potato psyllid. Adding different planting treatments to the potato psyllid integrated pest management arsenal could be very helpful as potato psyllid resistance has already been reported to imidacloprid (Liu and Trumble 2007), which is still being heavily used (Guenthner 2012). These results have motivated us to perform a larger scale planting time trial in 2013.

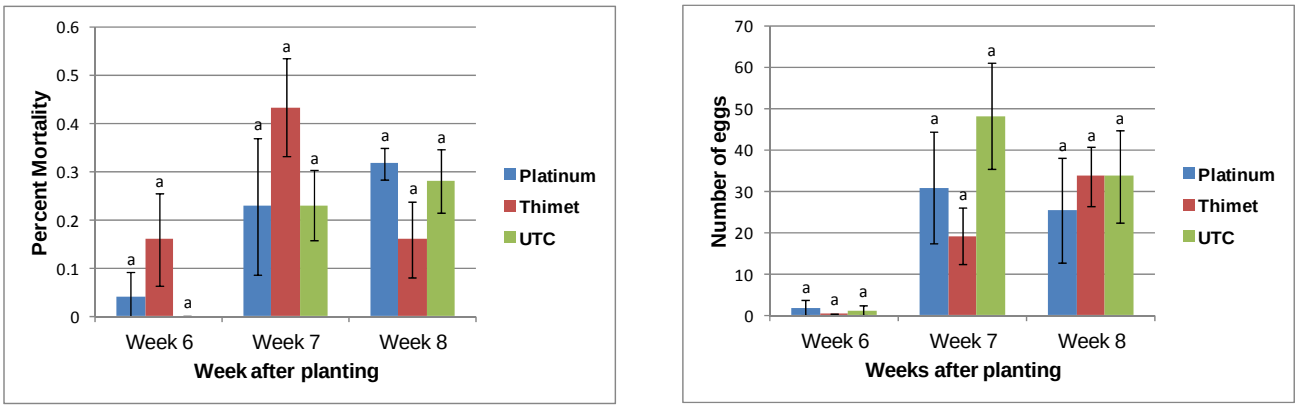


Figure 1. Preliminary planting time trial. Mortality (left) and oviposition (right). Means within a specified week followed by the same letter are not significantly different (Wilcoxon Rank Sum Test, $P<0.05$). Vertical black bars indicate standard error

Single insecticide and insecticide regime tests. Even though cages were infested with only eight infected potato psyllids (0.66 – 0.8 per plant), ZC incidence was unacceptably high in almost all treatments. We found no statistical differences in ZC incidence in the primary insecticide trial, probably due to the pathogen being transmitted before the insecticides were able to kill the potato psyllids. Pathogen transmission is reported to occur in less than two hours after potato plants are exposed to potato psyllids (Butler and Trumble 2012), which highlights the importance of properly timed preventative measures, such as planting time treatments and effective mid- and late-season insecticide applications.

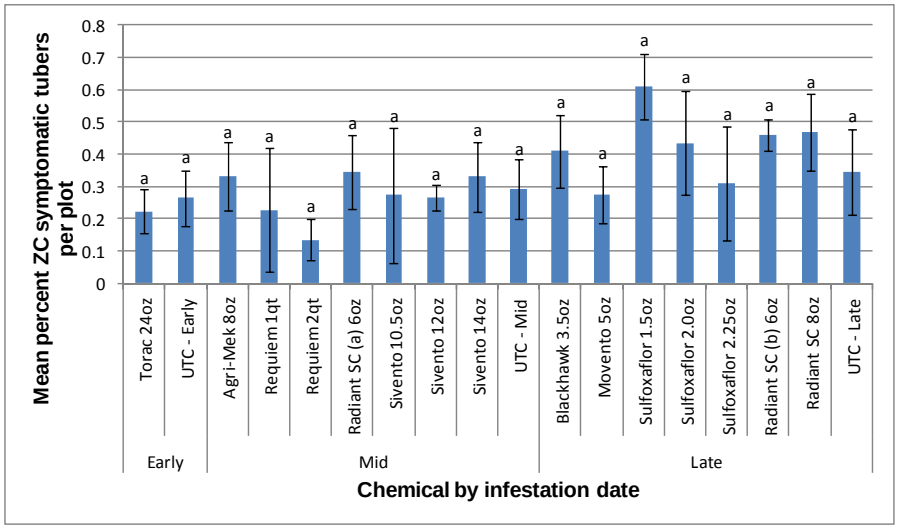


Figure 2. Single insecticide tests – ZC incidence.

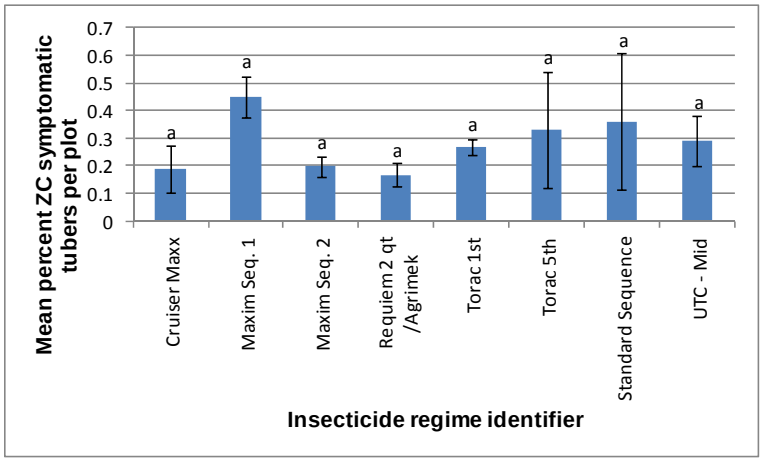


Figure 3. Regime tests – ZC incidence.

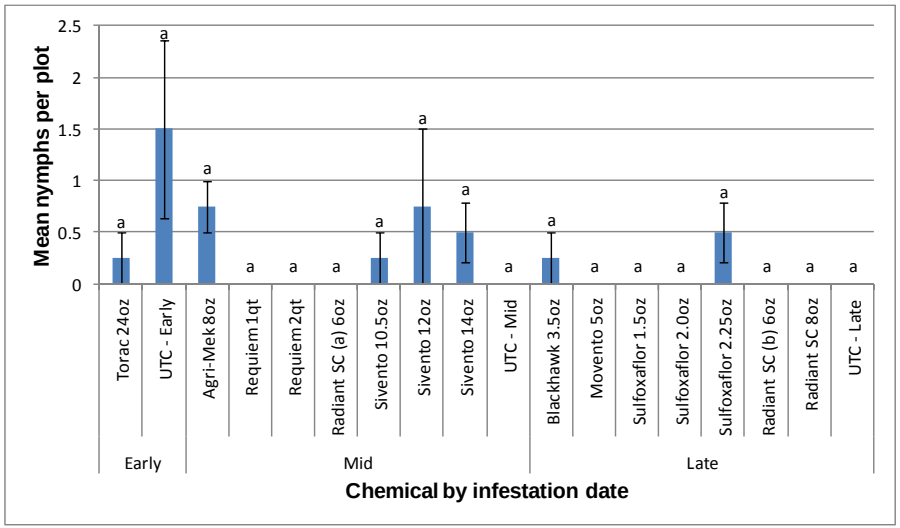


Figure 3. Single insecticide tests – end of season nymph counts.

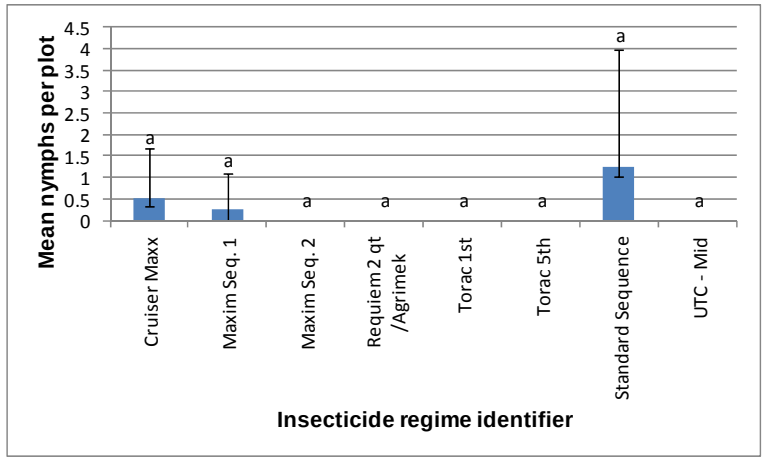


Figure 4. Regime tests – end of season nymph counts.

Figure Notes - Fig. 2 & 4: Significance letter "a" in "Early" is not statistically the same as "a" in "Mid" or "Late." "Early," "Mid" and "Late" were analyzed separately. Fig. 2 – 5: Treatments not connected by the same letter are significantly different (Wilcoxon Rank Sum Test. $P<0.05$). Vertical black bars indicate standard error.

Acknowledgement
This work was funded by the Texas Agriculture Zebra Chip Initiative and the USDA-AFRI-SCRI Zebra Chip Project

References
Butler, C. D., and J. T. Trumble. 2012. The potato psyllid, Bactericera cockerelli (Sulc) (Hemiptera: Triozidae): life history, relationship to plant diseases, and management strategies. Terrestrial Arthropod Reviews. 5: 87-111.

Guenthner, J., J. Goolsby, and G. Greenway. 2012. Use and Cost of Insecticides to Control Potato Psyllids and Zebra Chip on Potatoes. Southwest. Entomol. 37: 263-270.

Liu, D., and J. T. Trumble. 2007. Comparative fitness of invasive and native populations of the potato psyllid (Bactericera cockerelli). Entomol. Exp. Appl. 123: 35-42.

Martini, X., S. Seibert, S. Prager, and C. Nansen. 2012**.** Sampling and interpretation of psyllid nymph counts in potatoes. Entomol. Exp. Appl. 143: 103-110.

Effects of Planting Dates on ZC Incidence on Four Varieties in Pearsall, TX

G. Schuster, J. Trevino, A. Arp, and B. Bextine

Abstract
Zebra Chip (ZC) is a damaging disease of potatoes, causing an internal discoloration in tubers, rendering them unacceptable by potato chipping commercial standards. The disease is associated with the bacterium, "*Candidatus* Liberibacter solanacearum", transmitted by the potato psyllid, *Bactericera cockerelli* (Munyaneza et al. 2007). The objective of this research was to assess the impact of potato planting date on incidence of ZC using four potato varieties in Pearsall, TX: Frito Lay (FL) 1867, Red La Soda, Megas, and Harvey Blackwell in south Texas. The planting dates coincided with optimum and late planting and included January 15, February 1, and February 15. The plots (untreated) were monitored weekly for adult psyllid movement by using non-pheromone yellow sticky traps at each corner. All three planting dates had low psyllid activity until March 19 with significant peak activity within the first two weeks of April. ZC incidence was observed in the January 15 planting in Megas and Harvey Blackwell, in the February 1 planting in Red La Sodas, and in the February 15 planting in Megas only. Data are inconclusive and will be re-evaluated in 2013.

Introduction
Zebra chip (ZC) has caused millions of dollars to producers and potato processors. ZC is an emerging disease found in the southwestern United States (Munyaneza et al. 2007, Munyaneza et al. 2008). The disease is linked to *Bactericera cockerelli* (potato psyllid), which transmits the bacterium, "*Candidatus* Liberibacter solanacearum", associated with the disease. Failure to control this pest and pathogen could cause overwhelming losses in potato production, processing and retail industries (Rosson, 2009). Little is known on how the active psyllid populations correlate with the incidence and severity of ZC (Goolsby, J.A. et al. 2007). Documenting the relationship between migrating psyllid populations, planting dates, and pathogen occurrence may help us understand the spread of ZC (Munyaneza et al. 2007). Three different planting dates and four potato varieties were evaluated in Pearsall, TX. The objective of this research was to assess the impact of potato planting time on ZC incidence on four potato varieties.

Methods and Materials
This study was initiated at Black Gold Farms in Pearsall, TX. Four varieties evaluated included FL 1867, Red La Soda, Megas, and Harvey Blackwell. Three planting dates coinciding with optimum and late planting were used included January 15, February 1, and February 15. Seed pieces used were cut into four ounce pieces and treated with fir bark prior to planting. Each planting date had two rows per variety. Seed pieces were hand planted on 36" row spacing, spaced 10" inches apart. Each plot consisted of 8 rows by 100 feet. Plots were separated by wheat (20') to reduce psyllid movement. No insecticide was applied to the potatoes throughout the study to allow for psyllid colonization. Adult potato psyllids were monitored weekly by sticky traps. Plots were harvested by hand and incidence of infected tubers was determined by cutting tubers at both ends and visually scored using a simple yes or no based on discoloration. Percentage of incidence within each plot was determined.

Results
Potato psyllid populations were low for all three plantings until March 19, which followed by a significant increase, peaking during the first week of April (Figure 1). A second peak occurred two

weeks later for the January and February 15 planting only. Data indicates that the February 1 planting had the lowest adult population when compared to either January and February 15 plantings. More interestingly, in the February 1 planting total adults collected during the season was also the lowest with 29, compared to January 15 planting of 60 and February 15 planting of 57.

Zebra chip was not observed in all of the variety test plots (Table 1). Of the four potato varieties in the January 15 planting, the Megas and Harvey Blackwells were the only varieties with ZC incidence of 1.7%. Red La Sodas in the February 1 planting were significantly affected with a high ZC incidence of 15%. From the February 15 planting, Megas were the only variety with ZC incidence of 1.7%.

Figure 1. Total adults psyllids collected from yellow sticky traps from Pearsall, TX.

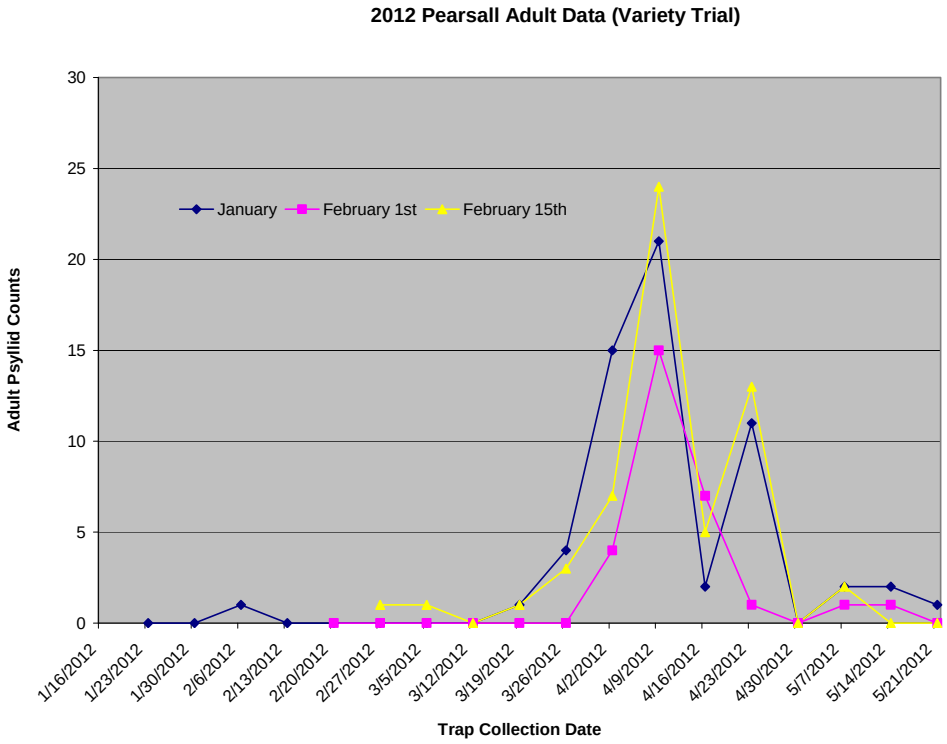


Table 1. Total ZC incidence observed from Pearsall, TX.

	FL 1867		Red La Sodas		Megas		Harvey Blackwell	
	Harvested	% Positive	Harvested	% Positive	Harvested	% Positive	Harvested	% Positive
Jan 15th	0/60	0%	0/60	0%	1/60	1.7%	1/60	1.7%
Feb 1st	0/60	0%	9/60	15%	0/60	0%	0/60	0%
Feb 15th	0/60	0%	0/60	0%	1/60	1.7%	0/60	0%

Acknowledgements
Financial support for this work was provided by the USDA-NIFA-SCRI (Project #2009-51181-20176). We are also grateful to Jack Wallace and Black Gold farms for providing potato seed. Special thanks to Black Gold Farms and their personnel for use of their land and assistance in the field throughout the project.

References
Goolsby, J.A, Adamezyk J., Bextine, B., Lin, D., Munyaneza, J.E., and Bester, G. 2007. Development of an IPM program for management of the potato psyllid to reduce incidence of zebra chip disorder in potatoes. Subtropical Plant Science, 59:15-23.

Munyaneza, J.E., Crosslin, J.M., and Upton, J.E 2007. Association of *Bacteria cockerelli* (Homoptera: Psyllidae) with "Zebra Chip," a New Potato Disease in Southwestern United States and Mexico. J. Econ. Entomol. 100(3):656-663.

Munyaneza, J.E., Buchman, J. L., Sengoda, V.G., Goolsby, J., Ochoa, A.P., Trevino, J., and Schuster, G. 2012 . Impact of Potato Planting Time on Incidence of Potato Zebra Chip Disease in the Lower Rio Grande Valley of Texas In Press, Southwestern Entomologist .

Rosson, P. 2009. Economic impacts of zebra chip on Texas. CNAS Issue Brief 2009-01, AgriLife Research, Texas A&M University.

2010-2012 Research Advances on Integrated Management of Potato Psyllid *Bactericera Cockerelli* (Sulc) in Chihuahua, Mexico

R. Galaviz, M. Barrera, G. Espinoza, C. Cedillo, G. Cerezo, J. González and F. Durazo.
PepsiCo Mexico Foods, Mexico City, Mexico

Abstract
Approximately 18% of total production cost is spent each season on insecticide applications in potato crops planted in Casas Grandes Chihuahua, Mexico. Despite this significant economic importance, there are no decision support tools available to improve spray timing effectiveness. From 2010 to 2012 we conducted a field study to understand the potato psyllid (PP), (*Bactericera cockerelli* Sulc) (Hemiptera: Triozidae), population dynamics by three different sampling methods; yellow sticky traps, net sweep and foliar nymph collection. Weekly sample collection demonstrated that PP overwinters in the Casas Grandes Chihuahua area. Adults appear in the crop in early April, as soon as potato emergence occurs. PP reach its highest population from July to September when high and low daily temperatures stabilize around 32°C and 17°C respectably. Accumulated degree-days was calculated for PP and correlated with nymph and adult population dynamics. The high correlations found in 2010 and 2011, r=0.81 and r=0.80, respectively, shows that despite agrochemical spray program, pest population grew directly proportional to accumulated degree-days. This indicates low agrochemical effectiveness. On the other hand, the 2012 PP chemical control was significantly improved (r=0.24). Based on the results discussed in this report, to improve agrochemical field effectiveness and reduce the agrochemical environmental impact, it is recommended to follow PP control programs based on regular monitoring to detect first arrivals and sprayings based on degree-days management criteria.

Introduction
Zebra chip (ZC) disease of potatoes (*Solanum tuberosum* L.) was first identified in potato fields near Saltillo, Coahuila, Mexico in 1994 (Cadena-Hinojosa and Guzman-Plazola 2003). In Northern Mexico, the disease~~s~~ has caused damage~~s~~ ranging from 20% to 60% of planted potato acreage, especially from 1994 to 2004 (Hernandez et al. 2006). Sabritas potato fields from Nuevo León and Coahuila were sampled from 2004 to 2007 by using sticky traps. This study demonstrated that potato psyllid (PP) population peaks occurs in May, so that ZC infection risks could be avoided by planting in late May and June (Galaviz et. al. 2010). Since the ZC appearance in 1994, Mexican potato growers have adopted PP management strategies based on heavy chemical spraying, usually on weekly calendar basis, more than on a pest population dynamic analysis basis. Early insect detection by effective monitoring, supported by degree-days (DD) calculations to improve spraying timing, may reduce the amount of agrochemical applied by season and at the same time increase its effectiveness in favor of productivity and environmental care (Nansen, et al. 2011). PP requires 356 DD to complete one biological cycle from eggs to adult stage (Becerra, 1989). DD is an important tool to support pest management strategies, and can be used to calculate the number of generations per season and predict~~s~~ future pest outbursts (McMaster, G. S. & W. Wilhelm, 1997).

Materials and Methods
In the region of Casas Grandes Chihuahua, Mexico, PP population dynamics was studied at the three local planting dates to identify the potential infection risk of *Candidatus liberibacter solanacearum* transmission. PP population dynamics was correlated with the accumulated DD from 2010 to 2012 to validate the technique as a tool to support agrochemical timing decisions and improve regional pest management.

Weekly monitoring programs during the 2010 to 2012 seasons was followed across Casas Grandes Chihuahua potato commercial fields using net sweep captures and yellow sticky traps to record potato psyllid population (PP) dynamics and correlate it with accumulated degree-days (DD).

Sweep net sampling method.
Adult population dynamics was sampled weekly across 20 irrigation pivots with a total acreage of 1200 cultivated hectares. In each irrigation pivot, a total of 400 net sweeps were done with 100 net sweeps per quadrant. (north, south, west, and east)

Sticky traps sampling method.
In each irrigation pivots, one yellow sticky trap was placed in each quadrant, for a total of 4 traps. Weekly counts and replacements were done to quantify adult population; these readings were recorded to identify first adult appearances in each pivot.

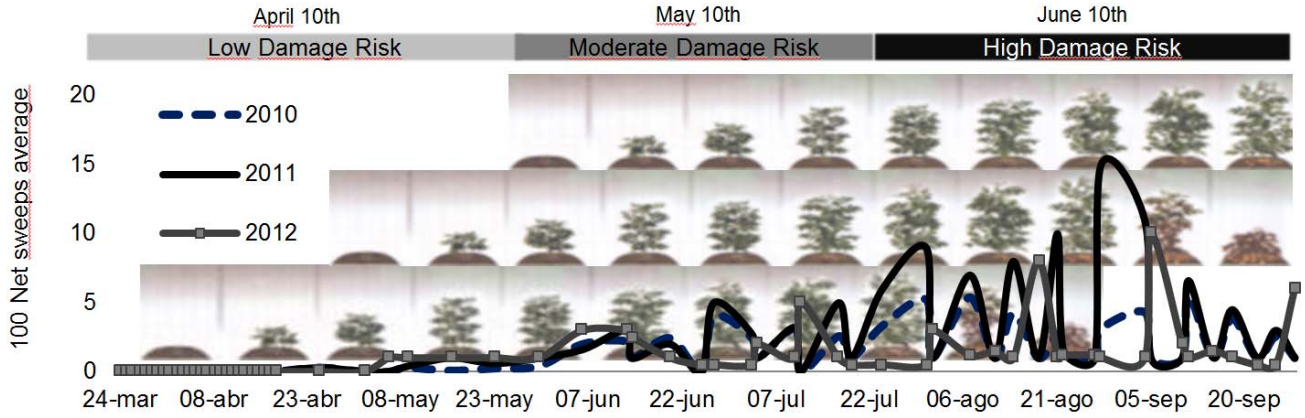


Fig. 1 Potato psyllid adult population dynamics compared with crop phonological stages of three planting dates in Casas Grandes Chihuahua, 2010, 2011 and 2012.

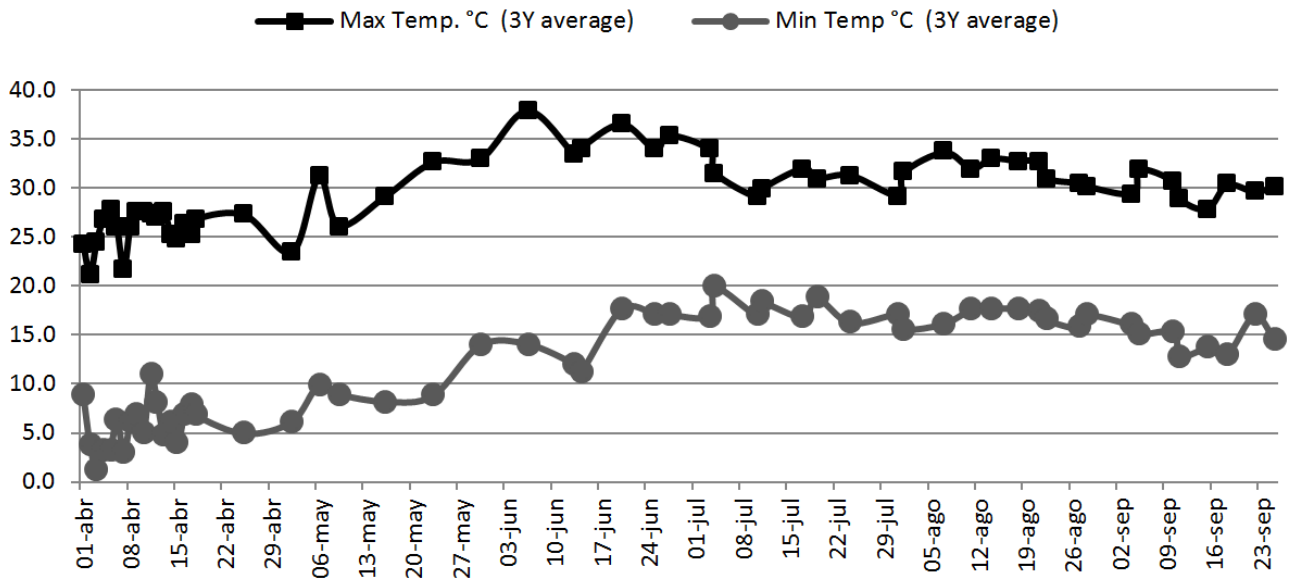


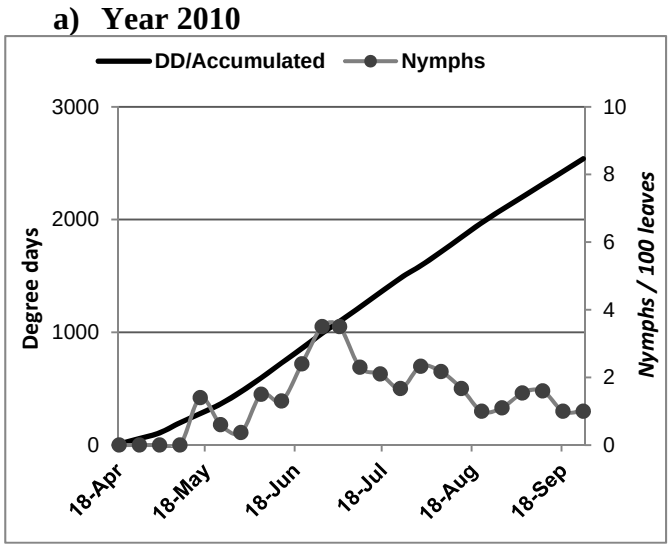
Fig. 2 Minimum and maximum temperatures (°C) recorded in Casas Grandes Chihuahua during 2010 to 2012.

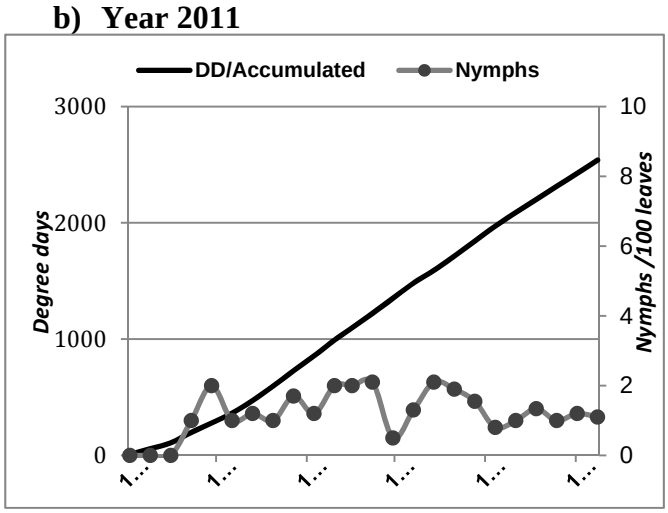
Temperature record and degree days analysis.
Temperature was recorded during each production season to calculate PP heat units. Heat units, expressed in degree-days (DD), were used to describe the timing of biological processes. The basic equation used is *DD = [(T °C max, + T °C min)/2] – T °C* threshold, where *T °C max and T °C min*: are daily maximum and minimum air temperature, respectively, and *T °C threshold* = is the base temperature for the species in study. In this case, (*Bactericera cockerelli*) T °C threshold is 7°C.

Results and Discussion
Collected data in 1200 hectares during 2010 to 2012 seasons suggests that potato psyllid overwinters in Casas Grandes Chihuahua region and migrates from other nearby host crops to potato fields. During the three years of the study, the PP consistently appears from late April to early May when first planting date starts vegetative stage, with peaks of psyllid adults from late August to early September just at the vine killing time (Fig. 1).

The results of the study also indicates that after its arrival, the PP rapidly reproduces. This is confirmed by population peaks of collected nymphs 2 to 3 weeks after adult appearance (Figs. 3a-3c) and 2 to 3 weeks before adult population starts to increase rapidly (Figs. 4a-4c). This suggests that the increase of collected adult PP is the result of rapid local reproduction of the pest.

Temperature conditions are suitable for PP reproduction (Fig. 2). The PP seems to be adapted to warm, but not hot weather. Optimum psyllid development occurs at approximately 27°C, whereas survival is reduced at 32°C and stops at 35°C. A single generation may be completed in 3 to 5 weeks depending on accumulated DD (Becerra, 1989, Medina & Covarrubias, 2007). Based on DD, from early April to early June, PP took 4 weeks to complete one generation while from middle June to September it took 3 weeks complete it (Fig. 4a – 4c). These results can be explained by the fact that this insect needs 356 calories to complete their cycle from egg to adult (Maya et al., 2003), considering that the minimum threshold for the PP is 7 °C.





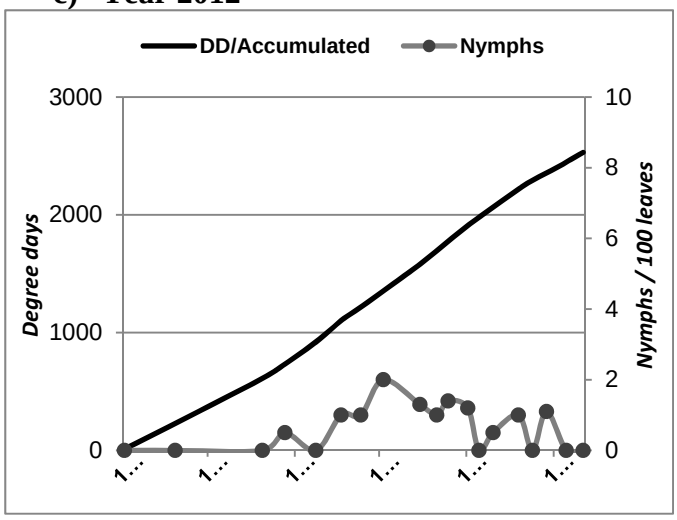
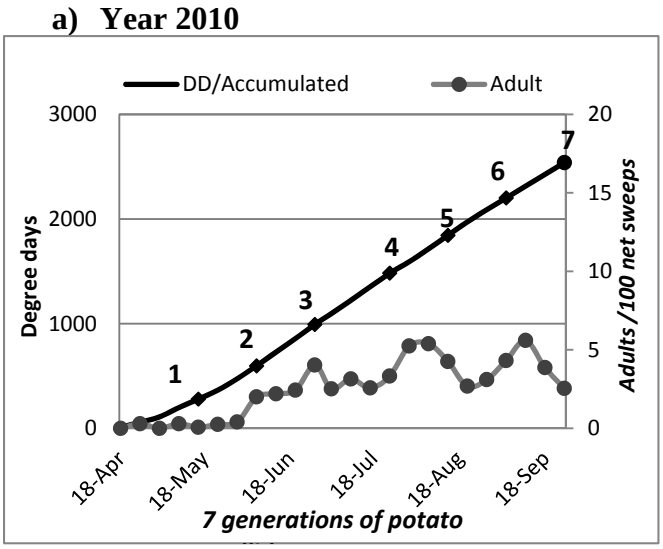
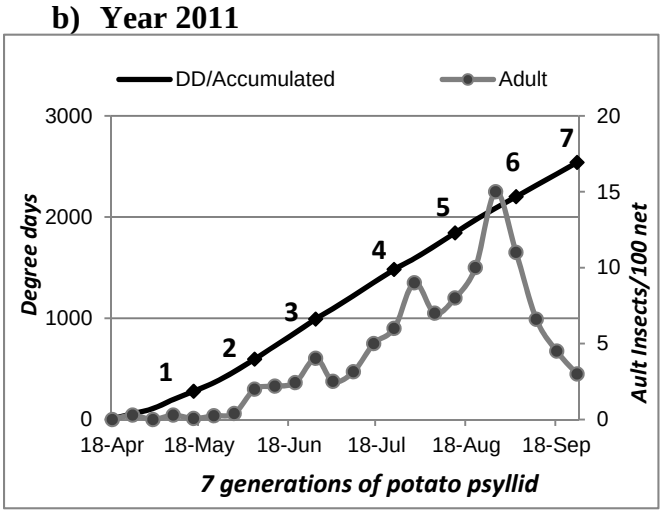


Fig. 3 Average number of potato psyllid nymphs collected from 100 foliar samples in 2010 (a), 2011 (b) and 2012 (c) at Casas Grandes Chihuahua





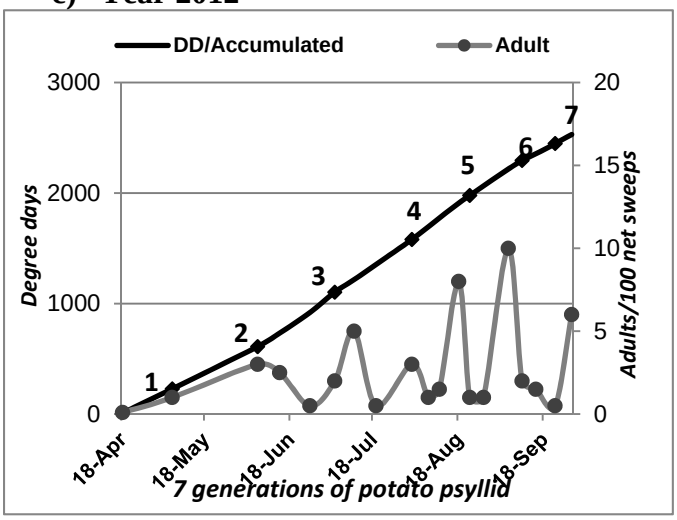


Fig. 4 Average number of potato psyllid adults collected by 100 net sweeps in 2010 (a), 2011 (b) and 2012 (c) at Casas Grandes Chihuahua

Effective monitoring and control of the PP are crucial for prevention and management of potential Zebra Chip outbreaks in this important potato growing region of the north of Mexico. Correlations between accumulated PP degree days and its population was r= 0.81, r=0.80 and r= 0.24 for 2010, 2011 and 2012 respectively, showing that during 2010 and 2011, PP were growing despite agrochemical spraying program. Low correlations observed in 2012 reflects that spraying timing was better targeted on generation outbreaks resulting in lower potato psyllid populations as a consequence of improved agrochemical management strategies (Fig.4 a – 4c).

Conclusions

In Casas Grandes Chihuahua Mexico, the potato psyllid overwinters in same geographical area, with first appearances occurring at the beginning of the season, and on average, seven generations area observed during the potato crop season. Potato psyllid degree days permits us to design a more effective agrochemical program and reduced the agrochemical environmental impact. The recommendation is to follow an active sampling program and a spraying plan based on potato psyllid degree-days calculation.

Acknowledgments

We thank and recognize the help of Mr. Guillermo Aguirre Borboa from. AGRICOLA RARAMURI, Mr. Ricardo Aguirre Borboa from AGROBO, Mr. Kelly Jones and Christian Jones from AG. VISTA DEL SOL. We also appreciate invaluable professional support of Ing. Luis Salazar from AGRICOLA RARAMURI, Ing. Manuel Beltrán and Ing. Jesús Terminel from AGROBO, & Ing. Manuel Salcido from AGROGROPPO.

Reference

Becerra, F.A. 1989. Biología de Paratrioza cockerelli (Sulc) y su relación con la enfermedad “permanente del tomate” en el Bajío. Tesis profesional, Universidad Autónoma de Querétaro, Facultad de Química. Querétaro, Méx. 55p.

Cadena-Hinojosa, M. A., and R. Guzman-Plazola. 2003. Distribution, incidence and severity of purple potato rot and abnormal sprouting of potato (Solanum tuberosum L.) tubers in the high valleys and mountains of the states of Mexico, Tlaxcala and the Federal District, Mexico. Rev. Mex. Fitopatolog. 21: 248D250.

Galaviz, R., V. Hernández, A. Sanchez, M. Barrera, G. Frías, and F. Durazo. 2010. Highlights on zebra chip: Mexican experiences. Proceedings of the 10th annual zebra chip reporting session. Hyatt DFW Airport Dallas, TX. November 7-10, 2010 42-46 p.

Hernández, G. V., Sanchez, A., Frías, G. y Padrón, E. 2006. Factores abióticos y su relación con el síndrome de punta morada de la papa. Tesis de Maestría en Ciencias. Universidad Autónoma Agraria Antonio Narro. Buenavista Saltillo, Coahuila, México. 62 p.

Munyaneza, J.E., J.M. Crosslin, and J.E. Upton. 2007a. Association of Bactericera cockerelli (Homoptera: Psyllidae) with “zebra chip”, a new potato disease in southwestern United States and Mexico. Journal of Economic Entomology 100: 656–663.

McMaster, G. S. & W. Wilhelm, 1997. Growing degree-days: one equation, two interpretations.Agricultural and Forest meteorology 87(1997) 291-300.

Maya, H. V.; Ramírez, O. J.; Cortéz, O. R.; Vega, M. R. y Moreno, Ch. G. 2003. Manejo integrado del pulgón saltador en jitomate en el estado de San Luís Potosí. Campo E. Palma de la Cruz, SLP. INIFAP. México. Folleto técnico. Núm. 22. 43 p

Medina, G. G. & J. M. Covarruvias. 2007. Reporte Meteorológico Marzo-2007. INIFAP. México. Folleto técnico. Núm. 34. 38. p

Nansen, C. Et. Al. 2011. A Decision-Support Tool to Predict Spray Deposition of Insecticides in Commercial Potato Fields and Its Implications for Their Performance. Journal of Economic Entomology. 104(4):1-8.

A Global Perspective on the Management of Tomato-Potato Psyllid, Zebra Chip and Other Key Potato Pests with the Use of the New DuPont™ Benevia™ and Verimark™ Insecticides

Juan Manuel Alvarez[1], Hector E. Portillo[1], I Billy Annan[1], Rachel A. Cameron[1], Clifton D. Brister[2], Jose del R. Munoz[3].
[1]DuPont Crop Protection Stine Haskell Research Center, 1090 Elkton Rd. Newark, DE 19711, USA; [2]DuPont Riogrand, Texas; [3]DuPont Morelos, Mexico.

Abstract
The causal agent of potato Zebra Chip (ZC) was recently identified as *Candidatus* Liberibacter solanacearum, which infects tomato and potato and the vector is the potato-tomato psyllid, *Bactericera cockerelli*. The ZC disease is currently causing severe yield and quality declines in Central and North America and in New Zealand. Insecticides are an important component in the management of ZC in potato. However, many growers in affected areas have not been able to reduce the disease transmission to satisfactory economic levels. Cyazypyr™ (DPX-HGW86, cyantraniliprole), is a novel cross-spectrum second generation anthranilic diamide insecticide that was discovered by the DuPont Company. Two Cyazypyr™ formulations (Verimark™ and Benevia™) will be registered in 2013 in several countries to be used in potatoes and many other crops. Field and greenhouse trials in the US, Mexico and New Zealand have shown that Verimark™ and Benevia™ are very effective insecticides against *B. cockerelli* and also reduced the transmission of ZC. These two insecticides have also proven effective against other key potato pests. The positive results indicate that Verimark™ and Benevia™ will be key tools for potato growers to be used in their management programs against the potato-tomato psyllid and ZC.

Introduction
Mode of Action is the basis for rapid feeding cessation and plant protection: Cyazypyr™ exhibits a novel mode of action, by selectively activating the ryanodine receptor in insect muscles, resulting in rapid cessation of feeding in affected pest insects. This causes reductions in the capability of the affected pest insects to vector plant diseases. Cyazypyr™ reduces viral and bacterial diseases transmitted by several Hemipteran species, including whiteflies, aphids, and psylli and non-Hemipteran insects. ZC is one of the diseases that Cyazypyr™ has been proven to reduce.
Two formulations of Cyazypyr™ will be registered in potatoes in the US: 1) Benevia™, which is designed for foliar applications and optimized for leaf penetration and locally systemic movement, improving coverage and availability of Cyazypyr™ to a cross-spectrum of pests. 2) Verimark™, which is designed for soil applications and optimized for root uptake and crop safety to tender roots and shoots, protecting both older leaves and new growth from a cross-spectrum of pests. Field and greenhouse trials in several countries have shown that Verimark™ and Benevia™ are very effective insecticides against *B. cockerelli* and also reduced the transmission of ZC. Information regarding these trials is presented below.

Materials and Methods

Field and greenhouse trials were conducted in the US (Texas and Oregon), Mexico and New Zealand to determine the efficacy of Benevia™ and Verimark™ to reduce populations of the potato-tomato psyllid and the incidence of ZC. Trials were conducted by DuPont research personnel at the DuPont Rio Grande Research Station in Donna TX, university researchers (Texas A&M University and Oregon State University in the USA and Universidad Autonoma Chapingo in Mexico), and private researchers in Tula

Mexico and Hermiston, Oregon, USA. Details about experimental design for individual tests are provided below.

Results and Discussion

Field Trial in Texas

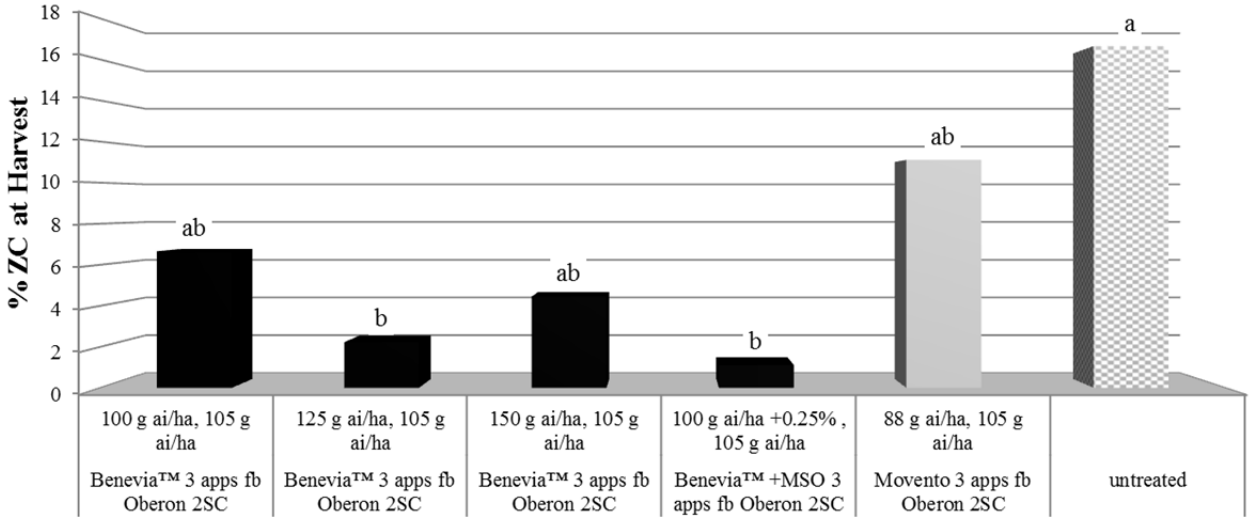


Fig. 1. Efficacy of Benevia™ foliar applications for reduction of zebra chip on potato. Treatment means (separated using LSD) with the same letters are not significantly different from each other (α = 0.05). Trial RGV11016.

This trial was conducted in Weslaco, TX in 2011 by Dr. John Goolsby. USDA-ARS. The potato variety was Atlantic and the plot size: 4.06 m x 12.19 m with three replicates in a RCBD design. Fig. 1 shows a reduction in percentage of ZC at harvest with all treatment regimens when compared to the untreated control. The percentage of ZC at harvest in all regimens that included Benevia™ applications were below 6.7%, whereas an 11.1% ZC infection was observed in the regime with three applications of Movento (spirotetramat) followed by an application of Oberon. The differences were not significantly different though. The treatment with the lowest percentage of ZC infection (1.1%) was three Benevia™ applications with MSO followed by an application of Oberon (spiromesifen).

Field Trials in Mexico

The efficacy of Benevia™ foliar applications for potato psyllid control and reduction of ZC on potato was evaluated in this trial conducted by an external cooperator, Peter Bruno, in Tula, Mexico (SWK-11-208). The experiment was planted on May 20, 2011 and replicated 4 times in a RCBD and the plot size was 10m x 15m. The potato cultivar was Vivaldi. Three foliar applications per treatment (July 28, Aug. 4 and 11) were conducted using a backpack sprayer (300 L/Ha). The table below shows results of the trial. Treatment means (separated using LSD) with the same letters are not significantly different from each other (α = 0.05). Benevia™ showed equal or superior control of tomato-potato psyllids when compared to the standard insecticide Movento. The Benevia™ treatments at 125 and 150 g ai/ha presented the lowest percentage of ZC at harvest and the difference between these treatments and the Movento treatment was statistically significant.

Treatment	rate g ai/ha	% Reduction of nymphs /100 leaves					% ZC
		5 DAT3	11 DAT3	21 DAT3	31 DAT3	41 DAT3	47 DAT3
Benevia™	100	100 a	100 a	94 b	95 b	96 a	19 a
Benevia™	125	100 a	100 a	98 a	98 a	95 a	3 b
Benevia™	150	100 a	100 a	98 a	98 a	97 a	3 b
Benevia™ + MSO	100 + 0.25%	100 a	100 a	97 ab	98 a	95 a	17 a
Movento	70	100 a	95 b	83 c	96 ab	95 a	16 a
Untreated	Numbers	103	245	305	361	430	91

The efficacy of an in furrow application with Verimark™ for potato psyllid control and reduction of ZC on potato was evaluated in this trial conducted in 2011 by Dr. Solis Aguilar, Universidad Autonoma Chapingo, Mexico (MXK-11-015). The experiment was replicated 4 times in a RCBD and the plot size was 3.6m x 6m. The table below shows results of the trial. Treatment means (separated using LSD) with the same letters are not significantly different from each other ($\alpha = 0.05$). The potato cultivar was Phianas. Verimark™ showed superior control of tomato-potato psyllids when compared to the standard insecticide Confidor (imidacloprid) and the differences were statistically significant for all Verimark™ treatments above 100 g ai/Ha.

Treatment	rate gai/ha	% Control of psyllid nymphs / plot				
		37 DAT1	44 DAT1	51 DAT1	58 DAT1	65 DAT1
Verimark™	80	93 a	91 bc	90 cd	87 bc	84 b
Verimark™	100	94 a	92 ab	91 bc	88 b	87 b
Verimark™	120	95 a	94 ab	94 ab	92 a	90 a
Verimark™	160	96 a	95 a	95 a	92 a	92 a
Confidor	525	58 b	62 c	65 d	49 c	60 c
Untreated (# nymphs)		30.9	26.1	26.7	26.4	28.7

Benevia™ and Verimark™ Control of Tomato-Potato Psyllid Adults

In a greenhouse trial conducted by Dr. Silvia Rondon, at the Hermiston Agricultural Research and Extension Center, Oregon State University in 2012, the adult mortality was assessed at 1 and 24 h after release. Releases of adult psyllids occurred immediately after foliar treatments had dried. The potato cultivar was Ranger Russet. Fig. 2 shows results of the trial.

A higher percentage of adult mortality was observed with the 150 g ai/Ha Benevia™ treatment when compared to Movento and Radiant (spinoteram) one h after release and the difference was significantly different to all other treatments. A higher percentage of adult mortality was also observed with the same Benevia™ treatment at 24 h after release. However, the difference was not significantly different from the other insecticide treatments.

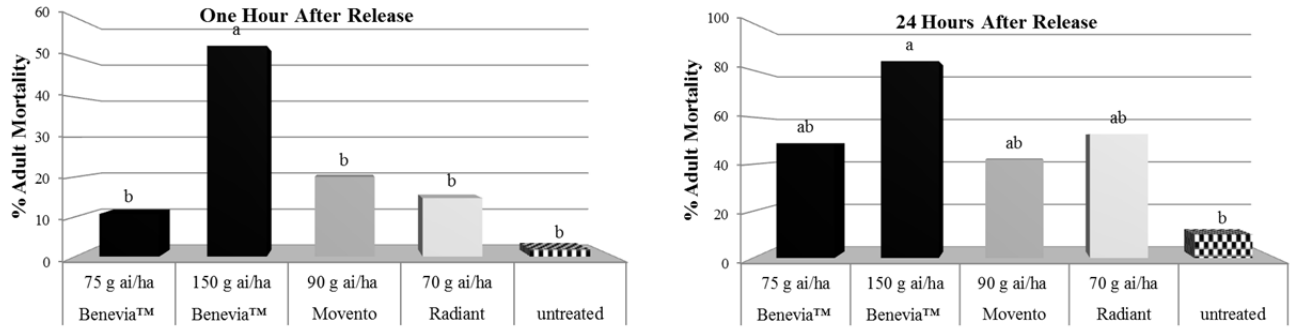


Fig. 2. Mean percentage mortality of adult psyllids per plant 1 h (left) and 24 h (right) after release. Release occurred immediately after foliar treatments had dried.

Pacific Northwest, United States

Control of the tomato-potato psyllid with In-Furrow (IF) Applications of Verimark™, Vydate® or Admire Pro followed by foliar insecticide programs was evaluated in a field trial conducted in Hermiston, OR in 2012. Results are presented in Fig. 3.

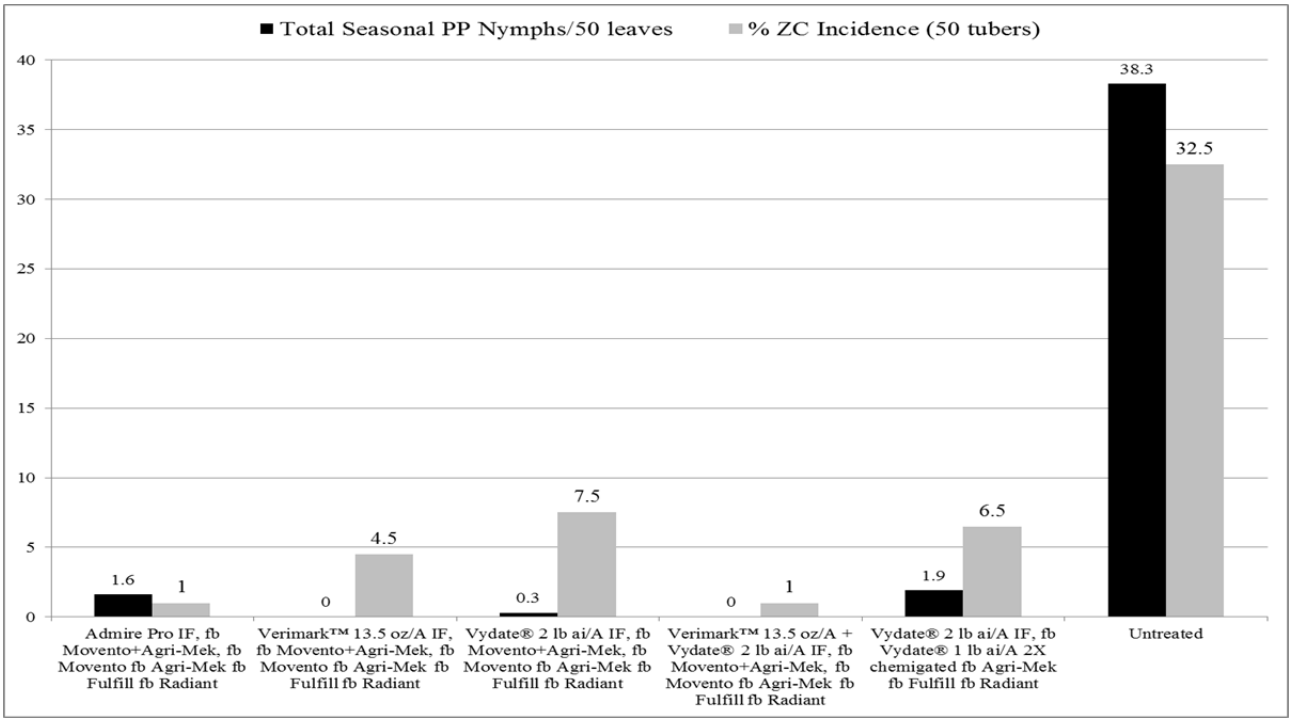


Fig. 3. Total seasonal tomato-potato psyllid nymphs per 50 leaves and zebra chip incidence under different management programs.

All treatments drastically reduced the number of psyllid nymphs and the incidence of zc when compared to the untreated control. No statistical significant differences were observed between treatments.

New Zealand

A field trial was conducted in 2009-2010 by the New Zealand Institute for Plant & Food Research Ltd in Pukekohe, New Zealand, to demonstrate the efficacy of three rates of Benevia™ for the control of

tomato-potato psyllid. The Benevia™ treatments were applied in a cluster of three 12-14 day applications within a full 7-8 day insecticide program. The table below shows results of the trial including psyllid control and postharvest tuber yields and size-grades and zebra chip evaluations. Benevia™ applied at 30-100 g ai/Ha completely controlled tomato-potato psyllid nymphs in potato (cv. Moonlight) foliage, resulted in higher yields and more 1st grade tuber and significantly reduced the incidence of ZC.

Treatment	Rate (g ai/ha)	Tomato-potato psyllids				Total Yield (Kg)	Yield 1st Grade (Kg)	Mean ZC Incidence (%)
		Pre treatment	7 d after spray 1	10 d after spray 2	8 d after spray 3			
Benevia™	30	0.5 ab	0.25 ab	0a	0a	38.8 b	35.1 c	16 ab
Benevia™ + Actiwett	30	0a	0a	0a	0a	40.5 b	34.6 c	16 ab
	50	0a	0a	0a	0a	40.6 b	34.8 c	15 ab
	100	0a	0a	0a	0a	34.9 b	30.7 bc	6.0 a
IPM reduced insecticide sprays		0.5 ab	1 ab	4.25 ab	2 b	32.2 ab	25.7 ab	31 b
Control		0.75 b	2 b	10 c	11.5 c	24.9 a	20.3 a	83 c

Treatment means with the same letters are not significantly different from each other ($\alpha = 0.05$).

Other Key Potato Pests Controlled by Verimark™ and Benevia™

Colorado potato beetle, *Leptinotarsa decemlineata;* Potato tuberworm, *Pthorimea operculella;* Aphids i.e. Green peach aphid, *Myzus persicae*, cooton-melon aphid, *Aphis gossypii;* Tomato fruitworm, *Helicoverpa zea*, and many other Lep pest species; Whiteflies, *Bemisia tabaci* and *Trialeurodes vaporariorum.*

Other Attributes of Cyazypyr™.

- Delivers a novel mode of action for sucking pests and controls pests that are resistant to other insecticides. Therefore, Cyazypyr™ is an excellent new tool for Integrated Resistance Management programs.
- Impacts multiple pest life stages and its selectivity to non-target arthropods helps conserve natural enemies. When used at the beginning of a pest infestation, these product features prevent or delay the population growth of highly prolific pests such as whiteflies, aphids, thrips, and psyllids.
- Has low toxicity to birds, fish, mammals, earthworms, and microorganisms, and it breaks down rapidly in the environment.

Acknowledgements

We thank all researchers and organizations that have contributed to the work presented here or that have helped further the characterization of Cyazypyr™ and its products.

Bugspotting Psyllids: Developing a Web-Based "Speed Scouting" and Survey Tool for Potato/Tomato Psyllids

Bradshaw, J. D.[1], Whipple, S. D.[1], Henne, D. C.[2], and McCornack, B. P[3]

University of Nebraska-Lincoln, Panhandle Research and Extension Center, Scottsbluff, NE[1]
Texans A&M, Texas AgriLife Research and Extension Center, Weslaco, TX[2]
Kansas State University, Manhattan, KS[3]

Abstract
Extension could be seen as facing a crossroads in communication. Our now digitally-connected world is quickly evolving tools and technologies that guide our communications and decisions across a vast relational network. Why should our data not do the same? Reported here is a project that aims to both stream-line data collection and share the information in real-time. This project attempts to build on an existing national scouting program for potato/tomato psyllids via the development of a web-based application. The application provides biological and management information about potato/tomato pysllids and begins to develop a binomial sampling method for potato/tomato psyllid monitoring. This sampling plan and delivery strategy attempt to borrow from the success of other binomial or "speed scouting" sampling plans (Giles et al. 2003, Hodgson et al. 2004, Butler and Trumble 2012).

Introduction
The way in which we approach pest monitoring is at a crossroads. The definition of "community" has evolved considerably in recent years; from a world dominated by face-to-face interactions and traditional mass media to today's more personalized social networking websites. Connectivity through cell phones, more specifically "smartphones" with internet capabilities, has played a pivotal role in this transition with adoption rates surpassing those set by early internet users. In addition to this connectivity, the mobile nature of smartphones allows "on-the-go" access of information during normal daily activities.

Given this movement towards wireless information delivery and increased use of social media platforms, Extension programs need to transform the way in which information is delivered and how we interact with stakeholders. Recent advances in mobile technologies and modes by which we access data have paved the way for more efficient, real-time approaches to connect pest monitoring and management information with pest management practitioners over wide geographic areas. Without easily-accessible information and IPM recommendations agricultural producers will fall back on non-sustainable, reduced-risk approaches that include prophylactic use of pesticides.

The goal of this project is to expand the accessibility of the existing potato/tomato psyllid monitoring program by using mobile technologies and web-based tools. Simply building a static website on a mobile device is not enough and does not adequately facilitate real-time decision making. In addition, a positive user-experience leads to increased use and rapid integration of relevant stakeholder inputs. Therefore, a major undertaking of this project is to define elements needed to sustain user interests in this monitoring system. We use open source software to develop the core program, which will ultimately lead to a more sustainable software platform that not only adapts to changing technologies and easily integrates added-value features with minimum cost. Our objectives were to 1) Develop a web-based platform for mobile data entry for potato/tomato psyllids and 2) Evaluate a "speed scouting" approach for monitoring potato psyllids.

Materials and Methods

Beginning in May 2012, potato/tomato psyllids were sampled using three methods at three potato fields (two, large production fields (MC11 and Jim Dye) and one, 1/3-acre field (PREC UTC) in Nebraska. The production fields used their standard pest-control program while the PREC UTC field was managed for weeds; however, no other pest-control program was implemented. Each location was sampled weekly for adults using 5 yellow sticky cards attached to the top of 1-meter wooden stakes. Sticky cards were sent weekly to Dr. Don Henne for psyllid species confirmation and counting. For nymphs and eggs, each location was sampled using two methods. One method (direct count) followed the nymph and egg sampling procedures described in Goolsby et al. (2012); 100 petioles per field were collected and sent to Dr. Henne to count nymphs and eggs. The comparative method ("speed scout") is based on a binomial sampling scheme similar to that used by Butler and Trumble (2012); petioles were collected in the field and if any psyllid egg or nymph was observed, the petiole was considered infested. Data for infested petioles were entered into a web-based app (www.thebugspot.org/potatopsyllids). The web-based app was programed, developed, and served by Dr. Henne's lab in Weslaco. Sample data from the binomial sampling scheme was entered into thebugspot.org by the number of infested petioles out of 100 and "tagging" the data according to location and whether the data were of eggs or nymphs. Data from the two egg and nymph sampling plans were evaluated by a simple fit to a natural log relationship.

Results and Discussion

TheBugSpot (www.thebugspot.org/potatopysllid/) provides A brief description of the pest and its damage to potato (Fig. 1A), facilitates data entry by field scouts (Fig. 1B), and allows for the real-time display of data (and associated metadata) (Fig. 1B and C). Using TheBugSpot allows for the field scout to have the latest information on identification and population levels at their fingertip. Simultaneously, a binomial sampling protocol allows for the scouting of population levels and sharing of that information in real time. Unlike, Butler and Trumble (2012), our sampling protocol is not intended (at this time) to provide action thresholds. Rather, the intent is to speed up the scouting procedure for monitoring psyllid populations.

Direct counts of potato psyllids revealed markedly different psyllid populations from field locations that were only 30-45 miles apart (Fig. 2A, B, and C). Most locations did not see an increase in potato psyllid abundance until mid-June to early July (Fig. 2A). Correspondingly, psyllid egg counts began to increase in early July (Fig. 1B) and nymphs in late July (Fig. 1C).

TheBugSpot was used throughout the season at each location and a sample of this data for egg counts at field MC11 is shown (Fig. 1C). To view more locations and data, visit www.thebugspot.org/potatopysllid/ and navigate to the links for the other data sets. To establish a baseline for evaluating the efficiency of the binomial sampling program implemented through TheBugSpot, simple correlations were constructed (Fig. 2D). Butler and Trumble (2012) indicated that a binomial sampling scheme fits best to direct counts by a natural log function. Thus, data for potato psyllid eggs and nymphs were fit to a natural log in relating average direct counts per 100 petioles to percent of infested petioles (Fig. 2D). Egg and nymph direct count and "infested" count correlated by a natural log by 82-84%. This indicates that this "speed scouting" sampling plan could be used to monitor psyllid nymph and egg populations in the field. Future work could continue to refine the web application to be more user-friendly and to incorporate the binomial sequential sampling plan of Butler and Trumble (2012).

References

Butler, D.C., and Trumble, J.T. 2012. Spatial dispersion and binomial sequential sampling for the potato psyllid (Hemiptera: Triozidae) on potato. Pest Manag. Sci. 68(6):865-869.

Giles, K.L., Jones, D.J., Royer, T.A., Elliott, N.C., and Kindler, S.D. 2003. Development of a sampling plan in winter wheat that estimates cereal aphid parasitism levels and predicts population suppression. J. Econ. Entomol. 96(3):975-982.

Goolsby, J.A., Adamcyk, J.J., Crosslin, J.M., Troxclair, N.N., Anciso, J.R., Bester, G.G., Bradshaw, J.D., Bynum, E.D., Carpio, L.A., Henne, D.C., Joshi, A., Munyaneza, J.E., Porter, P., Sloderbeck, P.E., Supak, J.R., Rush, C.M., Willett, F.J., Zechmann, B.J., and Zens, B.A. 2012. Seasonal population dynamics of the potato psyllid (Hemiptera: Triozidae) and its associated pathogen “Candidatus Liberibacter Solanacearum” in potatoes in the southern Great Plains of North America. J. Econ. Entomol. 105(4):1268-1276.

Hodgson, E.W., Burkness, E.C., Hutchinson, W.D., and Ragsdale, D.W. 2004. Enumerative and bionomial sequential sampling plans for soybean aphid (Homoptera: Aphididae) in soybean. J. Econ. Entomol. 97(6):2127-2136.

Fig. 1

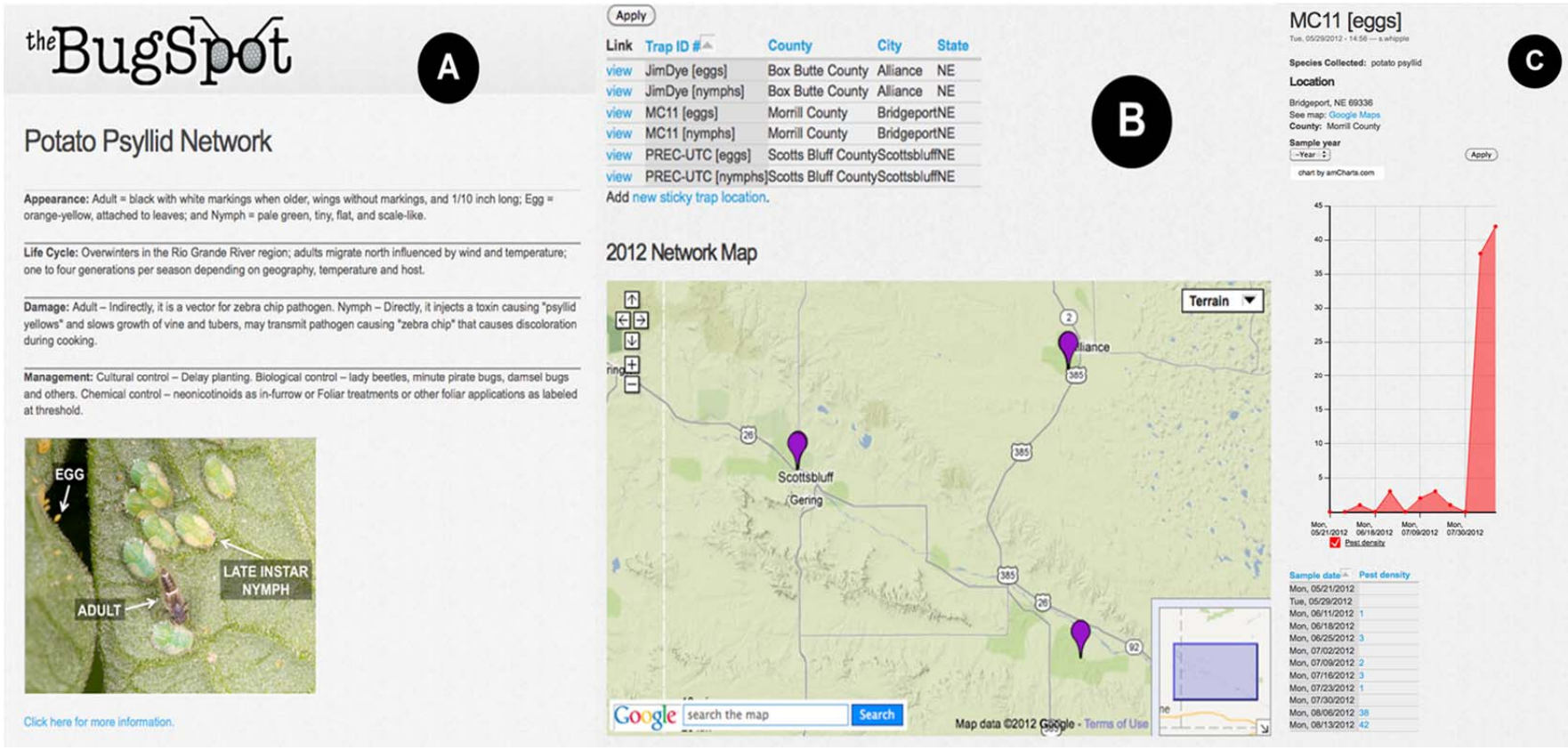
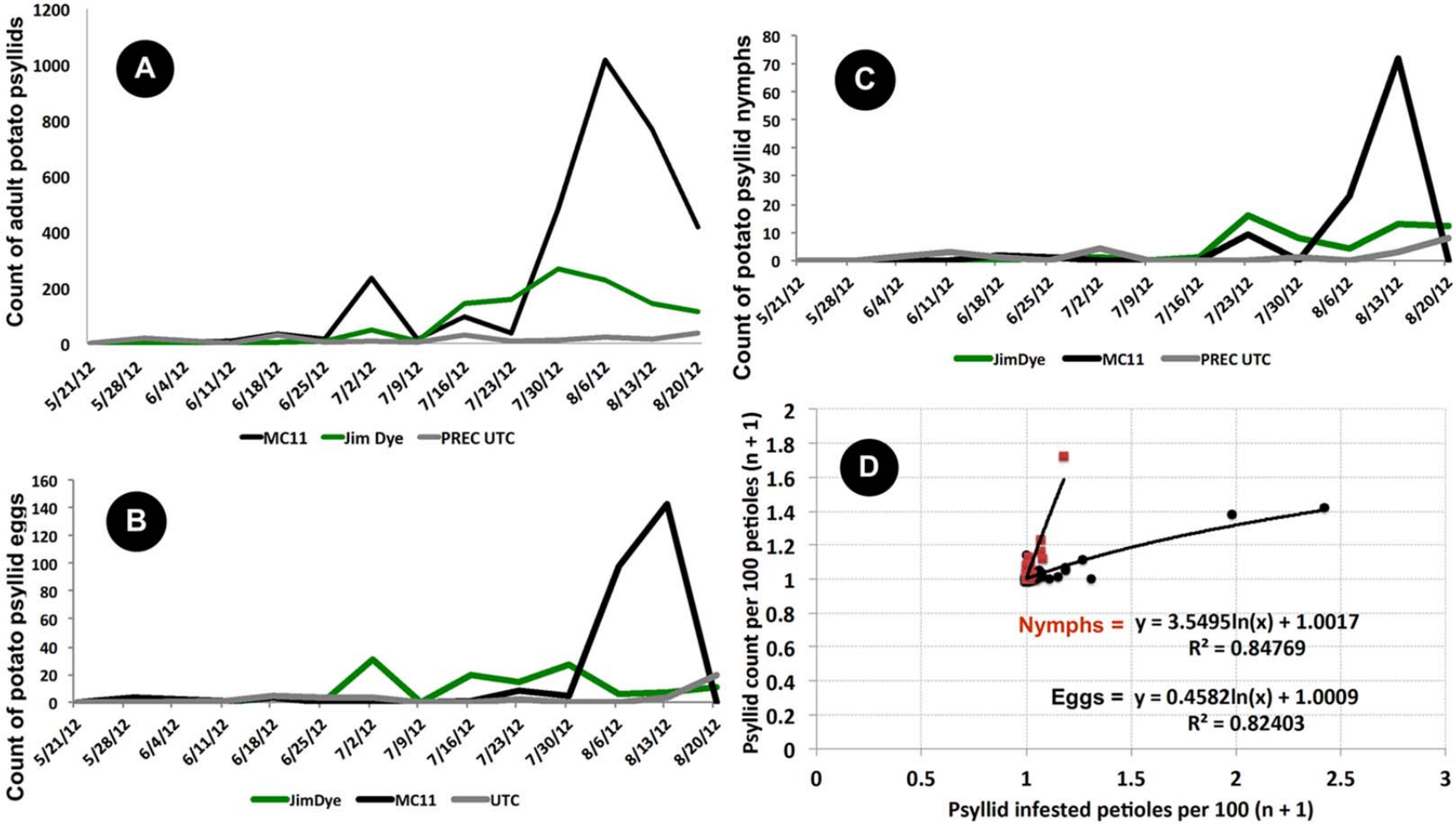

theBugSpot
A
Potato Psyllid Network
Appearance: Adult = black with white markings when older, wings without markings, and 1/10 inch long; Egg = orange-yellow, attached to leaves; and Nymph = pale green, tiny, flat, and scale-like.
Life Cycle: Overwinters in the Rio Grande River region; adults migrate north influenced by wind and temperature; one to four generations per season depending on geography, temperature and host.
Damage: Adult – Indirectly, it is a vector for zebra chip pathogen. Nymph – Directly, it injects a toxin causing "psyllid yellows" and slows growth of vine and tubers, may transmit pathogen causing "zebra chip" that causes discoloration during cooking.
Management: Cultural control – Delay planting. Biological control – lady beetles, minute pirate bugs, damsel bugs and others. Chemical control – neonicotinoids as in-furrow or Foliar treatments or other foliar applications as labeled at threshold.
EGG
LATE INSTAR NYMPH
ADULT
Click here for more information.
Apply
Link Trap ID # County City State
view JimDye [eggs] Box Butte County Alliance NE
view JimDye [nymphs] Box Butte County Alliance NE
view MC11 [eggs] Morrill County BridgeportNE
view MC11 [nymphs] Morrill County BridgeportNE
view PREC-UTC [eggs] Scotts Bluff County ScottsbluffNE
view PREC-UTC [nymphs] Scotts Bluff County ScottsbluffNE
Add new sticky trap location.
B
2012 Network Map
Terrain
Scottsbluff
Gering
Google search the map Search
Map data ©2012 Google - Terms of Use
MC11 [eggs]
Species Collected: potato psyllid
Location
Bridgeport, NE 69336
See map: Google Maps
County: Morrill County
Sample year
Apply
chart by amCharts.com
Mon, 05/21/2012
Mon, 06/18/2012
Mon, 07/09/2012
Mon, 07/30/2012
Pest density
C
Sample date Pest density
Mon, 05/21/2012
Tue, 05/29/2012
Mon, 06/11/2012 1
Mon, 06/18/2012
Mon, 06/25/2012 3
Mon, 07/02/2012
Mon, 07/09/2012 2
Mon, 07/16/2012 3
Mon, 07/23/2012 1
Mon, 07/30/2012
Mon, 08/06/2012 38
Mon, 08/13/2012 42

Fig. 2


A
Count of adult potato psyllids
1200
1000
800
600
400
200
0
5/21/12
5/28/12
6/4/12
6/11/12
6/18/12
6/25/12
7/2/12
7/9/12
7/16/12
7/23/12
7/30/12
8/6/12
8/13/12
8/20/12
MC11
Jim Dye
PREC UTC
B
Count of potato psyllid eggs
160
140
120
100
80
60
40
20
0
JimDye
MC11
UTC
C
Count of potato psyllid nymphs
80
70
60
50
40
30
20
10
0
JimDye
MC11
PREC UTC
D
Psyllid count per 100 petioles (n + 1)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
3
Psyllid infested petioles per 100 (n + 1)
Nymphs = y = 3.5495ln(x) + 1.0017
R² = 0.84769
Eggs = y = 0.4582ln(x) + 1.0009
R² = 0.82403

Psyllids, Whiteflies and Environmental Factors Affecting Potato Trials in S. Texas: 2010-2012

Raul T. Villanueva and Gabriela Esparza-Diaz, Texas A&M AgriLife Extension Service; Weslaco, TX 78596, USA

Abstract

Studies presented here show tallies of population densities of *Bactericera cockerelli* on insecticide trials conducted in 2010, 2011 and 2012 in Weslaco, and results on fried potatoes showing zebra chip disease (ZC). During 2010 and 2011 potato fields at the Texas A&M AgriLife Research and Extension Center in Weslaco had similar ZC damage levels. However, foliage of potatoes with necrotic edges and tubers showing ZC infection levels >65% were observed in 2012. We believe that the high abundance of potato psyllids, and whiteflies, and environmental pollutants caused an aggregate damage to potato plants. These damages were observed across all treatments and in all fields of the AgriLife Center in Weslaco in 2012.

Introduction

Zebra chip disease was detected on potato fields of the Rio Grande Valley in 2000 (Secor and Rivera-Varas, 2004). Since then, the disease has spread to northern states including Arizona, Colorado, Kansas, Nebraska, and Wyoming (Secor et al. 2006). The potato psyllid, *Bactericera cockerelli* is the vector of the bacterium *Candidatus* Liberibacter solanaceraum (*Ca*Lsol). We are presenting data showing population densities of *B. cockerelli* in insecticide trials conducted in 2010, 2011 and 2012. During 2010 and 2011 potato fields had similar ZC damage levels however, tubers showing ZC percentages were extremely high; and the foliage and entire potato plants were severely affected in 2012.
Here, we report results of several insecticide tests conducted in the Lower Rio Grande Valley of Texas from 2010 to 2012, and to discuss the damage observed on the foliage and tubers in 2012.

Materials and Method

2010 and 2011 trials

Small replicate trials (two rows/ treatment) were conducted in 2010 and 2011 using the potato cv. Atlantic. Sprays were conducted on 19 March and 13 April in 2010 with treatments shown in Table 1 and on 4 April and 18 April in 2011 using the treatments shown on Table 2. Psyllid tallies were conducted using a stereomicroscope.

Table 1. Insecticide rates used in 2010

Insecticides	Rate (prod/A)	Amt. Chem. (2 Gal@30gpa)
Radiant SC + DynAmic (0.25 %)	6 oz/AC	11.8 ml +19 ml
Movento +XL (0.25 %)	5 oz/AC	9.9 ml+19 ml
Oberon+ XL (0.25 %)	6 oz/AC	11.8 ml + 19.ml
Oberon+ XL (0.25 %)+UAN (1.5 qt/A)	4 oz/AC	7.9 ml +19 ml + 95 ml
Surround WP	50 lb /100 gal	1 lb
Control	-	-

Table 2. Insecticide rates used in 2011

Treatments	Rate(prod/ac)	Amt. Chem. 1.8 L @30gpa
Radiant SC	6 oz/AC	0.4073 mL
Tolfenpyrad 15 EC + NIS	24 fl oz + 0.25% v/v	1.6294 mL + 0.4 mL
Portal + Nis	32 fl oz + 0.25% v/v	2.1725 mL + 0.54 mL
Control	-	-

2012 trial

In 2012, two large replicate studies were conducted with 12 rows and 50 and 70 ft-long rows per plot. Results of these studies were similar, and in this report we show the results of one of these studies. Each treatment included insecticides that can be used in rotational program (Table 3). The first foliar application was delayed to observe the effects of treatments under high pressure and due to a rainfall that keep the tractors out of the field.

Table 3. Spray programs used in 2012. (Insecticides separated by comma were sprayed on different dates)

Treatments	Materials (Rate/Acre) (all foliar applied)	Application dates
1	HGW86 (13.5 oz), Agrimek (3.5 oz), Agrimek (3.5 oz), Fulfill (5.5 oz)	
2	HGW86 (17.5 oz), Agrimek (3.5 oz), Agrimek (3.5 oz), Fulfill (5.5 oz)	
3	HGW86 (20.5 oz), Agrimek (3.5 oz), Agrimek (3.5 oz), Fulfill (5.5 oz)	2/21, 3/21, 3/27, 4/3
4	Movento (5 oz), Agrimek (3.5 oz), Agrimek (3.5 oz), Fulfill (5.5 oz)	
5	Vydate (1 pt), Vydate (1 pt), Vydate (1 pt), Vydate (1 pt)	
6	Control	-

Results and Discussion

2010 trial

Significant differences were not observed for adult psyllid numbers (Fig. 1a). However, statistically significant reduction of psyllid nymphs were observed on most of treatments compared with the control (Fig. 1b).

Also, significant differences were observed in percentages of plants free of ZC symptoms, plants with yellows before harvest, and in tuber weights at harvest (not shown). However, significant differences were not observed on fried potato chips with ZC symptoms (Fig. 2). Surround showed the highest infection percentages (>14%).

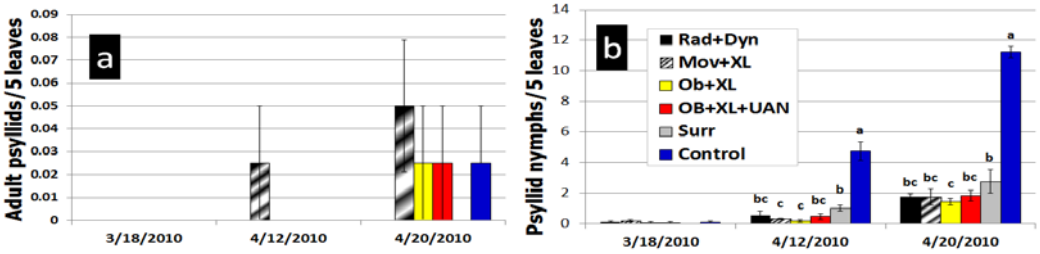


Figure 1. (a) Numbers (x±SEM) of potato psyllid adults and (b) nymphs in 2010

2011 trial

Significant differences were not found for most dates on the abundance of potatoes psyllid adults and nymphs based on ANOVA, Figs. 3a and 3b. At harvest, significant differences for ZC symptoms were not found on cut potato tubers (uncooked) (Fig. 4a) although, percentages between 11.2%±7.5% and 23.1%%± 10.8% were found for Portal and the control (Fig. 4a), respectively. However, the mean percentages of potato tubers showing symptoms after being deep fried were greater for all treatments compared to the uncooked (>40%) (Fig. 4b). In the latter, significant differences were found between all of the insecticide treatments compared with the control.

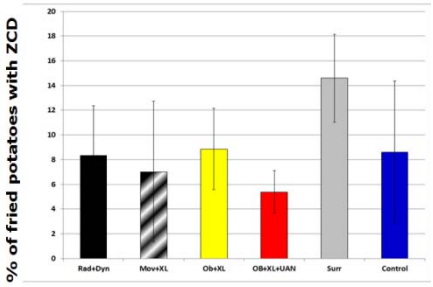


Figure 2. Percentages (x±SEM) of fried potatoes with symptoms of ZC, 2010

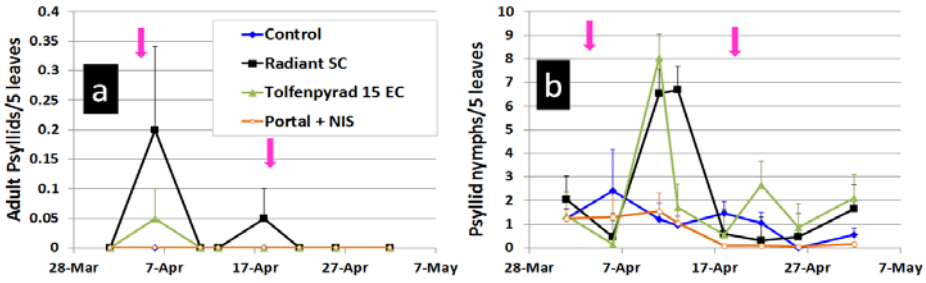


Figure 3. (a) Numbers (x±SEM) of potato psyllid adults and (b) nymphs in 2011 (pink arrow indicates spraying dates)

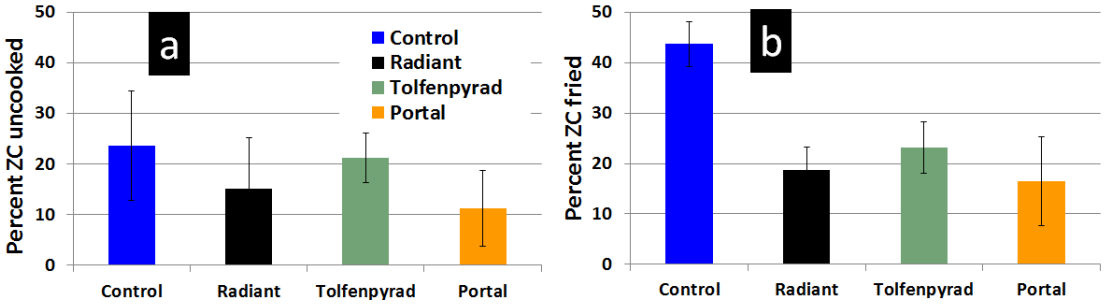


Figure 4. Percentages (x±SEM) of (a) uncooked and (b) fried potatoes with symptoms of ZC 2011

Potato psyllid population tallies were low during January 2012 in Weslaco. However, they increased rapidly in February and March (Figs. 5a and 5b). In addition, two major incidences occurred that affected potato fields in 2012. The first, related to leaf browning on edges, a typical symptoms shown due to environmental damage by pollutants on early February. This damage was observed in all potato fields in the Weslaco center, affecting plants and causing bronzing of leaves. This damage was previously described as ozone damage which causes necrosis of leave edges and early senescence of plants (Fig. 6a and 6b). The second episode dealt with the high abundance of white flies during February and March (Fig 5c). Probably, both pest species and the environmental damage caused an aggregating damage to plants. By mid-March almost all plants were severely damaged across all treatments and plants had few green leaves and symptoms of ZC were observed clearly (Figs. 6c and 6d).

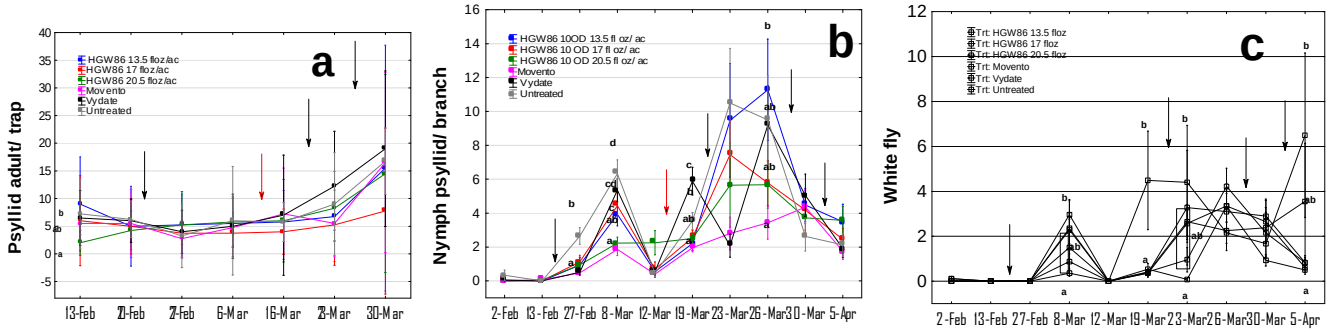


Figure 5. Mean numbers (±SEM) of (a) potato psyllid adults in yellow sticky traps, (b) psyllid nymphs, and (c) whitefly nymphs per leaf in 2012. Arrows show spraying dates.

The environmental damage observed on the foliage across all treatments might have facilitated the early plant senescence in 2012; this type of damage was not observed in 2010 or 2011. However, the cause of this latter damage in 2012 is puzzling and difficult to explain. Ground level ozone values in 2012 were

lower than in 2011 (Fig. 8a), but potato is susceptible to ozone damage (Treshow, 1970). Ozone peaks induce much stronger effects than constant concentrations via the identical total dose (Köllner and Krause, 2000). The mean ultraviolet index (UVI) was higher in 2012 (Fig 8b) than in 2011 for the period when plants were emerging to tuber formation however, these levels were not out of the normal levels to cause damage to potato foliage. Previous studies shown that UV-B radiation, decreased plant height, leaf area, and increased leaf thickness (i.e. Santos et al., 2004). Leaf reduction and plant stunt were observed in 2012, but these were caused by *Ca*Lsol.

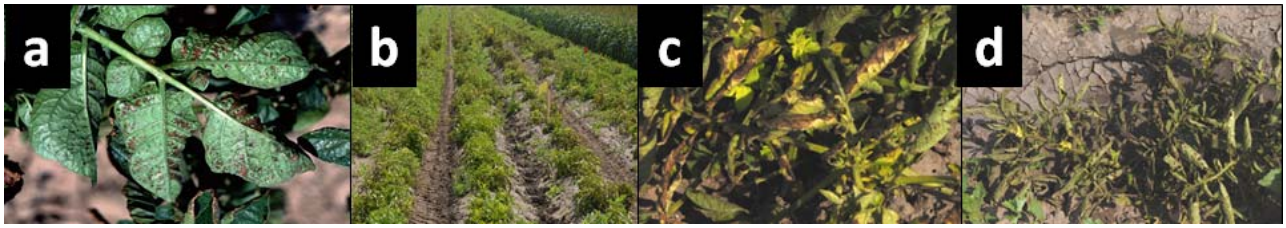


Figure 6. (a) Ozone damage in foliage, (early February), (b and c) patches of plant deteriorating rapidly by mid and late February, (d) plants by mid-March completely defoliated and with ZC symptoms on new leaves.

Whiteflies that appeard by the end of February 2012 might have effectively contributed to the early and rapid plant senescence on plants that were severely debilitated by the environmental damage and *Ca*Lsol. We considered that the potato psyllid and *Ca*Lsol were the major factor that affected potato fields in 2012 however it is important to notice that other factors mentioned above caused the rapid senescence of plants observed in 2012, the reason why tubers were not completely formed and tuber sizes were smaller as it is shown on the comparison of tuber weights for the three years (Fig 9). The behavior and phenology of the potato psyllid requires further research. It is important to investigate why there are some years of high *B. cockerelli* abundance, where *B. cockerelli* overwinters, what kind of hosts are in the southern areas of Texas and in Mexico.

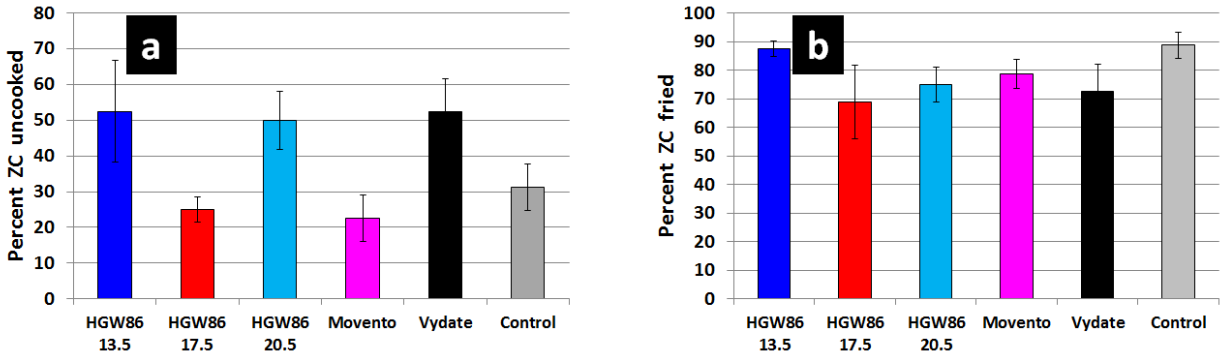


Figure 7. Percentages (±SEM) of (a) uncooked and (b) fried potatoes with ZCD symptoms 2012

The studies conducted in the Rio Grande Valley in S. Texas are important due to the previous reports that shown migration patterns of these insects from the southern regions of TX to the northern areas of the US (Wallis, 1955). Also, several recent studies have shown the presence of potato psyllids and ZC in some northern states (Washington, Idaho and Oregon) in 2012. As shown in Figs. 2, 4 and 7, the ZC percentages on fried potatoes were higher in 2012 than in 2010 and 2011. The main causes of these were the high densities of potato psyllids in 2012.

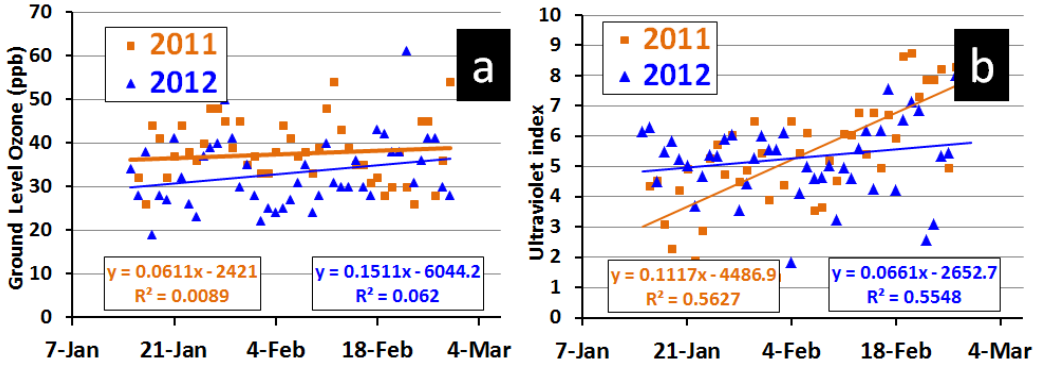


Figure 8. (a) Mean ozone values in Mercedes and Mission, TX (Texas Commission on Environmental Quality), and (b) Mean UVI between McAllen and Brownsville (NOAA National Weather Service)

Acknowledgements

We thank the technical support of Frank Garza, Alma Olguin, and Robert Gonzalez. UVI data for McAllen and Brownsville was provided by Craig Long, NOAA National Weather Service, NCEP Climate Prediction Center. We also want to acknowledge the support from the industry collaborators for providing funds to conduct these studies.

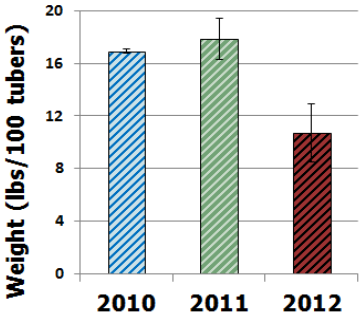


Figure 9. Comparison of tuber weights: 2010 to 2012

References

Köllner, B., Krause, G. H. M. 2000. Changes in carbohydrates, leaf pigments and yield in potatoes induced by different ozone exposure regimes. Agriculture, Ecosystems and Environment 78: 149–158.

Munyaneza, J. E., Goolsby, J. A., Crosslin, J. M., and Upton, J. E. 2007. Further evidence that zebra chip potato disease in the lower Rio Grande Valley of Texas is associated with *Bactericera cockerelli*. Subtropical Plant Sci. 59:30-37.

Santos, I., Fidalgo, F., Almeida, J. M. 2004. Biochemical and ultrastructural changes in leaves of potato plants grown under supplementary UV-B radiation. Plant Sci. 167:925–935

Secor, G. A., Lee, I. M., Bottner, K. D., Rivera-Varas, V., Gudmestad, N. C. 2006. First report of a defect of processing potatoes in Texas and Nebraska associated with a new phytoplasma. American Phytopathological Society (APS Press). Plant Disease. 90(3): 377.

Secor, G. A., and V. V. Rivera-Varas. 2004. Emerging diseases of cultivated potato and their impact on Latin America. Rev. Latinoamericana de la Papa (Suppl.) 1: 1-8.

Texas Commission on Environmental Quality. 2010-2012. Peak Daily One-Hour Ozone Averages. http://www.tceq.state.tx.us/cgi-bin/compliance/monops/peak_monthly.pl.

Treshow, M. 1970. Ozone damage to plants. Environ. Pollut. (1): 155-161.

Wallis, R. L. 1955. Ecological studies on the potato psyllid as a pest of potatoes. Vol. 1107. US Dept. of Agriculture.

Zebra Chip Economics

Guenthner, J. *, G. Greenway *, J. Goolsby** and D. Henne ***
* Department of AERS, University of Idaho, Moscow, ID 83844-2334, USA
** USDA ARS, Weslaco, TX 78596, USA
*** Department of Horticultural Sciences, Texas A&M University, Weslaco, TX 78596

Abstract
The focus of the economic analysis has been on estimating grower costs of controlling zebra chip (ZC) and psyllids. Growers in Texas, Kansas and Nebraska have provided data on insecticide applications in their commercial potato fields in 2009, 2010, 2011 and 2012. The number of different insecticides used in Texas declined from 16 in 2009 to 10 in 2011, then up to 14 in 2012. The most commonly used insecticides in all three states were Admire and Movento. For all locations in Texas growers spent an average of $318 per acre for the four year period while those in Kansas spent $345 and those in Nebraska $220. Those costs were stable for the four years in Texas but more variable in the other two states.

Introduction
The economic analysis part of the project focuses on the project's primary goal of the development of a comprehensive, environmentally responsible ZC disease management program. The three specific objectives of the economic analysis part are:
5.1. Estimate grower losses due to costs of current ZC control practices
5.1. Estimate ZC losses due to poor tuber quality
5.3. Evaluate the economics of alternative disease management strategies.
The focus of the work through fall 2012 has been on Objective 5.1.

Materials and Methods
To estimate ZC control costs we needed the following data:
(1) insecticides growers applied
(2) number of applications,
(3) application rates
(4) insecticide prices
(5) insecticide application costs
For items (1) and (2) we relied on data provided by co-author Dr. John Goolsby with USDA ARS in Weslaco, TX. He collected information regarding insecticide use among his cooperating growers for the 2009-2011 crops. In 2012 Dr Don Henne took over the project. During the four crop years the data included insecticide applications for 69 fields in Texas, Kansas and Nebraska.
The grower data did not include actual application rates. We analyzed insecticide labels for information on recommended application rates and used the highest label rate for individual applications. When total application limits were relevant, we reduced subsequent application rates to comply with maximum allowances. We obtained insecticide prices and pesticide application costs from the following sources:
(1) University of Idaho (Patterson & Painter 2010, Patterson & Painter 2011)
(2) North Dakota State University (2010)
(3) phone calls to agricultural chemical dealers

Results and Discussion

Cooperating growers in Texas used 22 different insecticides for the 2009-2012 crops (Table 1). The number of materials used decreased from 16 products in 2009 and 2010 to 10 in 2011, then up to 14 in 2012. The two most widely used materials in 2011 and 2012 were Movento and Admire. Movento was used in 100% of the fields in 2011 and 2012, moving up from 70% in 2009 and 92% in 2010. Admire use followed a similar upward trend.

Cooperating growers in Kansas and Nebraska used 26 different insecticides for the 2009-2012 crops (Table 2). Most of the materials used in Kansas and Nebraska are the same as those used in Texas. Movento was the most frequently used insecticide in the first three years, but use dropped in 2012. Trends are less clear for Kansas and Nebraska because of variation in the number of fields from 3 in 2009 to 12 in 2010 and 4 in 2011 and 2012. The number of insecticides used dropped from 20 in 2010 to 11 in 2011, then up to 16 in 2012.

Insecticide material and application costs exceeded $100 per acre in all but one of the 69 fields in the four year period (Table 3). The highest cost was $594 per acre at McAllen, Texas in 2012. The average cost in all Texas locations for all four years was $318 per acre. The four-year average for Kansas was $345 per acre, while in Nebraska it was $220 per acre.

The 2009-2012 trend for average insecticide costs is flat to slightly upward for Texas (Figure 1). For Kansas the upward trend for the first three years was broken in 2012. For Nebraska the was no clear trend. For some locations costs varied over a wide range in the same year. For example, 2010 costs in six fields at McAllen, Texas ranged from $176 to $499 per acre. Costs for the three fields at Pearsall, Texas varied from $154 to $401 per acre in 2011. Although average costs per acre seem to have stabilized, the costs in some fields continue to be well above averages.

Additional grower-level costs include yield and quality losses. We conducted a survey of experts who attended the 2011 zebra chip meeting in San Antonio to help estimate yield losses. We asked the respondents to estimate the percent yield loss due to ZC/psyllids, assuming the following:

- growers use best management practices
- typical growing season
- average for all varieties
- locations where ZC/psyllids are currently a problem

Twenty scientists, growers and other industry experts completed the survey. Estimates for yield loss ranged from 0.5% to 75%. The average was 18%. Comments included:

“We have had success recently. It appears to be due to a change in chemical use which allows for survival of beneficials (soft chemistries).”

“The impact on quality is equally important.”

“Averages are misleading because variety is a big factor.”

“Psyllids are a sporadic pest.”

References

North Dakota State University. 2010. Insecticide price list. Retrieved from: http://www.ag.ndsu.nodak.edu/aginfo/entomology/entupdates/ICG_09/25_price_list09.pdf

Patterson, P. and K. Painter. 2011. Idaho custom rates for Idaho agricultural operations 2010-2011. Extension Bulletin 729. University of Idaho.

Patterson, P. and K. Painter. 2010. Idaho crop input price summary. Agricultural Economics Extension Series No 10-02. University of Idaho.

Table 1. Insecticide use to control ZC and psyllids, Texas, 2009-12

Insecticide		Fields treated (%)			
Common Name	Active Ingredient	2009	2010	2011	2012
Admire Pro	Imidacloprid	40%	75%	92%	92%
Agri-Mek	Abamectin	40%	67%	25%	58%
Asana	Esfenvalerate	30%	17%		17%
Assail	Acetamiprid				8%
Baythroid	B-Cyfluthrin	10%	8%		8%
Belay	Clothianidin		8%		
Beleaf	Flonicamid	20%	17%	25%	50%
Epi-Mek	Abamectin	30%	33%	58%	58%
Fulfill	Pymetrozine	70%	42%	50%	50%
Leverage 360	Imidacloprid + beta-cyhalothrin	10%	8%		
Macho	Imidacloprid				8%
Movento	Spirotetramat	70%	92%	100%	100%
Oberon 2 SC	Spiromesifen	40%	58%	42%	75%
Platinum	Thiamethoxam	30%	8%		
Perm UP	Permethrin				8%
Radiant SC	Spinetoram	10%			
Requiem	chenopodium ambrosioides				8%
Thimet	Phorate	10%		8%	
Thiodan	Endosulfan	10%	8%	8%	
Venom (foliar)	Dinotefuran	30%	8%	17%	33%
Venom(soil)	Dinotefuran	10%	8%		
Vydate C	Oxamyl		8%		
Number of fields in sample		10	12	12	12
Total number of insecticides used		16	16	10	14
Average number of insecticides used per field		5.3	5.0	5.3	6.2
Average number of insecticide applications		8.7	7.9	7.9	13.1

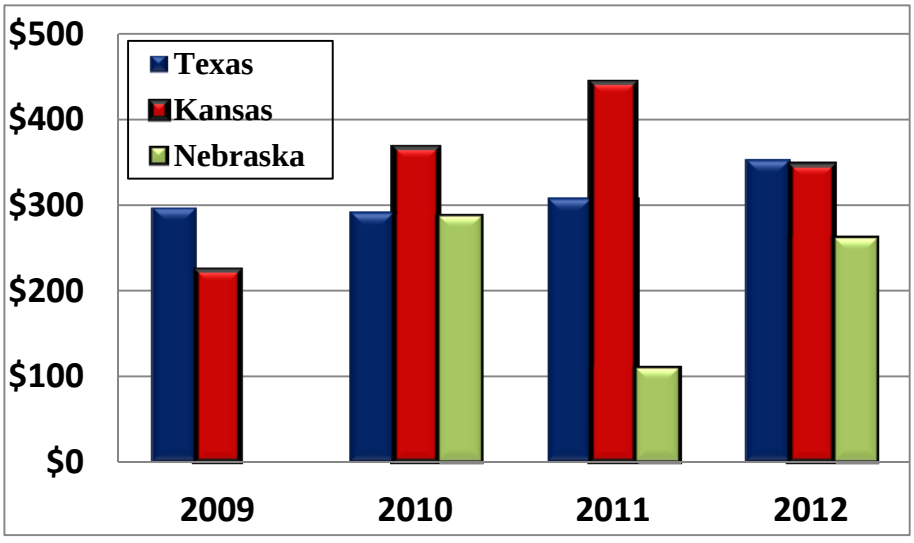
Table 2. Insecticide use to control ZC and psyllids, Kansas and Nebraska, 2009-12

Insecticide		Fields treated (%)			
Common Name	Active Ingredient	2009	2010	2011	2012
Abacus	Abamectin		8%		
Admire Pro	Imidacloprid	100%	58%	50%	13%
Agri-Mek	Abamectin		42%	25%	19%
Asana	Esfenvalerate	100%	25%	25%	
Baythroid	B-Cyfluthrin	33%	75%		
Beleaf	Flonicamid			25%	6%
Dimate	Dimethoate		25%		
Endigo	Lambda-cyhalothrin + Thiamethoxam		25%		13%
Endosulfan	Endosulfan		8%		
Epi-Mek	Abamectin		25%		6%
Fulfill	Pymetrozine		33%	25%	6%
Imidacloprid	Imidacloprid				13%
Leverage 360	Imidacloprid		8%	25%	
Lannate	Methomyl				6%
Movento	Spirotetramat	100%	75%	75%	13%
Mustang	s-cyano(3 phenoxyphenyl) methyl, ±,2,2 dimethylcycloprane carboxylate				6%
Oberon 2 SC	Spiromesifen		33%	25%	19%
Platinum	Thiamethoxam		42%		
Pounce	Permethrin		25%	25%	13%
Regent	Fipronil		17%		
Rimon	Novaluron				13%
Requiem	chenopodium ambrosioides				6%
Scorpion	Dinotefuran		33%		
Thimet	Phorate	100%	25%		13%
Thiodan	Endosulfan		17%	25%	13%
Vydate C	Oxamyl		8%		
Number of fields in sample		3	12	4	4
Total number of insecticides used		5	20	10	16
Average number of insecticides used per field		4.3	6.3	4.0	7.5
Average number of insecticide applications		7.7	9.5	6.0	13.3

Table 3. Insecticide costs for ZC and psyllid control, 2009-12

Year/Locations	Fields	Low ($/acre)	High ($/acre)	Average ($/acre)
2009				
Kansas, Garden City	3	$214	$241	$223
Texas, Dalhart	2	$286	$292	$289
Texas, McAllen	4	$296	$344	$319
Texas, Olton	1	$223	$223	$223
Texas, Pearsall	3	$214	$452	$358
2010				
Kansas, Garden City	3	$303	$399	$367
Nebraska, Alliance	3	$296	$354	$315
Nebraska, Imperial	3	$321	$515	$397
Nebraska, Minden	3	$131	$191	$153
Texas, Dalhart	2	$323	$388	$355
Texas, McAllen	6	$176	$499	$362
Texas, Olton	1	$270	$270	$270
Texas, Pearsall	3	$151	$226	$180
2011				
Kansas, Garden City	1	$443	$443	$443
Nebraska, Alliance (Angora, Bridgeport)	2	$146	$231	$188
Nebraska, Minden	1	$31	$31	$31
Texas, Dalhart	3	$252	$358	$304
Texas, McAllen	4	$229	$338	$274
Texas, Olton	2	$240	$516	$378
Texas, Pearsall	3	$154	$401	$279
2012				
Kansas	2	$339	$355	$347
Nebraska	2	$183	$341	$262
Texas, Dalhart	2	$313	$425	$369
Texas, McAllen	5	$147	$594	$317
Texas, Olton	2	$328	$531	$429
Texas, Pearsall	3	$250	$340	$297

Figure 1. Insecticide costs for ZC and psyllid control, 2009-12



Extension Activities for the Zebra Chip SCRI Program 2010-2012

Harveson, R.[1], Bradshaw, J.[1], Whipple, S.[1], French, R.[2], Schuster, G.[2], and Appel, D[2].
[1]University of Nebraska, Panhandle REC, Scottsbluff, and [2]Texas Agri-Life Extension.

Abstract
This report summarizes the activities to date for the extension participants in the SCRI program. These efforts include the production, distillation, and dissemination of information generated by the research community in the form of field days, informal and formal extension meetings, and written publications published as both electronic and hard copy formats. Additional information has also been generated by extension personnel through research and demonstration plots and applied research projects at both the field and lab/greenhouse levels. Further transfer of knowledge has occurred in the classroom ranging from the college graduate level to elementary school-age children.

Introduction
The Cooperative Extension Service (CES) was created by the Smith-Lever Act in 1914. It is the world's largest network (more than 16,000 educators in 50 states and four territories) with the goal of providing informal adult education as a service to help solve problems. As the name implies, it is a *cooperative* educational relationship among three levels of government.

Cooperative Extension is the educational branch partnering with the Land Grant Colleges and the Department of Agriculture in the U.S. It is a unique educational system that takes research-based knowledge developed through experimentation at research centers or grower fields. This information is then interpreted and disseminated ("extended") to individuals or industries who can then utilize it. It additionally attempts to modify prior behavior of people by offering programs and demonstrating more efficient methods for solving local problems, with the mission of "helping people help themselves". The purpose of this report will be to outline and summarize the efforts by the Extension participants of the SCRI zebra chip project.

Extension's Role in the SCRI Project
Extension member participants have contributed to this project in a number of unique ways. We have made presentations and conducted informal conversational meetings and workshops to numerous professional commodity boards and potato growers. This also includes presentations by all members at the Annual ZC Reporting Sessions for the last two years. Other activities outlined below also include: 1) designing and maintaining research/demonstration plots to address specific goals, 2) production of extension-oriented publications, both electronic and hard copy, and 3) the establishment of collaborative relationships with research personnel also involved with the SCRI project. As an example, oral presentations were made to grower, crop consultants, and commodity groups during Winter Extension Meetings, numbering more than 40 separate presentations to an estimated audience of approximately 900 contacts.

Demonstration/Research Plots (Field-Oriented Presentations)

- Planting dates and their effects on insect behavior and disease development
- Cultivar resistance evaluation and development
- Insecticide efficacy trials
- Psyllid sample monitoring with novel "tower traps" at multiple sites
- Evaluation of alternative chemical agents (biological/SAR compounds)

An estimated 800 contacts were exposed to current knowledge of the zebra chip/psyllid pathogystem through these field events.

Collaborative Projects:

- Psyllid monitoring as part of the overall National Psyllid Sampling Project
- Commercial field surveys to establish incidence and distribution of pathogen
- Established weather station monitoring sites and collected weekly environmental data
- Pathogen Diagnostics and Detection
    - Psyllids
    - Potato plants and tubers
    - Solanaceous weeds and alternate hosts

Publications and Information Delivery

- ZC website was developed and launched with the purpose of serving as a "one stop shop" for all things involving the zebra chip disease
- Three ZC fact sheets from Texas A&M, (one in Spanish) were produced electronically and included on multiple websites
- Two NebGuides and four Diagnostic Pocket Cards (University of Nebraska) were developed and are available on-line via several websites.
- Approximately 1500 hard copies were additionally printed and distributed to extension personnel in 12 states for distribution as they saw fit.
- These same publications were included in the registration packet for all participants of the 2012 ZC Reporting Sessions).
- More than 20 additional articles on various aspects of the disease and its vector have been published in trade journals and magazines, extension meeting proceedings, newsletters and newspapers with reader subscription
  estimated at >18,000 throughout the Midwest and Central High Plains
- Our psyllid sampling procedures have been integrated into mobile applications and delivered through thebugspot.org in collaboration with Kansas State University.
- Approximately 75 hard copies of the 2011 ZC Reporting Sessions have been distributed throughout Colorado, Nebraska, and Wyoming for those not attending reporting meetings.

Scanning Electron Microscopy of "*Candidatus* Liberibacter solanacearum" in Infected Tomato Phloem Tissue

Chen, J. USDA-ARS, Parlier, CA 93648, USA

Abstract
"*Candidatus* Liberibacter solanacearum" is an alpha-proteobacterium associated with potato zebra chip disease. The bacterium is currently non-cultureable. Very little is known about the bacterial morphology, an important characteristic of a complete bacterial description. In this study, "*Ca*. L. solanacearum" were examined by scanning electron microscopy in tomato phloem tissues where the bacterial cells were enriched. Bacillus, coccus, and pleomorphic bacterial cells were observed in phloem cells of infected tomato petioles. A preliminary analysis suggested that the different morphological types might reflect various life stages of "*Ca*. L. solanacearum". The new information increases our current understanding of the biology of "*Ca*. L. solanacearum" and will facilitate future efforts in the bacterial cultivation and host-bacterium interaction research.

Introduction
"*Candidatus* Liberibacter solanacearum" is currently regarded as the pathogen of potato zebra chip disease. Because of the challenge in *in vitro* cultivation, characterization of "*Ca*. L. solanacearum" has been exclusively DNA sequence-based. Detection of the bacterium heavily relies on PCR, a technique capable of amplifying "*Ca*. L. solanacearum" DNA under low titer situation. Primers flanking a small section of the bacterial genome with an amplicon ranging from hundreds to thousands bp were designed. The presence of "*Ca*. L. solanacearum" is represented by the observation of a target DNA band in conventional PCR, or appropriate Ct value readings in real-time PCR.

It is apparent that a complete description of "*Ca*. L. solanacearum" requires information beyond PCR results. As research on "*Ca*. L. solanacearum" deepens, many biological features of the bacterium need to be studied. One of them is the bacterial morphology, or what "*Ca*. L. solanacearum" looks like. Extensive knowledge of morphology could significantly facilitate our understanding of "*Ca*. L. solanacearum" and facilitate further scientific research including *in vitro* cultivation. In the history of fastidious prokaryote research, the recognition of spiroform morphology played a significant role in the success of *in vitro* cultivation of corn stunt spiroplasma (Chen & Liao, 1975; Williamson & Whibomb, 1975).

Direct microscopic observation of bacterial cells requires the availability of pure or enriched cell culture. Because "*Ca*. L. solanacearum" is currently not cultureable *in vitro*, an effort was made to enrich "*Ca*. L. solanacearum" cell titer using tomato plants (Chen, 2010, 2011). A California strain of "*Ca.* L. solanacearum" in tomato plants has been maintained in our greenhouse for over two years. PCR with primer set OA1/OA2c consistently detect the presence of "*Ca.* L. solanacearum". Symptoms of shoot stunting and leaf purpling occurred, but infected plants survived. On top of the previous efforts, this research focused on using the infected tomato plants to study the morphology of "*Ca.* L. solanacearum" with scanning electron microscopy.

Materials and Methods

Source of "*Ca.* L. solanacearum". "*Ca.* L. solanacearum" from a potato plant identified in California was originally transmitted to a tomato cultivar through grafting and has been maintained in a greenhouse located at the USDA-ARS San Joaquin Valley Agricultural Sciences Center, Parlier, California. The "*Ca*. L. solanacearum" infected tomato plants were subjected to standard greenhouse management (daily watering and weekly fertilizing). Greenhouse temperature setting ranged from 23° C to 29° C. Part of the bacterial maintenance was propagation of infected plants using young shoots. Infection of "*Ca*. L. solanacearum" was confirmed by PCR detection of tomato tissue using primer set OA2/OI2c (Liefting et al., 2009).

Scanning electron microscopy. Petioles of tomato leaves showing purpling symptoms and positive with PCR detection were fixed in 2.5% glutaraldehyde and 2.5% formaldehyde prepared from paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4°C. A mild vacuum was applied to ensure the rapid infiltration of fixative into the specimens. The fixed specimens were washed three times with 0.1 M phosphate buffer (pH 7.2), incubating at 23°C for 30 min each time. The specimens were infiltrated overnight with a cryoprotectant, 30% glycerol in water. Specimens were plunge-frozen in liquid N2, then cross-sectioned with a scalpel blade. Specimens were post-fixed in 1% OsO4 in 0.1 M phosphate buffer (pH 7.2) for 1 h, and then washed three times in deionized water incubating 30 min for each wash. The specimens were dehydrated in an ethanol series to 100% ethanol, then dried to the critical-point. The dried specimens were mounted on aluminum stubs and sputter-coated with gold. Observations and digital photos were made with a Hitachi S-3500N SEM at 15 kV.

Results and Discussion

All SEM observation began with the overview of vascular system (Fig. 1). Xylem vessels were easy to find for the presence of rings on the cell walls and also the large cell sizes. The information was used to assist the identification of phloem cells. Unlike the empty xylem vessels, phloem cells were filled with cellular matrix. closer examinations found that in many of the phloem cells, bacterial cells, characteristic by their micro-meter sizes, were frequently observed along with cellular matrix (Fig. 2). Because the tomato plants have been subjected to many times of selections or enrichments through specific PCR detection, it was assumed that the observed bacteria were "*Ca.* L. solanacearum".

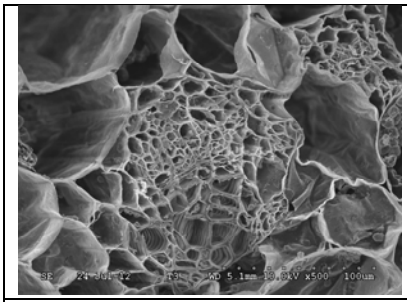


Fig. 1. Vascular tissue of an infected tomato leaf petiole.

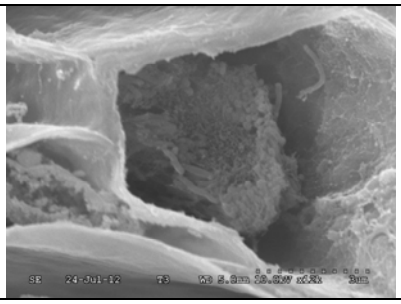


Fig. 2. Abundance of bacterial cells in a phloem cell.

In general, two types of bacterial cells were dominant: bacilli (Fig. 3) and cocci (Fig. 4). Bacillus cells could fills a phloem cell, whereas coccus type of bacterial cells were in general at a much lower titer. It was also common to observe the presence of both cell types simultaneously (Fig. 5). There was evidence that some coccus cells were actually produced from the extension of bacillus cells, suggesting that the

two morpholocial types were interchangeable, or there were two morphological stages of "*Ca*. L. solanacearum". More interestingly, "*Ca*. L. solanacearum" did not seem to be in typical bacillus form like *Bacillus*, or coccus form like *Staphylococus*. Instead, cells showed a level of polymorphism. Branching or budding cells could often been found (Fig. 6).

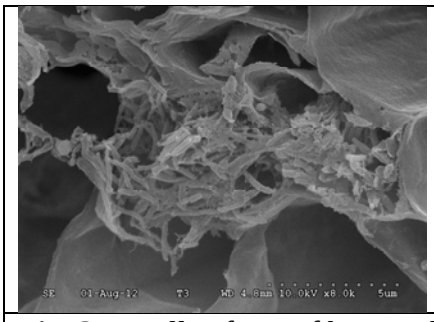


Fig. 3. Bacillus form of bacterial cells.

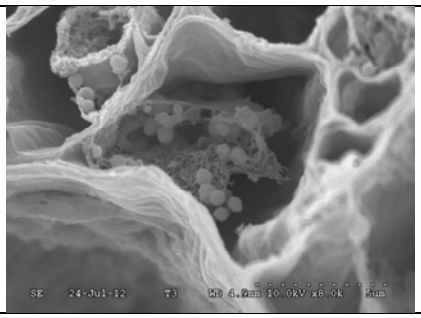


Fig. 4. Coccus form of bacterial cells

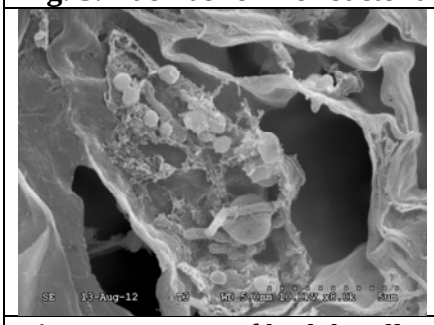


Fig. 5. Presence of both bacillus and coccus forms.

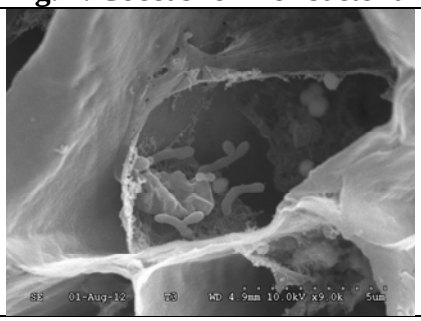


Fig. 6. Bacterial branching or budding forms.

The morphology of "*Ca*. L. solanacearum" was previously described by transmission electron microscopy (TEM) (Liefting et al., 2009; Secor et al., 2009). These reports showed evidence of bacillus type of bacteria. But some images could be interpreted as the presence of short bacillus or coccus cells. TEM primarily examines only sections of bacterial cells and a section of a coccus cell could not be differentiated from a transverse section of a bacillus cell. SEM is advantageous in its capacity to reveal a complete cell morphology. In addition, it also provide information about cell development and intracellular location of "*Ca*. L. solanacearum" in its hosts. The new information enriches our current understanding of "*Ca*. L. solanacearum" and will facilitate future research in the bacterial cultivation and host-bacterium interactions.

Acknowledgements

We thank Dennis Margosan, Rebecca Huerta, and Greg Phillips for their technical assistance. This research was supported by grants from SCRI (2009-34381-20036) and the Texas Department of Agriculture.

References

Chen, J., van Hest, P. A. and Civerolo, E. L. 2010. Establishing and fine-tuning an *in planta* system for potato Zebra chip disease research in California. Proceedings of the 10th Annual Zebra Chip Reporting Session, page 115-119.

Chen, J. 2011. Enrichment and characterization of "*Candidatus* Liberibacter solanacearum" in tomato host. Proceedings of the 11th Annual Zebra Chip Reporting Session, page 154-158.

Chen, T. A. and Liao, C. H. 1975. Corn stunt spiroplasma: isolation, cultivation, and proof of pathogenicity. Science 188:1015-1017.

Liefting, L. W., Weir, B. S., Pennycook, S. R., and Clover, G. R. G. 2009. '*Candidatus* Liberibacter solanacearum', associated with plants in the family *Solanaceae*. Int. J. Syst. Evol. Microbiol. 59:2274-2276.

Secor, G. A., Rivera, V. V., Abad, J. A., Lee, I.-M., Clover, G. R. G., Liefting, L. W., Li, X., and De Boer, S. H. 2009. Association of '*Candidatus* Liberibacter solanacearum' with zebra chip disease of potato established by graft and psyllid transmission, electron microscopy, and PCR. Plant Dis. 93:574-583.

Willamson, D. L. and Whitcomb, R. F. 1975. Plant mycoplasmas: a cultivable spiroplasma causes corn stunt disease. Science 188:1018-1020.

New Methods for Streamlining the DNA Extraction Process for Detection of "*Candidatus* Liberibacter solanacearum" from Insect Vectors

Levy, J.G., Hancock, J., Ravindran, A., Gross, D.C., Tamborindeguy, C., and Pierson, E.A. Texas AgriLife Research, College Station, TX.

Abstract

Accurate pathogen detection of *'Candidatus* Liberibacter solanacearum'(Lso) in insects and plants is essential for management of potato zebra chip (ZC) disease. Current methods rely on conventional polymerase chain reaction (cPCR) assays or quantitative real-time polymerase chain reaction (qPCR). Usually these methods necessitate extraction of pure DNA before amplification. Thus, current detection methods consist of four steps: 1. Sampling, 2. DNA extraction, 3. PCR amplification, and 4. Analysis of PCR results. Our efforts to improve detection methodology include previous studies on the movement and titer of bacteria in plant tissues to improve sampling and improvements in PCR-based amplification methods via a) the development of better conventional PCR (cPCR) primers, b) a more sensitive standard-curve based quantitative real time PCR (qPCR) methodology, and c) development of a loop-mediated isothermal amplification (LAMP), protocol, which does not require gel electrophoresis for analysis of PCR results (Levy et. al 2011, Ravindran et. al 2011, 2012). Reported here is a protocol for rapid DNA isolation from the psyllid vector *Bactericera cockerelli* that can be used directly with PCR or LAMP for the detection of Lso. The time required to complete the fast DNA extraction for 10 samples was on average approximately 5 minutes and required no special reagents or laboratory equipment. Detection of Lso in psyllids using the fast DNA extraction coupled with LAMP amplification can be performed *in less than 90 minutes* and is portable enough to be performed on the bed of a pick-up truck.

Introduction

Current protocols for the detection of Lso in psyllids rely on cPCR or qPCR assays. Recently, we introduced LAMP as a detection tool for Lso. Previously, it was thought that PCR-based methods required the extraction of purified DNA for reliable amplification. The existing emphasis on obtaining purified DNA stems from concerns over the possibility that phenolics or other PCR inhibitors might be present in crude extracts, reducing the efficacy of PCR-based amplification. In this study we, tested a protocol for the rapid extraction of DNA that would be of sufficient quality for direct, reliable amplification of DNA via cPCR or LAMP. If effective, this method would substantially improve the current detection protocols by reducing the time, equipment and cost per sample. To fully understand how this methodology would streamline the current detection protocols, it is important to understand each of four required steps: 1. Sampling, 2. DNA extraction, 3. PCR amplification, and 4. Analysis of PCR results.

A summary of the current methods relating to each step is given below:

1. Sampling: Current methods include the use of sticky traps or vacuum samplers in commercial fields or manual collection using an insect net.
2. DNA extraction: Current methods include the use of standard laboratory protocols or commercial kits. The CTAB method is the most commonly used standard laboratory method. It utilizes the surfactant CTAB and other chemical reagents including chloroform and isoamylalcohol, which must be used and discarded in a laboratory setting. The method also requires laboratory equipment including a water bath, centrifuge, and freezer and 3-4 hours (~20 steps) to complete 10 samples. Commercial kits available from various manufacturers (e.g. Qiagen, Promega, or MoBio) are typically the most expensive extraction method. They

also require the use of lab equipment, but are typically faster, requiring about 30 minutes to complete.

3-4. Amplification and Detection: These steps are typically accomplished using cPCR, qPCR, or LAMP. cPCR requires a thermocycler, 1-2 hours for set-up and amplification, and an additional 30-60 minutes for gel electrophoresis (typically requiring use and disposal of the carcinogen ethidium bromide) to analyze the PCR products. qPCR requires a real-time qPCR instrument, 60-90 minutes of set up of three replicates of 10 samples, ~2 h for amplification, and ~2 h for data analysis. Although qPCR is more sensitive that cPCR the reagents and technology are substantially more expensive. LAMP requires only a simple incubator; it does not require a thermocycler for amplification or agarose gel electrophoresis for resolution. Positive LAMP results are visualized directly as a precipitate in the reaction tubes and the entire process can be completed in 1 h. Although not as sensitive as qPCR, it is comparable to cPCR.

In this study, we present our Fast DNA extraction protocol that requires only sterile molecular grade water and a couple of minutes per sample. This process consists of three steps: sufficient grinding of the insect to release DNA, re-suspension of the homogenized material, and dilution. Given the potential for PCR inhibitors, we examined the importance of dilution for reliable PCR amplification and whether the extraction method could be used for multiple insect samples. We also present results on the efficacy of cPCR and LAMP amplification of insect and Lso targets from DNA extracts prepared using the Fast DNA extraction.

Materials and Methods

Fast DNA extraction method: Samples included either individual insects or multiple insect samples (3 or 5 insects/sample). The fast DNA extraction method was modified from (Gloor and Engels 1992). Briefly, live insects (nymphs or adults) from our laboratory colonies or dead insects obtained from yellow sticky traps (BioQuip) were placed into individual 1.5-ml microcentrifuge tubes and homogenized. Homogenized material was re-suspended in 75 µL of sterile water.

CTAB DNA extraction and purification method: Our standard method for the extraction and purification of DNA from psyllids utilizes CTAB (cetyltrimethylammonium bromide and was modified from (Reineke et al. 1998). The purified DNA was resuspended in 70 µL sterile water.

Conventional PCR: cPCR reactions (15 µL final volume) were performed using the GoTaq green Master Mix Promega Corp. (Madison, WI) and the following volumes of reagents: 1 µL of DNA extract, 7.5 µL of Master Mix, 1 µL of each primer (10 mM), and 4.5 µL sterile water. The PCR amplification was performed using an Eppendorf Thermocycler (Hamburg, Germany) using the following reaction conditions: (95°C for 3 min; 40 cycles of 95°C for 40 s, 58°C for 40 s, and 72°C for 1 min; and 72°C for 10 min. PCR products (all 15 µL) were resolved by electrophoresis in 1% agarose gels stained with ethidium bromide. For all analyses, a negative control (sterile water) and a positive control (DNA extracted and purified from a psyllid and previously shown to be Lso-positive via cPCR) were included. PCR primers were designed to target the *Bactericera cokerelli* 28S rDNA gene as previously published (Nachappa et al. 2011). The PCR primer sets used for detection of Lso were designed to target the Lso 16S-23S rDNA intergenic region (Lso TX 16/23 F/R) (Ravindran et al. 2011).

LAMP: LAMP amplification for detection of Lso was carried out in a final volume of 25 µL using the six LsoTX16SLAMP primers and other reagents exactly as published previously (Ravindran et al. 2012). The amplification reaction was conducted in a dry bath at the temperature 60°C for 60 min as described previously (Ravindran et al. 2012). LAMP amplification of the Lso target was determined by direct visualization of the formation of magnesium pyrophosphate during the DNA synthesis. Results were confirmed by electrophoresis (using all 25 µL) in 2% agarose gels stained with ethidium bromide. Positive and negative controls were as above.

Results and Discussion

Given the potential for PCR inhibitors in the FAST DNA extract, we examined the importance of dilution for reliable cPCR amplification. The cPCR amplification was conducted using 1 µL of either undiluted extract (1X) or extract diluted 1/10 and 1/100 in sterile water (Table 1). We found that cPCR amplification of insect and Lso target genes was reliable only when DNA was diluted 1:100 with sterile water.

Table1: Detection of insect DNA (positive control) using insect 28S rDNA primers and detection of Lso using Lso TX 16/23 primers in single insect and multiple insect (3 to 5 insects) samples. Samples were prepared using the FAST DNA extraction method.

Dilution (extract/water)	**MULTIPLE INSECT SAMPLES**		**SINGLE INSECT SAMPLES**	
	Insect (28S rDNA gene)	Lso (16S-23S rDNA gene)	Insect (28S rDNA gene)	Lso (16S-23S rDNA gene)
1X	0/8	0/8	3/25	2/25
1/10	0/8	0/8	13/25	2/25
1/100	8/8	8/8	25/25	8/25

We also tested the efficacy of cPCR amplification of the insect 28S rDNA target from single insect samples using insects in different life stages (adults vs. nymphs) or from different types of samples (alive vs. from sticky traps). cPCR amplification rates for adults and nymph were 94 and 88% respectively (Table 2), which is nearly identical to our success rates using purified DNA (typically the CTAB method: data not shown). Amplification rates for insects off yellow sticky traps were slightly lower and this could be related to their state of decay and our ability to correctly select psyllids when insects are in that state.

Table 2: Detection via cPCR of the insect 28S rDNA target from single insect samples using insects in different life stages (adults vs. nymphs) or from different sample types (alive vs. from sticky traps). The percentage of successful amplifications is given parenthetically for each type of sample analyzed. Samples were prepared from single insects using the FAST DNA extraction protocol and diluted 1/100 in sterile water.

Psyllid Samples	**Positive samples/total number (%)**
Live adults	90/92 (97.8%)
Live nymphs	14/16 (87.5%)
Dead adults (from sticky traps)	30/35 (85.7%)
Total from all samples	**134/ 143 (93.7%)**

To determine whether the FAST DNA extraction protocol also would work with LAMP, we prepared DNA samples using the FAST DNA amplification protocol and then amplified the same samples using both cPCR and LAMP (Fig. 1, Table 3). LAMP amplification of Lso targets from Lso-infected insect samples yielded strong positive results indicating this method is robust enough for using either with Taq (cPCR) or Bst (LAMP) DNA polymerase (Fig. 1). LAMP was more effective than cPCR in detecting Lso in insect samples prepared from randomly selected insects (Table 3), a finding previously reported for samples prepared using the CTAB method (Ravindran et al. 2012).

Figure 1: Comparison of LAMP and cPCR amplification of single insect samples prepared using the FAST DNA extraction protocol. Samples were diluted 1:100 in sterile water. LAMP results are visualized via a) in tube fluorescence (e.g. with calcein and $MnCl_2$ in the reaction mix) on a UV light stand b) gel electrophoresis with ethidium bromide staining. cPCR results are visualized c) via gel electrophoresis and ethidium bromide staining. Sample details: M= 100bp ladder, 1 to 4 = Lso-infected psyllids, 5= uninfected psyllid, 6= positive control, 7,8= negative control.

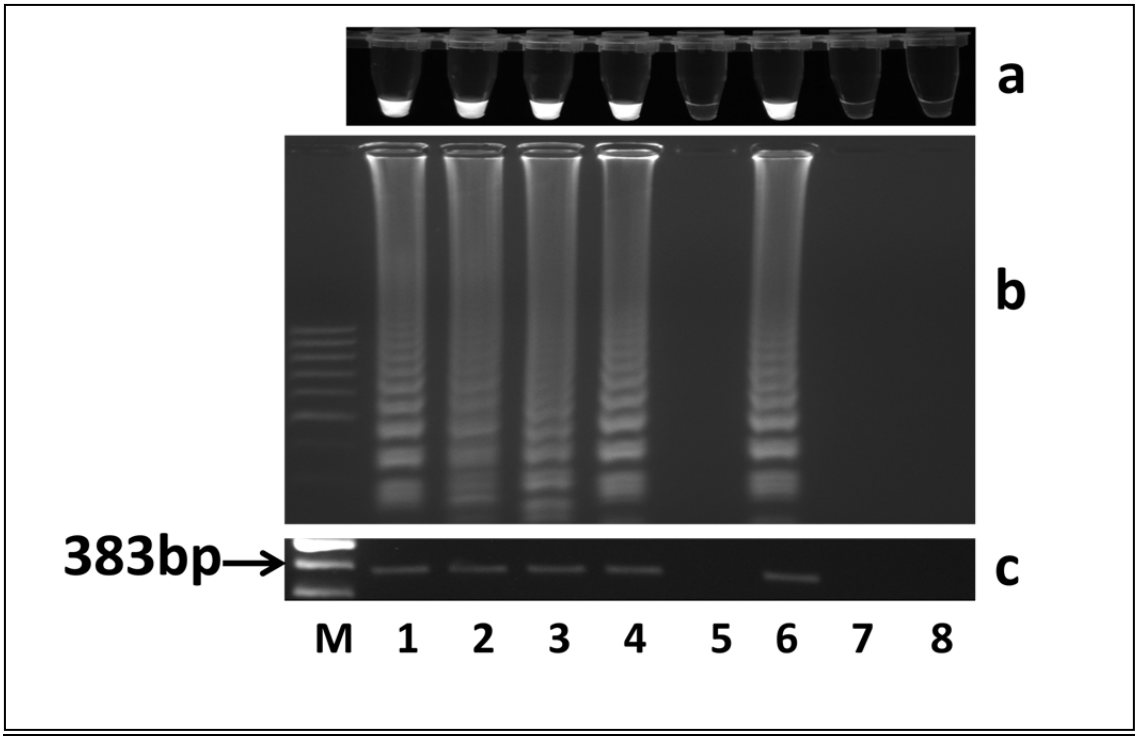


Table 3: Insect samples prepared using the FAST DNA extraction protocol and analyzed using both cPCR and LAMP. The percentage of samples in which Lso was detected is given in parenthesis.

Insect samples	cPCR	LAMP
Live adults from infected colony	17/28 (61%)	19/28 (68%)
Live adults from uninfected colony	0/17	0/17

Summary

We developed a method for the rapid isolation of DNA from insect vectors that can be used with cPCR or LAMP for reliable detection of Lso. The FAST DNA extraction protocol requires little time (*5 minutes for 10 samples*), labor (*three steps*) and no specific equipment. Samples prepared from single or multiple insect samples using the FAST DNA extraction protocol should be diluted 1/100 in sterile water for reliable cPCR or LAMP amplification. LAMP amplification requires no thermocycler or gel electrophoresis step for analysis, therefore coupling the FAST DNA protocol with LAMP greatly streamlines the current detection process by reducing the time and materials needed for DNA extraction, DNA amplification, and PCR analysis, making this process efficient, reliable and portable. Nevertheless LAMP is still a more expensive method than conventional PCR.

Acknowledgements

Work was supported by the Texas Department of Agriculture and the Specialty Crop Research Initiative. We thank Dr. Creighton Miller and members of his program for support of our research, Dr. Don Henne for insects, and Dr. Charlie Rush and the other PIs for the development of the Mini-Grant Program. We also thank the Texas Department of Agriculture for support through a Specialty Crop Block Grant. We thank especially the Texas potato growers for their interest and support of our work and their efforts to secure funding for specialty crop and ZC research.

References

Gloor, G and W Engels. 1992. Single-fly DNA preps for PCR. Drosophila Information Service. 71: 148-149.

Lévy, J, A Ravindran, D Gross, C Tamborindeguy, and E Pierson. 2011. Translocation of "*Candidatus* Liberibacter solanacearum," the zebra chip pathogen, in potato and tomato. Phytopathology, 101: 1285-1291

Nachappa P, J Lévy, E Pierson, and C Tamborindeguy. 2011. Diversity of endosymbionts in the potato psyllid, *Bactericera cockerelli* (Triozidae), vector of zebra chip disease of potato. CurMicrobiol. 62:1510-20.

Ravindran A, J Lévy, E Pierson, and D Gross. 2011. Developing primers for improved PCR detection of the potato zebra chip pathogen, "*Candidatus* Liberibacter solanacearum." Plant Dis 95: 1542-1546.

Ravindran A, J Lévy, E Pierson, and D Gross. 2012. Development of LAMP as a sensitive and rapid method for detection of "*Candidatus* Liberibacter solanacearum" in potatoes and psyllids. Phytopathology 102:899-907.

Reineke, A, P Karlovsky, and CP Zebitz. 1998. Preparation and purification of DNA from insects for AFLP analysis. Ins Mole Biol 7: 95-99.

A Fast-Track Breeding Methodology for Developing ZC Tolerant Potato Cultivars

J. C. Miller, Jr.[1], D.C. Henne[2], J.G. Levy[1], J.W. Koym[3] and, D.C. Scheuring[1]
Department of Horticultural Sciences, Texas A&M University, College Station,[1] Weslaco[2], and Lubbock[3].
Work was supported by funds from the Texas Potato Breeding and Variety Development Program, the Texas Department of Agriculture, and the Specialty Crop Research Initiative.

Abstract
Zebra Chip (ZC) tolerance/resistance is complicated, because it involves two major factors - an insect vector (*Bactericera cockerelli* (Sulc.)) and a bacterium (*Candidatus* Liberibacter solanacearum). While potato cultivars have been identified which vary in tolerance to the complex, no reports of true resistance have been found. Furthermore, tolerance/resistance is often found in unadapted germplasm, which is unsuited for commercial use and requires further breeding refinement. Cultivar improvement for ZC could be accomplished using tolerant parents in a conventional breeding program; however, such programs often take 12 or more years from hybridization to variety release. This investigation reports on a methodology for ZC tolerance/resistance breeding which could reduce the breeding procedure by three to five years.

Introduction
ZC is a serious concern for the potato industry in the western US, so producers and processors are seeking improved cultivars with tolerance (a contributor to IPM) and/or resistance. Cultivar improvement for this disease is very complex due to involvement of two major factors, an insect vector (*Bactericera cockerelli* (Sulc.)) and a bacterium (*Candidatus* Liberibacter solanacearum). Even without such complications, cultivar development using conventional breeding can take up to 12 or more years, involving a number of key steps. The Texas A&M Potato Breeding and Variety Development Program has evaluated these steps and identified areas where the breeding/cultivar development process can be shortened. This method allows for potential stacking of tolerance genes, as well as conventional tolerant by susceptible crosses. This investigation focused on the early identification of selections with good chip quality characteristics, as well as simultaneous introduction into tissue culture, virus cleanup, and selection maintenance.
Our breeding program has field screened more than 600 cultivars/advanced selections for ZC, including every market class. No **resistance** has been found; however, varying degrees of tolerance have been repeatedly observed. No studies on heritability of tolerance have been found in the literature; however, our approach assumes that the trait is heritable to some degree. Therefore, the objective of this investigation was to propose and evaluate a fast-track breeding methodology for developing ZC-tolerant potato cultivars.

Materials and Methods
To test the proposed methodology, 32 single-tuber progeny of the cross NY138 (tolerant) X Ivory Crisp (susceptible) were selected in Dalhart, TX in October, 2011. Selected tubers were washed, placed in

cold storage for 11 weeks, removed, and sprouted. In early January, sprouts from each tuber were introduced into tissue culture; virus tested and determined to be clean of seven tested viruses. Each of the 32 tubers was four cut, with two seed pieces planted in cages (along with checks Atlantic and NY138) on January 24, 2012 at the Texas A&M AgriLife Research and Extension Center at Weslaco. On the same day, the two remaining seed pieces of each tuber were field planted in a chip production field on the Jack Wallace Farm near Edinburg. Hot psyllids were placed in the cages on 13 March at the rate of five per plant. Cages were sprayed one week later with Movento and with AgriMek one week later. Tubers from cages and field were hand dug, washed, and chipped on 16 May. Chips were photographed, scored for ZC and chip quality, and yield per plant was recorded.

Results and Discussion

Table 1. Trial, selection, % chips with ZC, ZC chip rating, specific gravity, tuber number, total tuber weight, chip notes from field and cage trials in Weslaco, 2012.

Trial	Selection	% Chips with ZC	ZC Chip Rating*	Specific Gravity	Tuber Number	Total Tuber Weight (Lb)	Chip Notes
Field-Wallace	TX09403-2W	55	3	1.023	11	0.7	6 ZC
Cage-Weslaco	TX09403-2W	100	3	1.048	9	1.2	ZC, ZC
Field-Wallace	TX09403-15W	0	0	1.059	8	1.7	No ZC
Cage-Weslaco	TX09403-15W	0	0	1.052	6	1.6	No ZC
Field-Wallace	TX09403-21W	0	0	1.053	21	3.6	No ZC
Cage-Weslaco	TX09403-21W	25	1	1.050	8	1.2	2 Light ZC
Field-Wallace	Atlantic	0	0	1.073	9	24.3	
Cage-Weslaco	Atlantic	65	3	1.057	66	1	ZC
Field-Wallace	NY138	3	2	1.043	29	1	ZC
Cage-Weslaco	NY138	25	2	1.053	4	1	ZC

* 0=none, 1=very light, to 5= very dark

With this fast-track method, two selections (TX09403-15W and TX09403-21W) from the original 32 field selections were identified and increased for large scale evaluations.

Conclusion

A fast-track methodology for developing ZC tolerant cultivars was proposed and evaluated. In a one year period, 32 progeny from a cross of ZC tolerant and susceptible parents were selected, introduced into tissue culture, virus tested, ZC challenged, and chip and yield evaluated. During the same year, minitubers of the two surviving clones were produced for seed increase. This approach could save from three to five years in a conventional breeding program.

The Potato Psyllid (*Bactericera cockerelli*) Genome Project

Hail, D. A.[1], Hanson, J. D.[2], Hunter, W. B.[3], Tamborindeguy, C.[4], Johnson, C. P.[5], Gudmestad, N. C.[5], and Bextine, B.[1]

[1]University of Texas at Tyler 3900 University Blvd, Tyler, TX 75799
[2]Research and Testing Laboratory 4321 Marsha Sharp Fwy. Lubbock, TX 79407
[3]U. S. Horticultural Research Laboratory 2001 South Rock Road Ft Pierce, FL 34945
[4]Texas A&M University TAMU 2475 College Station, TX 77843-2475
[5]North Dakota State University 1301 12th St North Fargo, ND 58102

Abstract

The potato psyllid, *Bactericera cockerelli* (Hemiptera) is a competent vector of the phytopathogenic bacterium *Candidatus* Liberibacter solanacearum (*Ca*. L. solanacearum), the putative causal agent of Zebra Chip (ZC) in potatoes (*Solanum tuberosum*). Like many hemipteran pests, genomic information in publically available databases is limited. Advancements in next generation sequencing have made genome scale projects more feasible and this report documents the progress made in the potato psyllid genome project. To date, we have used the Ion Torrent sequencing platform to generate 12,599,070 reads. After bacterial reads were filtered the remaining reads (12,250,021) had an average length of 170 bp, Q20 at least of 83.2% (75.3% incl. trim), Q30 at least of 50.7% (45.1% incl. trim) and Q40 at least of 0.0%. Referenced assemblies of these reads to Asian citrus psyllid (*Diaphorina citri*, ACP) genome scaffolds showed that 98.16% of the reads did not align. Because of this, it is possible that *D. citri* will not be a sufficiently closely related species for the purposes of assembly. In any case, more data is being generated and more will need to be generated in the future to refine the organization of this genome.

Introduction

Psyllids are phloem feeding jumping plant lice (Order: Hemiptera) and around forty species are known to be of economic importance. These insects are pests of a wide array of plants including pistachio (*Agonoscena targionii*, Lichtenstein), avocado (*Trioza aguacate*, Hollis & Martin), pear (*Cacopsylla pyricola*, Förster), berry (*Trioza tripunctata*, Fitch) and many ornamentals.

Recently, three psyllid species have become subjects of intense research because of their ability to transmit various species of the phytopathogen *Candidatus* Liberibacter (α-Proteobacteria). One of these, the potato psyllid (*Bactericera cockerelli*) is a pest of solanaceous plants (potatoes, tomatoes, peppers) and has become a major limiting factor in the production of potatoes in both Central and North America (CNAS 2006). The insect is native to central regions of North America and has been reported as far north as southwestern Canada (Davidson et al 2008) and as far south as Nicaragua (Bextine 2012). New populations have been established in New Zealand (Liefting 2008) and Korea, Argentina, and Brazil have placed quarantine restrictions on US potatoes to prevent further spread (Center for Invasive Species Research – University of California Riverside).

Of the 26 open Hemipteran genome projects on NCBI only six have any data, only two (*Rhodnius prolixus* and *Acyrthosiphon pisum*) have an appreciable amount of sequence data and only two (*Pachypsylla venusta* and *Diaphorina citri*) are psyllids. Because of the increasing threat that psyllids are posing to economically viable plant species, a thorough understanding of their genomes is essential.

Materials and Methods

Insects

Potato psyllids were initially provided by Drs. Tong-Xian Liu and Xiangbing Yang (Texas AgriLife Research). These were used to set up a colony maintained on potato plants of various ages (grown from tubers) at 25° C, 40% humidity and a 12/12 day/night cycle. Adult psyllids were collected from the leaves by attaching a 1000 µl filter pipette tip to the end of a 25 ml serological pipette connected to a vacuum line. These were placed in 1.5 ml microcentrifuge tubes and stored at -80° C.

Genome/Transcriptome Sample Preparation

DNA was extracted from adult potato psyllids using Qiagen's DNeasy Blood and Tissue kit and genome amplification was accomplished using Qiagen' REPLI-g Mini kit. Total RNA was extracted from other adult potato psyllids using Qiagen's RNeasy Mini kit and mRNA from the total RNA was purified using Qiagen's Oligotex mRNA Mini kit. Transcriptome amplification was accomplished using Qiagen's QuantiTect Whole Transcriptome kit. All were performed according to the manufacturer's protocol.

Ion Torrent Library Preparation and Sequence Analysis

Samples were run on an Ion Torrent 318 sequencing chip following the manufacturers guidelines. After sequencing, the genome and transcriptome .qual and .sff files were imported into Geneious (www.geneious.com) for analysis. Ion Torrent reads from UT Tyler and NDSU were combined into one group and totaled just over 12.5 million reads. In a previous sequencing study 100+ bacterial genera were identified by bacterial tag-encoded FLX amplicon pyrosequencing (Hail et al 2012). These genera were used as filters in an attempt to remove as many bacterial reads from the initial set as possible. Each individual bacterial genera was the subject of an NCBI search and any genomes or sequences recovered were used as a reference for the assembly of the Ion Torrent reads.

Referenced (Geneious) and *de novo* assembly tools (Geneious, Newbler) were used to analyze the Ion Torrent reads both before and after bacterial filtration. Potato psyllid transcriptome 0074-L04_B01 (136K sequences), Potato Psyllid Transcriptome 0074-L04_B02 (109K sequences), Asian citrus psyllid Transcriptome 0.9 (33K sequences), Asian citrus psyllid Genome Scaffolds 1.0 (163K sequences), Asian citrus psyllid ORF Predications (25K sequences) and Asian citrus psyllid Transcripts (123K sequences) were all individually used as standard sets for referenced assemblies. The Ion Torrent sequences that aligned to the standard sets were then *de novo* assembled and the top 500 contigs were identified using BLAST. Hits were organized as No result, Mitochondria, rRNA, rProt and Other and separated into different folders.

Results and Discussion

Different analyses were performed both before and after removal of bacterial reads. Prior to filtering two *de novo* assemblies, one in Geneious and another using Newbler (454 Roche), produced 10,000 and 8,091 contigs respectively. Because Geneious reports the best contigs first, only the top 5,000 were taken for further review. Of the contigs produced by the Geneious *de novo* assembler 2,465 of them were various kinds of bacteria (many "Uncultured") and 2,164 of them were some kind of insect and many of those were microsatellite regions or transposons. The Newbler run assembled 8,091 large contigs (64,763 in total) and 5,866 of them gave no result in BLAST. 447 sequences resulted in BLAST returns that matched an insect species but only 73 of those aligned to the Asian citrus psyllid genome

scaffolds. 177 of them BLASTed as *Ca*. Liberibacter or related phages and 172 of those aligned to a *Ca*. Liberibacter solanacearum genome. 688 of them BLASTed as various low coverage animals including clams, algae and humans. 483 of them BLASTed as other kinds of bacteria. 419 of them BLASTed as *Wolbachia* or related phages and 191 of those aligned to a *Wolbachia* genome.

After bacterial reads were filtered (Table 1) the remaining reads (12,250,021) had an average length of 170 bp, Q20 at least of 83.2% (75.3% incl. trim), Q30 at least of 50.7% (45.1% incl. trim) and Q40 at least of 0.0%. A *de novo* assembly (Geneious) produced 1.5 million contigs. Referenced assemblies were against two potato psyllid transcriptomes and four sets Asian citrus psyllid sequences. The potato psyllid trancriptomes had 4.38 and 4.28 million reads align to them and the other four sets (from Asian citrus psyllids) had 44,571; 224,961; 50,291 and 257,053 reads align to them. All of these sets of assembled reads were independently *de novo* assembled. These assemblies produced 263,623 and 251,298 (potato psyllid); 2,421; 8,184; 2,925 and 6,874 (Asian citrus psyllid) contigs. From all these sets of contigs the top 500 were identified by BLAST (Table 2).

Table 1. Bacterial Genera and Species used as Filters for the Depletion of Reads from Ion Torrent Sequence Pool. Bacteria identified in a 454 16S sequence survey of all potato psyllid lifestages were used as filters to remove reads from the genome sets of sequences. When available, genomes were downloaded from NCBI; otherwise all available sequence data was compiled into a single file and used as a reference to which the Ion Torrent sequences were assembled.

	R&T	NDSU		R&T	NDSU		R&T	NDSU
Bacteria Filters	Count	Count		Count	Count		Count	Count
Acinetobacter sp	1	0	Enterococcus sp	0	1	Phenylobacterium sp	7	0
Alishewanella sp	51	6	Escherichia sp	22	1	Planctomyces sp	20	2
Alkanindiges sp	1	0	Filifactor sp	35	7	Polyangium sp	0	0
Anaerococcus sp	193	64	Flavobacterium sp	1548	626	Polynucleobacter sp	1	0
Aquabacterium sp	1163	3	Gemmata sp	106	26	Propionibacterium sp	78	34
Aranicola sp	0	0	Gordonia sp	7	3	Pseudomonas sp	17	1
Arcobacter sp	158	35	Hydrogenophaga sp	161	29	Pseudonocardia sp	7	1
Arthrobacter sp	7	0	Hymenobacter sp	2	0	Pseudoxanthomonas sp	8	1
Bacteroides sp	0	0	Hyphomicrobium sp	14	0	Ralstonia sp	3	3
Beijerinckia sp	33	322	Klebsiella sp	0	0	Rheinheimera sp	125	9
Brachybacterium sp	118	29	Lactobacillus sp	35	5	Rhodoferax sp	20	1
Bradyrhizobium sp	0	0	Legionella sp	66658	12716	Rhodoplanes sp	0	0
Brevibacillus sp	291	106	Leptothrix sp	8	2	Shigella boydii	0	0
Brevibacterium sp	700	0	Liberibacter	9864	127853	Sphingomonas sp	313	124
Brevundimonas sp	0	0	Methylobacterium sp	13	2	Sphingopyxis sp	6	0
Carsonella	8050	66	Microbacterium sp	202	19	Sporobacter sp	1	16
Cellulomonas sp	3	1	Micromonospora sp	12	1	Sporocytophaga sp	0	0
Chryseobacterium sp	355	114	Mitsuaria sp	12	11	Staphylococcus	18149	6366
Citrobacter sp	308	4	Morganella morganii	446	8	Stella sp	1	3
Clostridium sp	33	4	Nevskia sp	14	0	Sterolibacterium sp	57	4
Comamonas sp	20	43	Niastella sp	1	0	Streptococcus sp	17460	50342
Corynebacterium sp	16	2	Nocardioides sp	4	0	Streptomyces sp	9	1
Coxiella sp	1	0	Paenibacillus sp	0	1	Synechococcus sp	0	0
Curvibacter sp	1576	2	Pantoea sp	21	2	Tannerella sp	220	56
Cyanobium sp	0	0	Parkia sp	1	2	Tepidimicrobium sp	0	0
Delftia sp	21	4	Parvibaculum sp	4	3	Terrimonas sp	0	0
Denitratisoma sp	266	74	Pedobacter sp	86	17	Ulvibacter sp	4	0
Derxia sp	1	1	Pedomicrobium sp	171	12	Ureibacillus sp	172	53
Diaphorobacter sp	0	0	Pelomonas sp	0	0	Variovorax sp	19	1
Enterobacter sp	0	0	Peptoniphilus sp	718	0	Wolbachia	19459	6
							149687	199251

Table 2. Referenced Assemblies of all Ion Torrent Reads to Various Groups of Sequences.

Reference Groups	Sequences per Group	Total Align Sequences	*de novo* Assembled Contigs	No result	Mitochondria	rRNA	rProt	Other
0074-L04_B01	136,518	4.38 M	263,623	262	77	27	48	86
0074-L04_B02	109,983	4.28 M	251,298	248	78	31	54	89
ACP 0.9	33,894	44,571	2,421	367	10	99	1	23
ACP 1.0	163,023	224,961	8,184	346	11	75	35	33
ACP ORF Pred	25,058	50,291	2,925	300	4	21	80	95
ACP Transcripts	123,653	257,053	6,874	136	13	237	41	73

Acknowledgements
Funding was provided by The University of Texas at Tyler Research Development fund and USDA-SCRI Zebra Chip 2009-51181-20176.

References
Bextine, B., A. Arp, E. Flores, E. Y. Aguilar, L. Schindler, F. Soza, C. Powell, and A. Rueda. 2012. First Report of Zebra Chip and "Candidatus *Liberibacter* solanacearum" on Potatoes in Nicaragua. Plant Dis. http://dx.doi.org/10.1094/PDIS-09-12-0824-PDN

CNAS (Center for North American Studies). 2006. Economic Impacts of Zebra Chip on the Texas Potato Industry. Available: http://cnas.tamu.edu/Zebra%20Chip%20Impacts%20Final.pdf

Davidson, M. M., Teulon, D.A.J., Scott, I.A.W. and Workman, P. 2008. "A review of the potato psyllid (Bactericera cockerelli); a new insect pest of potato in New Zealand - A report prepared for Horticulture New Zealand. ." New Zealand Institute for Crop & Food Research Limited.

Hail, D., S. E. Dowd and B. Bextine. 2012. Identification and location of symbionts associated with potato psyllid (*Bactericera cockerelli*) lifestages. Environ. Ent. 41:98-107.

Liefting, L., L. Ward, et al 2008. "A New 'Candidatus Liberibacter' Species in Solanum betaceum (Tamarillo) and Physalis peruviana (Cape Gooseberry) in New Zealand." Plant Disease 92(11):1588-1588.

Cold Tolerance in Potato Psyllids

Whipple, S. D., Bradshaw, J. D., and Harveson, R. M.

University of Nebraska-Lincoln, Panhandle Research and Extension Center, Scottsbluff, NE 69361

Abstract

We subjected adult and nymphal potato psyllids from Wapato and Nebraska colonies to temperatures of -1, -2, -4, -6, -8, -10, -13, -15, -17, and -20°C for durations of 1, 2, 4, 6, 8, 10, and 24 hours to investigate cold tolerance. On average, 50% of psyllids were capable of surviving temperatures of -14°C for 24 hours, with 100% of individuals surviving 24 hours down to -10°C. Psyllids from the Nebraska colony had greater survival at colder temperatures, which may suggest local adaptation. Results support that psyllids are very cold tolerant, and suggest that overwintering in northern climates is possible.

Introduction

Potato/tomato psyllids (*Bactericera cockerelli*) are known to overwinter in Mexico and many areas of the southwestern United States, with an annual wind assisted northern migration. While the optimum temperature for psyllid development is 27°C, survival is reduced at higher temperatures (32°C) and 35°C is fatal (List 1939; Abdullah 2008; Yang and Liu 2009; Yang et al. 2010). Until recently, psyllids were not thought to be capable of overwintering in northern climates. However, research by Henne et al. (2010) found nymphs capable of surviving -15°C for short periods, with 50% of adults surviving over 24 hours at -10°C. Even in a state such as Nebraska that frequently experiences harsh winters, mean annual winter temperature is -3.7°C (Ratcliffe and Paulsen 2008). Given the dispersal capabilities and broad host potential of potato psyllids, further investigation of cold tolerance in psyllids is warranted throughout its' range.

Materials and Methods

Adult and nymphal psyllids from Wapato and Nebraska colonies were tested for cold tolerance following modified methods of Hall et al. (2010). Subsets of ten individual psyllids were placed in a petri dish in a Percival environmental chamber at -1, -2, -4, -6, -8, -10, -13, -15, -17, and -20°C and removed at 1, 2, 4, 6, 8, 10, and 24 hour intervals and placed at room temperature (25°C). Psyllids were monitored for 48 hours to determine survival, which was defined as walking upright within the observation period. Time to 50% mortality (LT_{50}) was calculated for each time period and temperature using R statistical software.

Results and Discussion

Psyllids from both the Wapato and Nebraska colonies are very cold tolerant (Table 1), with 100% survival for 24 hours at -10°C and 50% of individuals surviving temperatures of at least -13°C for 24 hours. Temperatures of -16°C for six hours are required to achieve 50% mortality in both nymphal and adult psyllids from these colonies.

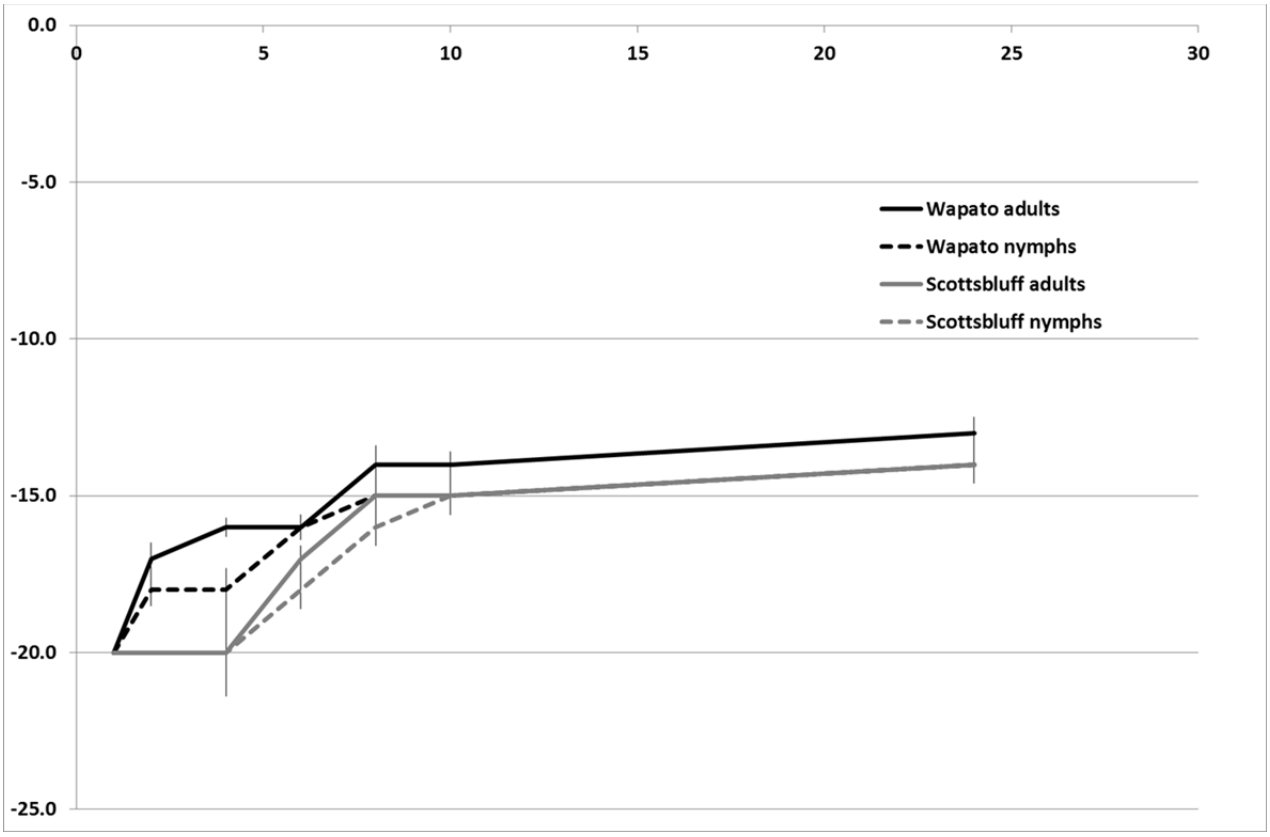
Table 1. Time to 50% mortality (LT_{50}) for nymphal and adult psyllids from the Wapato and Nebraska colonies.

Predicted LT_{50} estimates (SE) for tomotato/potato psyllid exposed to 0 to -20 degrees C

		Exposure (hours)						
		1	2	4	6	8	10	24
Wapato colony	adults	-17< > -20	-17 (0.5)	-16 (0.3)	-16 (0.4)	-14 (0.6)	-14 (0.4)	-13 (0.5)
	nymphs	-17< > -20	-18 (0.5)	-18 (0.7)	-16 (0.4)	-15 (0.6)	-15 (0.5)	-14 (0.6)
Scottsbluff colony	adults	< -20	< -20	-20 (1.4)	-17 (0.4)	-15 (0.5)	-15 (0.4)	-14 (0.5)
	nymphs	< -20	< -20	-20 (1.0)	-18 (0.6)	-16 (0.6)	-15 (0.6)	-14 (0.6)

Nymphal psyllids from both colonies often required colder temperatures to reach the LT_{50} than adults (Figure 1). Differences in survival between the colonies are best observed at the extreme cold temperatures for short durations. Both adults and nymphal Nebraska psyllids readily survived extreme cold temperatures of -20°C for up to 2 hours, while the Wapato psyllids reach LT_{50} at approximately -18°C for 2 hours. Over 50% of psyllids from the Nebraska colony survived -20°C for 4 hours.

Figure 1. Plotted LT_{50} for nymphal and adult psyllids from the Wapato and Nebraska colonies.



Our results support the findings of Henne et al. (2010) that potato psyllids are very cold tolerant, and are likely capable of overwintering in many areas of the northern United States. Psyllids from the Nebraska colony survived colder temperatures for short durations, which may suggest local adaptation to cold climates, as the Wapato colony originated in Texas.

Psyllids in the field, which would potentially have the added advantage of leaf litter and alternate host plants as insulation from the elements, may be better able to survive cold temperatures. This is especially important considering both nymphal and adult psyllids are capable of surviving direct exposure to temperatures of -13°C for 24 hours. That being said, it is unclear whether Liberibacter can persist in its vector at these cold temperatures. It is also unclear where psyllids might overwinter and how they might access host plants in the spring.

More research is needed to determine the overwintering potential of potato psyllids in northern climates. Additional laboratory and field studies should be conducted on more colonies from throughout the U.S. to determine if psyllids are overwintering locally.

Acknowledgements
We thank Susan Harvey, Kathy Nielsen, Rick Patrick, and Jeanna Jenkins for their help conducting experiments and maintaining psyllid colonies. We also thank Joe Munyaneza for providing us with psyllids from the Wapato colony. This project was partially funded by the Nebraska Potato Council and the Specialty Crop Research Initiative (SCRI).

References
Abdullah, N.M.M. 2008. Life history of the potato psyllid *Bactericera cockerelli* (Homoptera: Psyllidae) in controlled environment agriculture in Arizona. Afr. J. Agr. Res. 3:60–67.

Henne, D.C., Paetzold, L., Workneh, F., and Rush, C.M. 2010. Evaluation of potato psyllid cold tolerance, overwintering survival, sticky trap sampling, and effects of liberibacter on potato psyllid alternate host plants, pp.149–153. In Proceedings of the 10th Annual Zebra Chip Reporting Session, ed. F. Workneh and C.M. Rush. Dallas, TX (November 7–10, 2010).

List, G.M. 1939. The effect of temperature upon egg deposition, egg hatch and nymphal development of *Paratrioza cockerelli* (Sulc). J. Econ. Entomol. 32:30–36.

Ratcliffe, B.C., and Paulsen, M.J. 2008. The Scarabaeoid beetles of Nebraska (Coleoptera: Scarabaeoidea). Bull. Univ. Nebr. State Mus. 22: 1-570.

Yang, X.B., and Liu, T.X. 2009. Life history and life tables of *Bactericera cockerelli* (Homoptera: Psyllidae) on eggplant and bell pepper. Environ. Entomol. 38:1661–1667.

Yang, X.B., Zhang, Y.M., Hua, L., and Liu, T.X. 2010. Life history and life tables of *Bactericera cockerelli* (Hemiptera: Psyllidae) on potato under laboratory and field conditions in the Lower Rio Grande Valley of Texas. J. Econ. Entomol. 103:1729–1734.

Can Carrot Psyllid (*Trioza apicalis*) Transmit '*Candidatus* Liberibacter solanacearum' to Potato?

Anne Nissinen[1], Satu Latvala[1], Marika Rastas[1], Anne Lemmetty[1], Asko Hannukkala[1], Minna Pirhonen[2]
[1]MTT Agrifood Research Finland, Plant Production Research, FI-31600 Jokioinen, Finland
[2]University of Helsinki, Department of Agricultural Sciences, Box 27, 00014 University of Helsinki, Finland

Abstract
'*Candidatus* Liberibacter solanacearum' (CLs) has been previously found in several solanaceous plants and in a psyllid, *Bactericera cockerelli,* as well as in psyllid-affected carrots and carrot psyllid (*Trioza apicalis*). However, the haplotype of the bacterium occurring on carrot is different from those occurring on potato. The aim of this study was to investigate if CLs haplotype C occurring in carrot can be transmitted to potato by carrot psyllid to evaluate the risk to potato industry by this haplotype of the bacterium. In 2 greenhouse experiments carrots and potatoes were exposed to either greenhouse reared or field-collected psyllids (20 or 30 individuals per cage) for 7 or 11 days. No transmission of CLs to potatoes was detected by conventional PCR using primer pair OA2/O12c. Transmission of CLs to carrots was confirmed by PCR analysis, but only half of the exposed plants were positive, probably due to variable percentage of CLs positive psyllids in different populations used in 2012. Exposure with higher number of psyllids and longer growth time to follow the symptom development on potatoes would be needed to verify this result.

Introduction
'*Candidatus* Liberibacter solanacearum' has been previously found in several solanaceous plants and in a psyllid, *Bactericera cockerelli,* (e.g. Hansen et al. 2008, Liefting et al. 2008; 2009a,b; Lin et al. 2009; Wen et al. 2009) and it is associated with a potato disease called 'zebra chip' as well as some other diseases in solanaceous crops (Abad et al. 2009; Lin et al. 2009; Liefting et al. 2009a,b; Secor et al. 2009; Wen et al. 2009). Zebra chip causes millions of dollars losses to potato industry in the USA and Central America (e.g. Munyaneza et al. 2007; Abad et al. 2009). CLs has also caused severe losses in other solanaceous crops in New Zealand (Leifting et al. 2009a; Teulon et al. 2009). The carrot psyllid damage in plants, that is leaf curling and stunted growth, was for a long time associated with a toxin excreted by the psyllid. The nature of the toxin, however, has not been discovered (Markkula & Laurema 1971). Quite recently it was observed that carrot psyllid–damaged carrots showed strong discoloration symptoms in the leaves, which could indicate association of a plant pathogen with psyllid damage (Nissinen et al. 2007). Subsequently, association of CLs to both carrot psyllid-damaged carrots and carrot psyllids was shown (Munyaneza et al. 2010a,b). This pathogen was also recently added to the list of pests recommended for regulation as quarantine pests by European and Mediterranean Plant Protection Organization (EPPO). The aim of this study was to investigate whether CLs haplotype C occurring on carrot (Nelson et al. 2011) can be transmitted to potato by carrot psyllid to evaluate the risk caused by this haplotype of the bacterium to potato industry.

Materials and methods
Plant material for Experiment 1
To investigate CLs transmission 3-5 carrot (cv Fontana) seeds were sown in 3-l pots in a commercial peat-sand mixture 'Kylvöseos' (Kekkilä Oyj, Eurajoki, Finland) and thinned to one seedling per pot after germination. At the same time one potato tuber (cv Bintje) was planted into each of 5-l pots. All plants were fertilized 6 times with 1‰ solution (1g/l) of Vihannes-Superex (9:5:31, N:P:K) (Kekkilä Oyj, Eurajoki, Finland) during their growth period. The exposure was started when the plants were 4

weeks old. The experiment was run in a greenhouse unit with light and temperature regimes L18:D6, 21/16 ºC day/night in April-June 2011.

Plant material for Experiment 2
To investigate CLs transmission success 5-10 carrot (cv Fontana) seeds were sown in 1.1-l pots filled with commercial peat-sand mixture 'Kylvöseos' (Kekkilä Oyj, Eurajoki, Finland) and thinned to 2 seedlings per pot at 1-leaf stage. One small potato tuber (greenhouse propagated cv Bintje) was planted in each of 3.5-l pots filled with commercial peat-sand mixture 'Ruukutusseos' (Kekkilä Oyj, Eurajoki, Finland). Both potatoes and carrots were confined in insect cages (33x33x60 cm) immediately after sowing to prevent contamination with any other insect species. The psyllid exposure was started 9 days after planting the potatoes. All plants were fertilized 2 times a week with 1‰ solution (1g/l) of Vihannes-Superex (9:5:31, N:P:K) (Kekkilä Oyj, Eurajoki, Finland) over a six-week period. The experiment was run in a greenhouse unit with light and temperature regimes L20:D4, 21/16 ºC day/night in June-August 2012.

Sources of insects
In experiment 1, carrot psyllids, which were originally collected from Haukivuori (61°58'28" N, 27°11'00" E) in June 2000, were used. They were continuously reared in a greenhouse (L20:D4, 20/15 ºC day/night, 50% RH) at Jokioinen, as described in Nissinen et al. (2007). In experiment 2, in addition to the greenhouse reared carrot psyllids (colonies collected from Haukivuori in 2000 and from Forssa in 2008) field-collected (June 2012) individuals were used in the exposure. Psyllids were starved 14-18h before starting the exposure. During the starvation period, the psyllids were provided with water source to prevent the insect mortality.

Psyllid exposure
In experiment 1, ten carrot seedlings were exposed to 5 carrot psyllid females for 7 days at 2-leaf stage. Five seedlings serving as controls were confined in an empty insect cage. Similar kinds of cylinder insect cages and similar releasing procedure were used on carrots as described in Nissinen et al. (2012). Simultaneously with the exposure on carrots, ten potato seedlings were exposed to 20 adult carrot psyllids for 7 days. Psyllids were released after a starvation period of 14-18 h. Potatoes were confined in a hoop cage. Each frame was made of 2 metallic wires, which were bent over the pot and stuck into the soil at the ends. The cage was covered with fine white cloth, which was sealed around the pot by an elastic band. The psyllids were released from Eppendorf-tubes at the base of the plants. Five potato seedlings were confined to similar kind of hoop cages without psyllids to serve as controls. Adult psyllids were removed from carrots with forceps and stored in 95% ethanol until DNA extraction and CLs detection. Psyllids from the potatoes were removed by an insecticide spray.

In experiment 2, one potato seedling (ten days after plating) and one pot of carrots (2 seedlings at 1-leaf stage) were confined in an insect cage (33x33x60 cm). 15 females and 15 males from the greenhouse-reared colonies were used per cage and 25 females and 5 males from field collected population were used (the field population was strongly female biased). Psyllids were released after a starvation period of 17-18 h. Six Eppendorf-tubes each containing 5 psyllids (either females or males) were stuck on the soil around the potato seedling and the lids opened. The behaviour of the psyllids was observed once a day for 11 days. The mortality rose quickly in the field collected population after three days, suggesting that the psyllids feeding on potato died thereafter. The psyllids were removed manually after 11 days. Thereafter, the plants were taken out from the insect cages and sprayed with insecticide to remove the remaining insect. The nymphs were removed from the carrots with 2 subsequent insecticide sprays starting 2 and 3 weeks after the removal of adults.

Plant and insect material for CLs detection
In experiment 1, all the living psyllids on each carrot plant were collected to a sample after 7 days and stored in 95% ethanol until DNA extraction and CLs testing. The plants were grown for 1.5 months after the psyllid removal. Thereafter, 0.5 g samples were cut from carrot petioles, potato petioles and potato tubers from both control and psyllid-exposed plants and stored in freezer until DNA extraction.

In experiment 2, four randomly selected psyllids were collected for PCR analysis from each of the insect cages and analyzed individually, to ensure that the psyllids were CLs infected. The plants were grown for 2 months after removing the psyllids. Tubers were washed, weighted, counted, cut to half, and photographed. No symptoms of necrotic flecking were observed when the tubers were cut. Thereafter, 0.5 g samples were cut from carrot petioles, potato tubers and stolons from both control and psyllid-exposed plants and stored in freezer until DNA extraction. Continuously psyllid-exposed greenhouse grown plants showing discoloration symptoms were used as positive controls for CLs analysis of carrots. As a positive control for potatoes, was used a potato plant exposed to feeding of CLs infected *Bactericera cockerelli* in UDSA, Wapato, USA. As negative controls both unexposed potato from USA and unexposed potato and carrot from our own experiments were used.

DNA-extraction and PCR analyses for CLs detection
In carrot samples, DNA from plants was extracted from mid veins and petioles of healthy and symptomatic plants. For potato plants leaves and tuber tissues were used for DNA extractions. Extractions were conducted using DNeasy Plant Mini Kit (Qiagen, Hilden Germany), according to the manufacturer's instruction with minor modifications. Prior to DNA extraction 0.1g of dissected plant tissue was ground with FastPrep-24tissue and cell homogenizer (MD Biomedicals, Ohio, USA). Total DNA from insect samples was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's supplementary protocol. DNA was extracted from batches of 2 to 5 carrot psyllid individuals in experiment 1, and individually in experiment 2. The presence of CLs was confirmed by PCR assay (Munyaneza et al. 2010b) using primer pair OA2/O12c (Liefting et al. 2009).

Results and Discussion
In experiment 1, none of the psyllid-exposed potatoes showed any specific symptoms compared to control plants, six weeks after exposure. None of the psyllid-exposed potato samples either cut from petioles or from potato tubers yielded an expected sized amplicon. All the psyllid samples collected from the carrot plants, following a 7–day exposure, however, yielded expected sized amplicon with primer pair OA2/O12c. Five out of the 10 psyllid-exposed carrots tested positive for CLs (cPCR) . None of the control plants tested positive for the pathogen, using this primer pair.

In experiment 2, the carrot psyllids were observed on potatoes for up to 11 days. Individuals from both greenhouse-reared and field-collected populations of carrot psyllids were observed on potato even though there was also a pot with 2 carrot seedlings in the same insect cage at the same time, i.e. the psyllids had the opportunity to choose a host plant instead of a non-host. Feeding or at least probing apparently occurred since some psyllids were found dead on the potato leaves within few days. However, none of the psyllid-exposed potatoes were tested positive for CLs. While 80% of insect samples from the field-collected population tested positive for CLs, only 44% and 29% tested positive from 2008 and 2000 greenhouse-reared populations respectively. None of the unexposed control samples (carrot and potato) yielded amplicon with this primer pair. None of the carrots in the cages exposed to the greenhouse-reared populations yielded amplicon. Those carrots did not show discoloration symptom either. In contrast, all of the carrots, which were exposed to the field-collected carrot psyllids, tested positive for CLs and they also showed the discoloration symptom.

In experiment 1, we showed that CLs was transmitted to carrot seedlings by infected carrot psyllids from the greenhouse-reared colony, however, this population was not able to transmit the bacterium to the carrots in 2012. This was despite the fact that some of the insects were tested positive for this bacterium. Both of the laboratory colonies suffered from an infection caused by an insect pathogenic fungus just before starting the experiment in 2012. Thus the percentage of positive psyllids was much lower in cages than in 2009, when the percentage of CLs-infected psyllids in the laboratory colony was 70% (Munyaneza et al. 2010b). In 2009, in field-collected psyllids the percentage CLs-infected psyllids was 61%, which however, was for pooled samples (Munyaneza et al. 2010b). Although the percentage of CLs positive psyllids in the field was higher in 2012 than in 2009, the transmission of CLs to potatoes by these psyllids was not shown in greenhouse experiment. Neither the transmission to potato was shown by the greenhouse-reared populations in 2011 or 2012. In 2012 we used newly sprouted potato seedlings instead of four weeks old potatoes since Gao et al. (2009) suggested that in the older plants the shoot structures could be less palatable to psyllid feeding. Obviously potato is not a suitable host for carrot psyllids since the psyllid mortality started to rise in a few days. Previously, Rygg (1977) has observed that carrot psyllids are able to live on non-hosts for 4 to 6 days, which suggests that the psyllids are able to some extent feed on non-hosts; psyllids would not survive any longer than 24 hours without plants (Rygg 1977). Whether short feeding time is adequate for some carrot psyllid individuals to transmit the bacterium to potato needs to be verified by using higher number of psyllids and longer growing time to follow the symptom development on potatoes.

Acknowledgements
We thank Outi Järvinen, Panpan Jiang, Senja Räsänen and Senja Tuominen for their technical assistance and Dr. Joseph Munyaneza from USDA for providing us with the CLs positive potato samples.

References
Abad, J.A., Bandia, M., French-Monar, R.D, Liefting, L.W., and Clover, G.R.G. 2009. First report of the detection of '*Candidatus* Liberibacter' species in zebra chip disease-infected potato plants in the United States. Plant Dis. 93: 108.

Gao, F., Jifon, J., Yang, X., and Liu, T.X. 2009. Zebra chip incidence in potato is influenced by timing of potato psyllid infestation, but not by the host plants on which they were reared. Insect Sci. 16: 399-408.

Hansen, A.K., Trumble, J.T., Stouthamer, R., and Paine, T.D. 2008. A new Huanglongbing species "Candidatus Liberibacter psyllauros" found to infect tomato and potato is vectored by the psyllid *Bactericera cockerelli* (Sulc). Appl. Environ. Microbiol. 74: 5862-5865.

Liefting, L.W., Rez-Egusquiza, Z.C., Clover, G.R.G, and Anderson, J.A.D. 2008. A New '*Candidatus* Liberibacter' Species in *Solanum tuberosum* in New Zealand. Plant Dis. 92: 1474.

Liefting, L.W., Sutherland, P.W., Ward, L.I., Paice, K.L., Weir, B.S., Clover, G.R.G. 2009a. A new '*Candidatus* Liberibacter' species associated with diseases of Solanaceous crops. Plant Dis. 93: 208-214.

Liefting, L.W., Weir, B.S., Pennycook, S.R., Clover, G.R.G. 2009b. '*Candidatus* Liberibacter solanacearum', associated with plants in the family Solanaceae. Int. J. Syst. Evol. Microbiol. 59: 2274-2276.

Lin, H., Doddapaneni, H., Munyaneza, J.E. Civerolo, E.L., Sengoda, V.G., Buchman J.L. and Stenger D.C. 2009. Molecular characterization and phylogenetic analysis of 16SrRNA from a new "Canditatus Liberibacter" strain associated with Zebra chip disease of potato (*Solanum*

tuberosum L.) and the potato psyllid (*Bactericera cockerelli* Sulc). J. Plant Pathol. 91: 215-219.
Markkula, M. and Laurema, S. 1971. Phytotoxaemia caused by *Trioza apicalis* Först. (Hom. Triozidae) on carrot. Ann. Agr. Fenn. 10: 181-184.
Munyaneza J.E., Buchman J.L., Upton J.E., Goolsby J.A., Crosslin J.M., Bester G., Miles G.P., Sengoda V.G. 2008. Impact of different potato psyllid populations on zebra chip disease incidence, severity and potato yield. Subtrop. Plant Sci. 60: 27-37.
Munyaneza, J.E., Crosslin, J.M. and Upton, J.E. 2007. Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with “zebra chip”, a new potato disease in southwestern United States and Mexico. J. Econ. Entomol. 100: 656-663.
Munyaneza, J.E., Fisher, T.W., Sengoda, V.G., Garcynski, S.F., Nissinen, A. and Lemmetty, A. 2010a. First report of "*Candidatus* Liberibacter solanacearum" associated with psyllid-affected carrots in Europe. Plant Dis. 94, 5/2010: 639.
Munyaneza, J.E., Fisher, T., Sengoda, V., Garczynski, S., Nissinen, A. and Lemmetty, A. 2010b. Association of "*Candidatus* Liberibacter solanacearum" with the Psyllid, *Trioza apicalis* (Hemiptera: Triozidae) in Europe. J. Econ. Entomol. 103: 1060-1070.
Nelson, W.R., Fisher, T.J., and Munyaneza, J.E. 2011. Haplotypes of “*Candidatus* Liberibacter solanacearum” suggest long-standing separation. Eur. J. Plant Pathol. 130: 5-12.
Nissinen, A., Vanhala, P., Holopainen, J. K., Tiilikkala and K. 2007. Short feeding period of carrot psyllid (*Trioza apicalis*) females at early growth stages of carrot reduces yield and causes leaf discolouration. Entomol. Exp. Appl. 125: 277-283.
Nissinen, A. I., Lemmetty, A., Pihlava, J.-M., Jauhiainen, L., Munyaneza, J. and Vanhala, P. 2012. Effects of carrot psyllid (*Trioza apicalis*) feeding on carrot yield and content of sugars and phenolic compounds. Ann. Appl. Biol. 161: 68-80.
Rygg, T. 1977. Biological investigations on the carrot psyllid *Trioza apicalis* Förster (*Homoptera, Triozidae*). Meld. Norg. Landbruks. 56: 1-20.
Secor, G.A., Rivera, V.V., Abad, J.A., Lee, I.M., Clover, G.R.G., Liefting, L.W., Li, X. and de Boer SH. (2009): Association of '*Candidatus* Liberibacter solanacearum' with zebra chip disease of potato established by graft and psyllid transmission, electron microscopy, and PCR. Plant Dis. 93: 574-583.
Teulon, D.A.J., Workman, P.J., Thomas, K.L., and Nielsen, M.C. 2009. *Bactericera cockerelli*: incursion, dispersal and current distribution on vegetable crops in New Zealand. New Zeal. Plant Prot. 62: 136-144.
Wen, A., Mallik, I., Alvarado, V.Y., Pasche, J.S., Wang, X., Li, W., Levy, L., Lin, H., Scholthof, H.B., Mirkov, T.E., Rush, C.M., and Gudmestad, N.C. 2009. Detection, distribution, and genetic variability of ‘*Candidatus* Liberibacter’ species associated with zebra complex disease of potato in North America. Plant Dis. 93: 1102-1115.

Host Selection by the Potato Psyllid, *Bactericera Cockerelli* (Sulc) (Hemiptera: Triozidae)

J. Thinakaran[1], C. Tamborindeguy[2], E. Pierson[3], and D. Henne[1]
[1]Texas A&M AgriLife Research, Weslaco, TX, 78596; [2] Department of Entomology, TAMU, College Station, TX 77843; Department of Horticultural Sciences, TAMU, College Station, TX 77843.

Abstract

The potato psyllid, *Bactericera cockerelli* is an oligophagous pest of several cultivated and non-cultivated solanaceous hosts and transmits the pathogen '*Candidatus* Liberibacter solanacearum' (Lso) in the process of feeding. Experiments were conducted to determine the settling behavior and assess preference of adult *B.cockerelli* to different solanaceous hosts. Results showed that *B.cockerelli* preferred eggplant followed by pepper, potato, silverleaf nightshade (SLN) and tomato. It was observed that host size influenced the choice made by *B.cockerelli*, and that *B.cockerelli* always preferred the larger host. However, under field conditions, *B.cockerelli* preferred potato plants, as potato plants grew much faster compared to other hosts. Experiments to test preference of *B.cockerelli* for Lso-infected and uninfected hosts revealed that adults initially settled on Lso-infected potatoes, but quickly moved to uninfected plants. However, no preference was exhibited for Lso-infected tomatoes, eggplants and peppers three weeks after infection**.**

Introduction

The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), is a serious pest of several solanaceous plant species, as it transmits the phloem-restricted bacterium, '*Candidatus* Liberibacter solanacearum' (Lso) (Munyaneza et al. 2007) which causes zebra chip disease of potatoes. Although, the biology of *B. cockerelli* has been studied on cultivated solanaceous hosts, information is lacking on how this information translates into practical field applications. Several non-cultivated weed hosts have been reported as alternate hosts of *B. cockerelli* (Wallis 1955) and Lso (Henne et al. 2010). However, little knowledge is available concerning solanaceous weeds present in the Lower Rio Grande Valley of Texas that might serve as reservoir hosts of Lso, which may enable carryover of the pathogen in the absence of potatoes and other solanaceous hosts.

Preferential responses exhibited for feeding and oviposition by *B. cockerelli* (Butler et al. 2011) motivated the present study on host selection behavior. Although insecticides play a pivotal role in the management of *B. cockerelli*, a more rational approach would be to incorporate ecologically sound methods that work in tandem with insecticides to contain the pest and the pathogen. Physical methods such as trap cropping can be effectively incorporated into an integrated pest management system. For practical considerations, the trap crop should be more preferable than the main crop either as a food source, or for oviposition, or both. The significance of this study originates from the idea of utilizing alternate hosts of *B. cockerelli* as attract and kill trap crops near potato fields, if the host is highly or the least equally favored from among the different hosts tested.

Materials and Methods

The host plants used were potato, *Solanum tuberosum* cultivar ('Atlantic'), tomato, *Lycopersicon esculentum* cultivar ('Lance'), bellpepper, *Capsicum frutescens* cultivar ('Capistrano'), eggplant, *Solanum melongena* cultivar ('Italian') and silverleaf nightshade, *Solanum elaeagnifolium* (SLN).

A. Settling behavior of adult *B. cockerelli* in the lab: Three to four week-old plants of potato, tomato, eggplant, pepper and SLN of uniform size were used in lab experiments. They were organized into pairs in all possible comparisons; each pair was then placed in separate cages (Fig. 1a). Thirty Lso-positive adult *B. cockerelli* were released in each cage. The settling response of adult *B. cockerelli* was assessed

1, 4, 8, 24, 48 and 72 hours after release. The cages were rearranged every day to minimize location effects. The adults were removed from cages one week after release.

B. Settling behavior of adult *B. cockerelli* in the field: A field experiment was conducted during December 2011 at the Texas A&M AgriLife Research Center, Weslaco, TX (Fig. 2a). Four to five week-old plants (approximately 5-6 leaf stage) of the five solanaceous plant species were planted in a randomized complete block design with four replications. Each treatment comprised of a paired combination. Plant pairs were planted in close proximity to each other, and were separated from other plant pairs by at least 10 feet. The number of adults settling on each plant was recorded approximately one month after transplant.

C. Preference for Lso-infected and uninfected hosts: Three week-old plants were infected with Lso by using 5-6 Lso-positive adult *B. cockerelli* enclosed in 1" dia. clip cages (Fig. 1b). The clip cages were removed after one week, along with the portion of the leaf that was clipped, to avoid incidental infestation. PCR was performed on the infected plants three weeks later to test the presence of the pathogen. Preference for Lso-infected and uninfected hosts were assessed in the laboratory by releasing thirty Lso-positive adult *B. cockerelli* and settling response was recorded 1, 4, 8, 24, 48 and 72 hours after release.

Results and Discussion

A. Settling behavior of adult *B. cockerelli* in the lab: It was observed that *B.cockerelli* adults preferred eggplants to peppers, which were preferred to potatoes and SLN (Fig. 3). Tomato was the least preferred host. It was also shown that plant size influenced the settling behavior of *B.cockerelli (*Fig. 4). They always preferred the larger plant, regardless of the host. When two different host plants of similar size were provided a choice was made, but adults settled uniformly when host plants of similar size and cultivar were provided.

B. Settling behavior of *B. cockerelli* in the field: The field experiment conducted during December 2011 revealed that more *B.cockerelli* adults settled on potatoes than the other hosts. A total of 37 adult *B.cockerelli* settled on all the potato plants, with significantly less numbers on tomato, pepper and eggplant (Fig. 2b). Only one adult settled on SLN. It was also observed that the plants were not growing uniformly in the field: Potatoes grew much faster than the other host plants, whereas SLN grew much more slowly. Therefore, differences in the settling behavior in the field and lab could be attributed to differences in plant sizes.

C. Preference for Lso-infected and uninfected hosts: Lso-infected potato, tomato, eggplant and pepper plants were tested for settling behavior of Lso-infected adult *B. cockerelli* (Fig. 5). With the exception of potatoes, Lso-infected plants were non-symptomatic three weeks after inoculation. It was demonstrated that *B. cockerelli* settled uniformly on infected and uninfected eggplants, peppers or tomatoes up to three days after release. Only in the case of potatoes was it observed that *B. cockerelli* significantly preferred the Lso-infected potato plants, but quickly moved to the uninfected plant for feeding/oviposition. Despite being symptomatic, approximately 30% of the potato plants failed to test positive for Lso at the start of the experiment. However, they were later tested and proved positive for Lso. Future work is directed at testing the host plants in an olfactometer assay for their olfactory cues.

Acknowledgements

Funding provided by USDA RAMP grant (2009-51101-05892), and USDA SCRI grant (2009-34381-20036) is gratefully acknowledged. The author wishes to thank J. Martinez, C. Bonczyk, T. Salazar, M. Dominguez, A. Villarreal, M. Oyervides, B. Warfield, A. Yanez, A. Silva, A. Alvarez, A. Jacques, A. Pena, and E. Huerta and Drs. E. Mirkov, J. Jifon, and M. Damaj, for their technical support.

References

Butler, C. D., Gonzalez, B., Manjunath, K. L., Lee, R. F., Novy, R. G., Miller, C. J., and Trumble J. T. 2011. Behavioral responses of adult potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), to potato germplasm and transmission of Candidatus *Liberibacter psyllaurous.* Crop Prot. 30:1233-1238.

Henne, D. C., Paetzold, L., Workneh, F., and Rush. C. M. 2010. Evaluation of potato psyllid cold tolerance, overwintering survival, sticky trap sampling, and effects of liberibacter on potato psyllid alternate host plants. Proceedings of the 10th Annual zebra chip reporting session. Pp.149-153.

Munyaneza, J. E., Crosslin, J. M., and Upton, J. E. 2007. Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "zebra chip", a new potato disease in southwestern United States and Mexico. J. Econ. Ent. 100:656-663.

Wallis, R. L. 1955. Ecological studies on the potato psyllid as a pest of potatoes. Tech. Bull. U.S. Dep. Agric. 1107: 1-24.

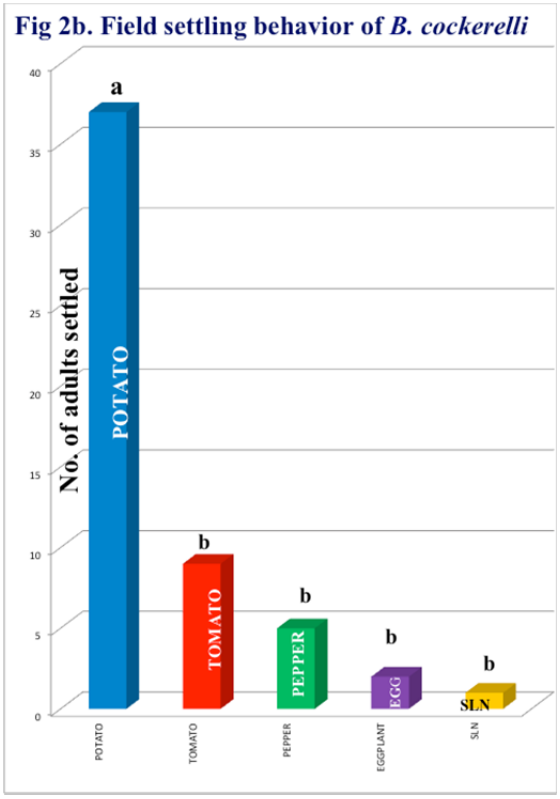
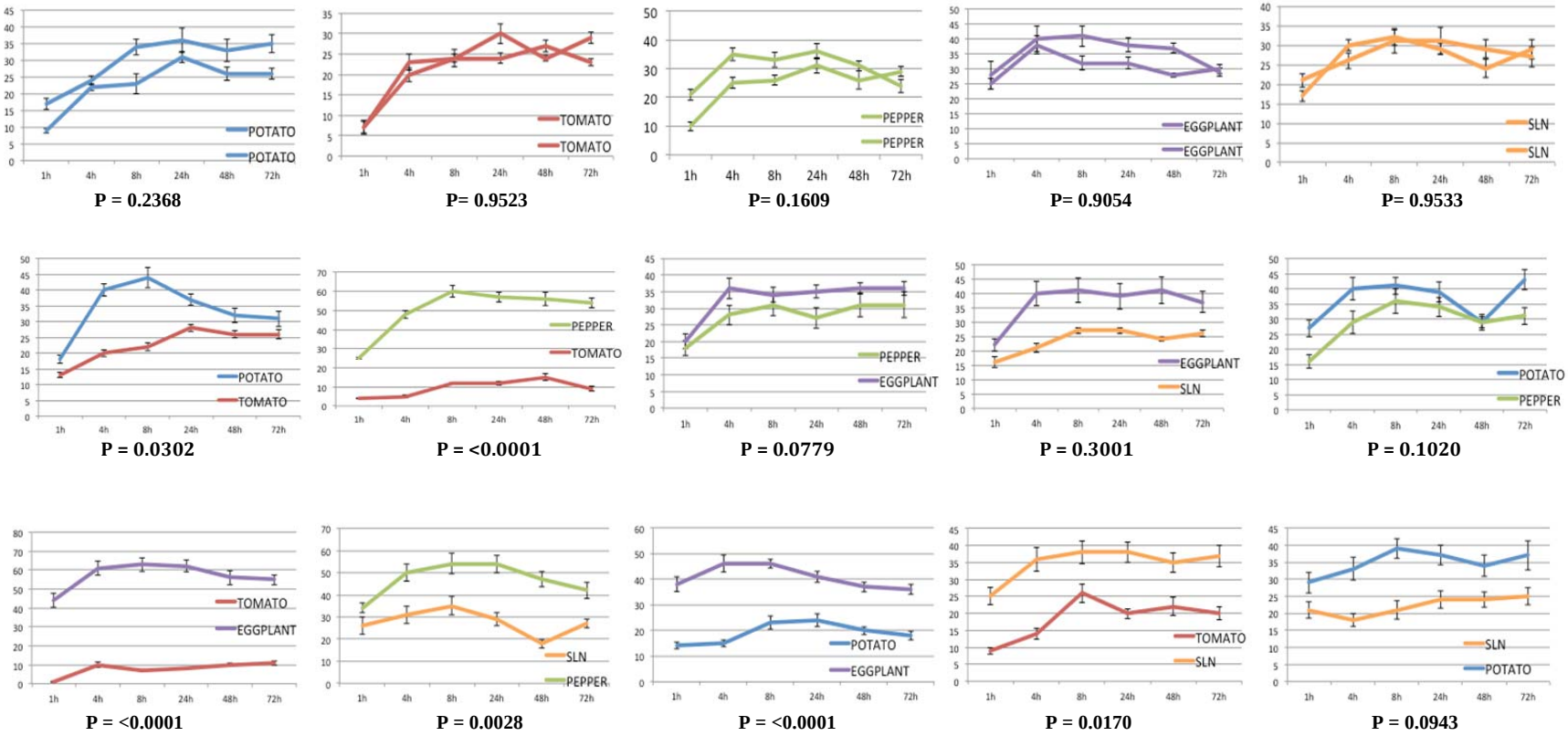


Figure 2b represents the number of adult *B. cockerelli* that settled on each of the five hosts. A Kruskal-Wallis test showed the differences to be statistically significant (p=< 0001). Bars with same letters are not significantly different from each other.

Figure 3. Settling behavior of *B.cockerelli* on solanaceous hosts



The graphs represent the number of adult *B. cockerelli* (mean of four replications ± S.E) that settled on fifteen paired comparisons in cages under laboratory conditions. The top row of five graphs represent comparisons between the same plant species, and *B. cockerelli* settled uniformly (lines overlap each other and a non-significant p-value). When they were provided with two different host species, *B. cockerelli* adults exhibited choice, as seen by the lines diverging from each other (and significant p-value) in several of the paired comparisons.

Figure 4. Plant size comparison

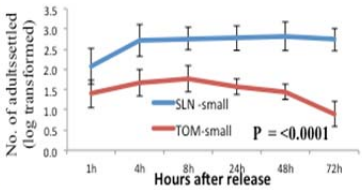


Fig. 4a

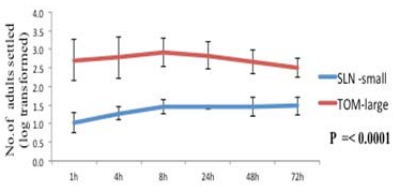


Fig. 4b

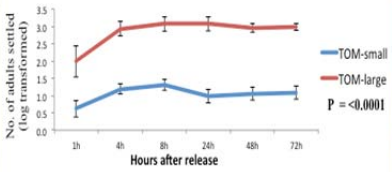


Fig. 4c

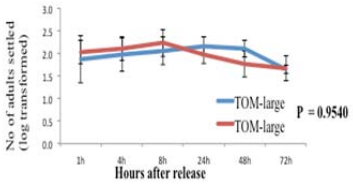


Fig. 4d

When tomato plants were compared with SLN plants of the same size, significantly more ($p<0.0001$) *B. cockerelli* adults settled on SLN as indicated by the p-value in Fig. 4a. When large tomato plants were compared with small SLN plants, *B.cockerelli* preferred the large tomato plants (Fig. 4b). Fig. 4c shows the comparison between large and small tomato plants, where significantly more ($p<0.0001$) adults settled on the large plant. While comparing similar size tomato plants (Fig. 4d), *B.cockerelli* adults settled uniformly on both plants as indicated by a non-significant p-value.

Figure 5. Settling behavior of *B. cockerelli* on Lso-infected and uninfected hosts

POTATO (3 weeks after infection)

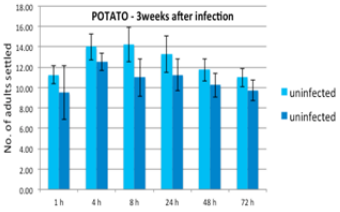


Fig. 5a

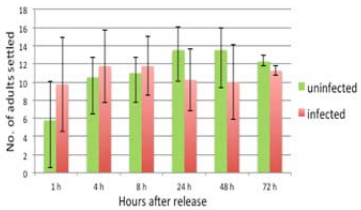


Fig. 5b

TOMATO (3 weeks after infection)

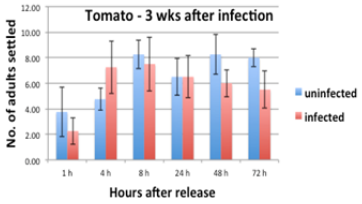


Fig. 5c

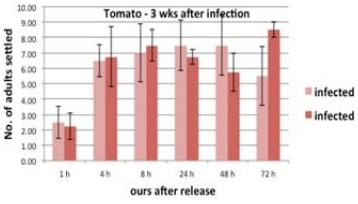


Fig. 5d

Lso-infected *B. cockerelli* adults were initially attracted to the Lso-infected potatoes (Fig. 5b) but quickly moved to the uninfected plants. However, no such behavior was observed on tomatoes (Fig. 5c), peppers and eggplants. It was observed that Lso-infected tomatoes, eggplants and peppers were asymptomatic three weeks after inoculation and *B. cockerelli* settled uniformly on infected as on uninfected plants.

Tritrophic Interactions between Psyllids, *Liberibacter,* and Solanaceous Plants: Effects on Aphids and *Hippodamia Convergens*

Kay, S., and Tamborindeguy, C.

Abstract
Vector-borne plant pathogens can affect their vector's fitness directly or indirectly through modification of the host plant upon disease development. Indirect effects can also affect the fitness of co-occurring herbivores, such as aphids. Similarly, vector-borne plant pathogens may also affect the responses of natural enemies to insect vectors as a direct consequence of the insects' harboring the pathogen or indirectly through host plant modifications that affect vector fitness or enemy attraction. We studied the effect of *Bactericera cockerelli* infestation and/or '*Candidatus* Liberibacter solanacearum' (Lso) infection on the fitness of *Myzus persicae* and *Macrosiphum euphorbiae* on solanaceous hosts (potato, tomato and bell pepper). The effect of plant infection alone on *M. euphorbiae* survival, development time and fecundity was also quantified on potato. The effect of psyllid infection on psyllid consumption by *Hippodamia convergens* was evaluated on bell pepper. There was no significant difference in *M. euphorbiae* survival or development time across treatments on potato. The presence of psyllids in combination with Lso may allow *M. persicae* to survive on an otherwise unsuitable host (tomato). Plant infection alone appeared to improve the survival of *M. persicae* on tomato, but not on pepper. Individual *H. convergens* consumed fewer infected than uninfected psyllids on uninfected pepper in a 24-h period, although the difference was not significant.

Introduction
Herbivorous insects and plant pathogens can modify plant defenses and plant composition (Walling 2000). Plant modifications and their effects on herbivores vary according to plant species (Walling 2000). Interactions among phytophagous Hemiptera, which transmit most plant pathogens, are common in nature (Denno et al. 1995). Understanding the dynamics of interactions between phytophagous Hemiptera and plant pathogens on various host plants may help in predicting pest outbreaks and subsequent disease incidence. We are studying a novel interaction between *Myzus persicae* (Hemiptera: Aphididae) and *Bactericera cockerelli* (Hemiptera: Triozidae) on tomato (*Solanum lycopersicum*). In our laboratory, a *M. persicae* clone that cannot normally survive on tomato was observed to persist for several weeks on tomato plants infested with psyllids harboring '*Candidatus* Liberibacter solanacearum' (Lso).

Plant pathogens may also affect the responses of natural enemies to insect vectors as a direct consequence of the insects' harboring the pathogen or indirectly through host plant modifications that affect vector fitness or enemy attraction. It has been shown for other systems that infection of either plants or insect vectors by plant pathogens may confer protection from natural enemies (Richmond 2007; Stumpf & Kennedy 2007). Resistance to natural enemies could have serious implications for vector control. *Hippodamia convergens* (Coleoptera: Coccinellidae) is a native generalist predator known to prey upon *B. cockerelli* (Hoffmann & Frodsham 1993). It is often found in association with *B. cockerelli* in potato-growing regions in Texas. The impact of Lso infection of either the psyllid or the host plants on psyllid predation by this beetle is currently unknown.

The objectives of this work were to: 1) characterize the interaction between *M. persicae* or *Macrosiphum euphorbiae*, and *B. cockerelli* on potato, tomato, and bell pepper, in the presence or absences of plant infection by Lso; 2) quantify survival, development time and fecundity of *M. euphorbiae* and *M. persicae* on uninfected and Lso-infected potato; 3) evaluate *H. convergens*

consumption of uninfected or Lso-infected *B. cockerelli*, on uninfected or Lso-infected potato, pepper, and tomato.

Materials and Methods

Plant material

Potato (var. Atlantic), tomato (var. Moneymaker) and bell pepper (var. Calwonder) were 48-55, 63, and 70-107 days old, respectively at the start of experiments. Plants infected with Lso were obtained by allowing 1 week feeding of two 3^{rd}-4^{th} instar *B. cockerelli* nymphs from Lso infected colonies. Plants were allowed a 2-3 week period for Lso infection before experiments.

Insects

Macrosiphum euphorbiae used in these experiments were maintained on potato (var. Atlantic). *Myzus persicae* used in these experiments were maintained on tomato and bell pepper plants. Potato psyllids used in these experiments were maintained on tomato and bell pepper plants. Adult *H. convergens* were purchased from Carolina Biological Supply Company®.

Interactions between psyllids and aphids on different hosts

Myzus persicae were monitored on bell pepper (var. Calwonder) and tomato (var. Moneymaker) with four treatments (3 plants per treatment): (1) uninfected, (2) Lso-infected, (3) uninfected + psyllids, and (4) Lso-infected + psyllids. Plants in treatments 2 and 4 were infected as previously described. Three days prior to the start of experiments, plants in treatments 3 and 4 were each pre-infested with ten 3^{rd}-4^{th} instar psyllid nymphs.

One week before beginning experiments, up to 25 adult aphid apterae were collected, isolated on uninfected, non-infested plants, and allowed to nymphoposit for 24 h, or until at least 75 nymphs had been nymphoposited. The adults were removed, and the nymphs of known age were allowed to mature to adulthood before being used in experiments.

Twenty-four hours before the start of experiments, five adult *M. persicae* were placed on each plant in all treatments. To prevent aphid or psyllid escape, pepper and tomato plants were individually caged in 12” cube cages (BioQuip); potato plants were individually caged in 22’ tubular cages made from 8.5x11” mylar sheets and masking tape, topped with thrips-proof mesh held in place by micro binder clips. All experiments were conducted at room temperature under artificial light on a timer (16:8 L/D). The number of adult aphids, aphid nymphs, and psyllid nymphs per plant were recorded daily for a 1-week period. Psyllid nymphs that molted to adulthood during the experiment were removed and replaced.

Aphid life history on potato

The survival, development time, and total fecundity of *M. euphorbiae* were measured on uninfected and Lso-infected potato plants (4 plants per treatment). Three weeks prior to the experiment, plants were infected as previously described.

Adult apterae were individually confined to one leaf of each potato plant using clip cages (BioQuip) for 24 h or until at least one nymph had been nymphoposited. All but one 1-day old nymph were subsequently removed from the cages. Caged nymphs were monitored every other day, and the instar

number was recorded until they reached adulthood. Then, adults were monitored every other day, and the nymphs produced per adult were counted and removed until death of the adults. The experiment was conducted in a growth chamber (Percival, constant 23°C, 16:8 L:D).

Beetle consumption of uninfected and Lso-infected psyllids on pepper

Eight to 12 hours prior to experiments, leaves of bell pepper (var. Calwonder) were excised, their petioles inserted through Parafilm® into water-filled 1.5 ml eppendorf tubes, and were placed in 15x150 mm plastic Petri dishes with a thrips-proof mesh window in their lids. Each leaf was infested with ten 3^{rd}-5^{th} instar *B. cockerelli* nymphs. There were two treatments (3 leaves per treatment): Uninfected Psyllids and Infected Psyllids.

A single adult *H. convergens* was added to two dishes per treatment. One dish per treatment replicate was left sealed (without a beetle) to reference psyllid survival under experimental conditions. Dishes were held at room temperature under artificial light on a timer (16:8 L/D) for 24 hours. After 24 hours, the percent mortality of psyllids was assessed. Eight replicates were performed.

Statistical analyses

The Wilcoxon and Kruskal-Wallis tests in JMP 9.0 were used to analyze the mean numbers of adults and nymphs per plant per treatment, development time and the mean longevity (days) of aphids per plant per treatment, and percent mortality of psyllids. Fecundity data for the life history bioassay was not analyzed (see below).

Results and Discussion

Interactions between psyllids and aphids on different hosts

On tomato plants, adult survival of *M. persicae* (Figure 1, left panel) did not differ significantly across treatments (prob>X^2 = 0.2006), but nymph count (Figure 2, right panel) was significantly different (prob>X^2 <0.0001). Nymph count on infected tomato plants with psyllids was significantly higher than on infected tomato plants without psyllids (prob>X^2 = 0.0002), uninfected tomato plants with psyllids (prob>X^2 <0.0001) and uninfected tomato plants without psyllids (prob>X^2 <0.0001). Nymph count on infected plants without psyllids was also significantly higher than on uninfected plants with or without psyllids (prob>X^2 = 0.0460 and 0.0207, respectively).

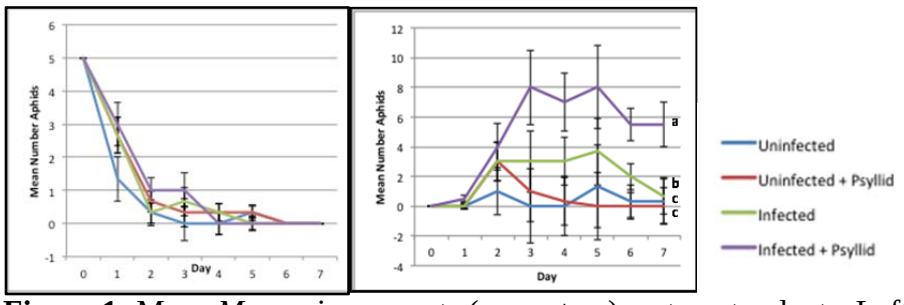


Figure 1: Mean *M. persicae* counts (mean ± se) on tomato plants. Left panel: adults, right panel: nymphs. Lines not connected by the same letter are significantly different.

On bell pepper plants, both adult survival and nymph count of *M. persicae* were significantly different across treatments (adults: prob>X^2 = 0.0004; nymphs: prob>X^2 = 0.0237). Adult survival (Figure 2, left panel) was significantly higher on uninfected plants with psyllids than on uninfected plants without psyllids (prob>X^2 = 0.0113) and infected plants without psyllids (prob>X^2 <0.0013). Adult survival was also significantly higher on infected plants with psyllids than on infected plants without psyllids (prob>X^2 = 0.0196). Nymph count (Figure 2, right panel) on uninfected plants with psyllids was also significantly higher than on uninfected plants without psyllids (prob>X^2 = 0.0379) and on infected plants without psyllids (prob>X^2 = 0.0006). Nymph count on infected plants with psyllids was significantly higher than on infected plants without psyllids (prob>X^2 = 0.0151). Nymph count on uninfected plants without psyllids was also significantly higher than on infected plants without psyllids (prob>X^2 = 0.0013).

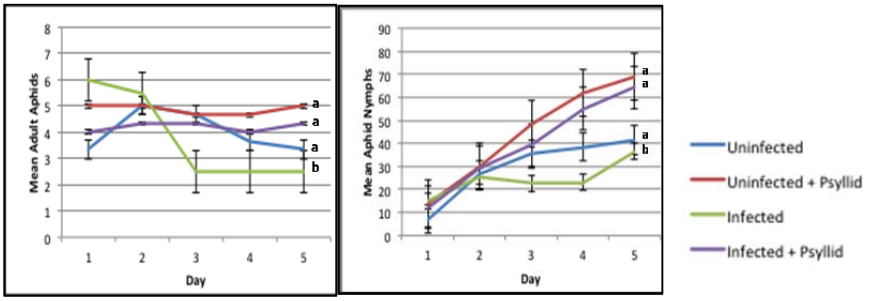


Figure 2: Mean *M. persicae* counts (mean ± se) on pepper plants. Left panel: adults, right panel: nymphs. Lines not connected by the same letter are significantly different.

Plant infection alone appears to improve the survival of *M. persicae* on tomato, but not on bell pepper. In addition, the presence of psyllids in combination with Lso may allow *M. persicae* to survive on tomato, thereby increasing this aphid's host range. These bioassays will be repeated and completed to have both aphid species' fitness studied in the three hosts.

<u>Aphid life history on potato</u>

The mean longevity of *M. euphorbiae* on uninfected and Lso-infected potato was 25 and 9.67 days, respectively, although the difference was not significant (prob>X^2 = 0.0745). Development time from 1st instar to adult (between 8-10 d) was not significantly different across treatments (Figure 3).

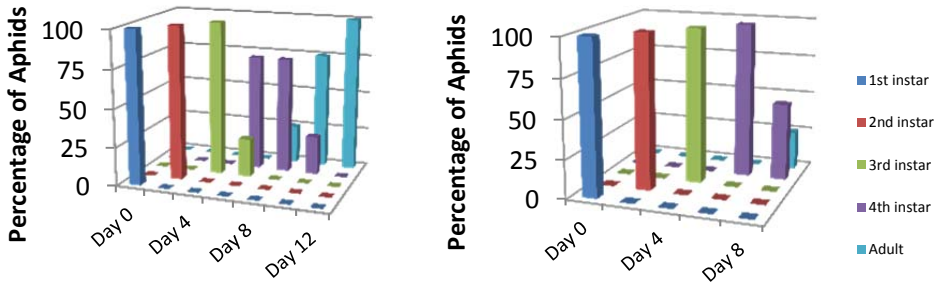


Figure 3: Aphid development time potato. Left panel: uninfected plants, right panel: infected plants.

Aphids on uninfected plants began nymphoposition at 10 days. Fecundity data for aphids on uninfected plants was confounded by large numbers of nymphs that escaped the clip cages before they could be counted, starting 14 days into the experiment. On infected plants, aphids began nymphoposition at 12 days; daily fecundity was estimated to be 4.25 nymphs. Infected plants quickly succumbed to Lso (PCR

confirmed), showing symptoms of infection (chlorosis) 2 weeks following the end of the 1-week infection period, and dying 12-16 d after the start of the experiment.

Plant infection by Lso does not appear to affect the development time of *M. euphorbiae* on potato. Lso may not confer fitness advantages to this aphid in the field. This experiment will be repeated with both *M. euphorbiae* and *M. persicae* in the three host plants. In future experiments, a sleeve cage may be employed to prevent nymph escape.

Beetle consumption of uninfected and Lso-infected psyllids on pepper

Individual *H. convergens* consumed more uninfected than Lso-infected psyllid nymphs in 24 h, although the difference was not significant (prob>X^2 = 0.9762) (Figure 4). However, with increasing numbers of replicates, the difference may become significant. In addition, this experiment will be repeated on tomato and potato leaves, which may reveal differences in beetle consumption on different host plants.

If beetles consistently consume fewer Lso-infected psyllids, the pathogen would be seen as conferring a fitness advantage to the psyllids in the form of resistance to predation.

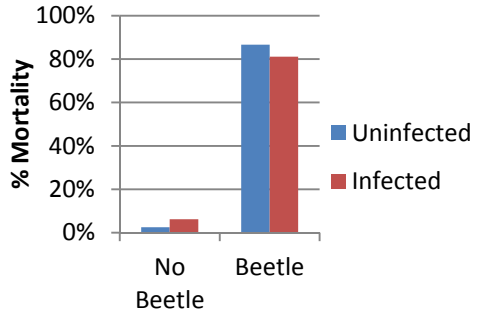


Figure 4: Percent Mortality of Psyllids in Petri Dishes

Acknowledgements

The authors thank Dr. Keyan Zhu-Salsman (Texas A&M University) for providing *M. persicae*, and Dr. Fiona Goggin (University of Arkansas) for providing *M. euphorbiae*.

References

Denno, R.F., McClure, M.S. and Ott, J.R. 1995. Interspecific Interactions in Phytophagous Insects: Competition Reexamined and Resurrected. Annu. Rev. Entomol., 40: 297-331.

Hoffmann, M.P. and Frodsham, A. 1993. Natural enemies of vegetable insect pests. Cornell University.

Richmond, D.S. 2007. Mediation of herbivore-natural enemy interactions by Neotyphodium endophytes: The role of insect behavioral response. Proceedings of the 6th International Symposium on Fungal Endophytes of Grasses (eds. A. J. Popay and E. R. Thom), 313–319. New Zealand Grasslands Association, Christchurch, New Zealand.

Stumpf, C.F. and Kennedy, G.G. 2007. Effects of tomato spotted wilt virus isolates, host plants, and temperature on survival, size, and development time of Frankliniella occidentalis. Entomologia Experimentalis et Applicata, 123: 139-147.

Walling, L.L. 2000. The myriad plant responses to herbivores. Journal of Plant Growth Regulation, 19: 195-216.

AUTHORS INDEX

NAME	PAGE
Adams, J.	40
Alvarez, J.M.	36,153
Aniciso, J.	1
Annan, I.B.	36,153
Appel, D.	173
Archer, C.	58
Arp, A.	40,145
Barrera, M.	148
Barrett, S.	1
Beard, S.	26
Berry, N.A.	26,74
Bester, G.	122
Bextine, B.	114,145,186
Bible, J.	140
Bradshaw, J.D.	1,65,158, 173,191
Bralley, J.B.	58
Brazille, J.	140
Brister, C.D.	36,153
Buchman, J.L.	23,122
Butler, C.D.	48,118
Butler, R.	26
Cameron, R.A.	153
Carpio, L.	1
Carroll, B.	140
Castner, E.P.	36
Cedillo, C.	148
Cerezo, G.	148
Chen, J.	175
Crites, S.	140
Crosslin, J.M.	1,5,65,109
Deroo, K.	1
Diaz-Montano, J.	118
Duan, Y.P.	99
Durazo, F.	148
Echegaray, E.	63
Esparza-Diaz, G.	163
Espinoza, G.	148
Footle, D.	140
French-Monar, R.D.	43,173
Galaviz, R.	148
Gonzalez, B.	118
González, J.	148
Goolsby, J.A.	1,5,65,168
Gray, J.	58
Greenway, G.	168
Gross, D.C.	179
Gudmestad, N.C.	79,99, 104,186
Guenthner, J	168
Hail, D.A.	186
Hamlin, L.	1
Hancock, J.	179
Hannukkala, A.	194
Hanson, J.D.	186
Harveson, R.M.	65,173,191
Henne, D.C.	1,5,23, 65,125, 158,168, 184,199
Hunter, W.B.	186
Huot, O.	84
Jensen, A.S.	63
Johnson, C.P.	79,99, 104,186
Jones, E.	140
Jorgensen, N.	26,74
Karasev, A.	10
Kay, S.	204
Koym, J.W.	125,184
Kral, C.W.	36
Kund, G.	31,130
Lange, R.	140
Latvala, S.	194
Lee, R.F.	118
Lemmetty, A.	194
Lévy, J.G.	84,125, 179,184
Lewis, M.	140
Lin, H.	94
Ludwig, S.	40
Manjunath, K.L.	118
Mathews, J.M.	1
McCornack, B.P.	158
McKinley, N.D.	36
Medlin, C.R.	36
Michels, J.	140
Miller, J.C., Jr.	122,125,184
Munoz, J.R.	153
Munyaneza, J.E.	23,109, 118,122
Murphy, A.F.	53,63
Nissinen, A.	194
Nolte, P.	10
Nordgaard, J.	122
Novy, R.G.	118,122
Nwugo, C.C.	94
Olsen, N.	10
Paetzold, L.	18,58,65
Pattison, S.	58
Patton III, A.F.	43
Pierson, E.A.	125,179,199
Pirhonen, M.	194
Pitman, A.R.	26
Porter, P.	1
Portillo, H.E.	36,153
Prager, S.M.	31,48,130
Ramirez, H.T.	36
Rashed, A.	18,58,65,89
Rastas, M.	194
Ravindran, A.	179
Read, N.S.	74
Read, S.	26
Richmond, J.	1
Rondon, S.I.	53,63,70
Rush, C.M.	18,58,65,89
Samler, J.	40
Scheuring, D.C.	125,184
Schuster, G.	1,145,173
Seger, P.	1
Serrato-Diaz, L.M.	43
Shah, F.	26
Swisher, K.D.	109
Tamborindeguy, C.	84,125,179,186, 199,204
Taylor, N.	26,74
Teulon, D.A.J.	13
Thinakaran, J.	199
Thompson, S.	26
Thornton, M.	10
Tran, L.T.	13
Trevino, J.	145
Trumble, J.T.	31,48, 118,130
Van Hest, P.	122
Vereijssen, J.	13,74
Villanueva, R.T.	163
Walker, M.	26
Walker, S.	1
Wallingford, A.	89
Wallis, C.M.	89,122
Walston, A.	40
Wen, A.	79,104
Wenninger, E.J.	10
Whipple, S.D.	1,65,158, 173,191
Willett, J.	1
Wilson, G.D.	36
Workneh, F.	18,58,65
Worner, S.P.	13
Wright, P.	26
Yilma, S.	122