INVESTIGATING THE TRANSCRIPTOME OF THE POTATO PSYLLID (*BACTERICERA COCKERELLI*): TOWARD AN RNAI BASED MANAGEMENT STRATEGY

Hail[1], D.A., Dowd[2], S., Hunter[3], W.B., and B. Bextine[1]

1. University of Texas, Tyler, TX 75799, USA
2. Research and Testing Laboratory, Lubbock, TX 79407, USA
3.USDA ARS, U.S. Horticultural Research Lab, Ft. Pierce, FL 34945, USA

Summary

The potato psyllid is the vector of the α-Proteobacteria *Candidatus* Liberibacter psyllaurous, the causal agent of Zebra Chip in potatoes. The disease is a major limiting factor in both the production and processing of potatoes into chips and there is currently no cure. RNA interference (RNAi) has the potential to limit the spread and severity of Zebra Chip by reducing the insect load per field per season. Pyrosequencing was used to identify target genes and synthetic dsRNA constructs were designed to block the activity of heat shock protein 70 and heat shock cognate 70. These molecular chaperones are intimately involved in essentially all life processes by folding and stabilizing other proteins. In this study these constructs were tested on potato psyllid cell cultures and morphological evidence showed shriveling and dispersal into the media. We plan to target other genes and deliver RNAi to insects via the plant through a root soaking procedure.

Citation: **Hail, DA, Dowd, S, Hunter, WB, Bextine, B. Investigating the transcriptome of the potato psyllid (*Bactericera cockerelli*): Toward an RNAi- based management strategy. Proceedings of the 10th Annual 2010 Zebra Chip Reporting Session. (Workneh, F, and C.M. Rush, Eds). Pg: 183-186. Nov. 7-10, Dallas, TX.**
http://agrilife.org/zebrachip/files/2011/06/PROCEEDINGS_2010.pdf

Introduction

Zebra chip (ZC) is a disease of the potato (*Solanum tuberosum* L.) (Abad et al 2009) characterized by chlorosis, yellowing, curling and scorching of leaves, swollen nodes and aerial tubers. When below ground tubers are sliced and processed into potato chips, alternating light and dark bands along the medullary rays become prominent due to the increased amount of soluble sugars in infected plants (Gao et al 2009). The disease is caused by an intracellular infection by the recently implicated phloem-limited α-Proteobacteria *Candidatus* Liberibacter psyllaurous (Hansen et al 2008; Liefting et al 2008, 2009). The potato psyllid (*Bactericera cockerelli*), a phloem feeding insect pest of solenaceous plants, has been associated with the disease (Munyaneza et al 2007) and is now a known vector of the putative causal agent.

Once referred to as papa manchada or stained potato, Zebra chip was originally identified in potato fields surrounding Saltillo, Mexico, in 1994 (Secor, unpublished). Since then the disease has caused millions of dollars in losses (CNAS 2006; Secor and Rivera-Varas 2004) and continued to be a major threat to both producers and processors (Hernandez-Garcia et al 2006; Salas-Marina et al 2006), having spread north into the United States and south into Guatemala. In 2004 the disease was so prevalent that in the northeastern states of Coahuila and Nuevo Leon as many as 80% of plants in affected fields showed symptoms. Many fields with particularly heavy infestations of *Candidatus* Liberibacter infected potato psyllids have had to be abandoned entirely (Flores et al 2004).

When genes are active, or are coding, they produce mRNA which instructs the cell to manufacture a protein derivative of the gene. RNA interference (RNAi) is a method of down regulating specific genes in a cell by the application of synthetic double stranded RNA (dsRNA) molecules designed to be complementary to a gene's mRNA (Fire et al 1998). When the dsRNA taken into the cell, a protein called Dicer cleaves it into small segments. These segments are incorporated into the RNA induced silencing complex (RISC) and the cell's mRNA, using the synthetic RNA as a template, is enzymatically degraded thereby blocking protein production in a dose dependent manner. In an alternative pathway, the cleaved segments of dsRNA are used as templates for the RNA dependent elongation enzyme RdRP; in this way multiple off-target genes can be down regulated by a single dsRNA construct. Since its discovery, RNA interference has proved promising in determining a gene's function, controlling cancer and

slowing viral replication (Huvenne and Smagghe 2010). Many studies have indicated that RNAi has great potential in managing insect pest – especially those that are capable of causing damage or disease in plants (Borovsky, 2005; Gordon and Waterhouse, 2007; Price and Gatehouse, 2008). In this study we used pyrosequencing to compile cDNA libraries for the adult and 5th instar lifestages of the potato psyllid. With this information we identified several targets for an RNAi-based management strategy.

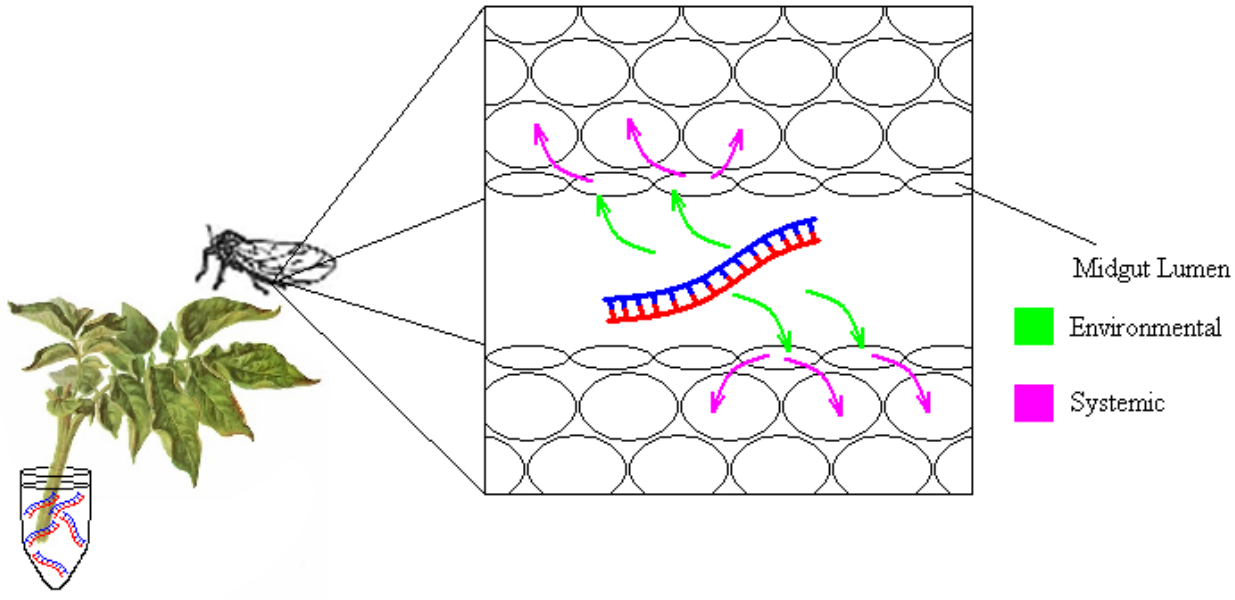

Figure 1. A novel dsRNA delivery mechanism for the RNAi in the Potato Psyllid. The roots of the plant are soaked with the constructs and drawn up into the plant to be delivered upon feeding.

Materials and Methods

Potato psyllids used to start a colony were provided by Drs. Tong-Xian Liu and Xiangbing Yang (Texas AgriLife Research) and maintained on potatoes (25°C; 40% humidity). Adult and 5th instar psyllids of mixed genders were collected and total RNA was extracted using the RNeasy mini Kit (Qiagen). Poly-A mRNA was isolated with the Oligotex mRNA mini kit (Qiagen), retrotranscribed using Stratagene's ZAP-cDNA synthesis kit and sent to the Research and Testing Laboratory of the Medical Biofilm Research Institute for pyrosequencing. Double-stranded cDNA was quantified and nebulized to 300-550bp fragments and the pyrosequencing

library was created according to manufacturer's instructions (Roche 454). Resulting sequences were assembled using DNAstar's NGEN assembler (Madison WI) and annotated using BLASTx - W.ND BLAST (Dowd et al. 2005) and cross referenced to functional annotations using DAVID (Huang et al. 2009).

Results and Discussion

Sequences homologous to previously identified cellular function and metabolic activity genes were recovered from the libraries. Sequences related to ribosomal functions, organelle construction, and muscular, neurological, and reproductive developmental processes were recovered. Stress response, ion transporters, nucleic acid binding, and primary metabolism sequences were also recovered. This information can be used to identify gender and life- stage-specific genes for RNAi-based management strategies, providing new direction for targeting single pest insects as opposed to the current broad spectrum insecticide regimes. Genes of interest include vitellogenin, an egg yolk precursor protein, ejaculatory bulb protein, a male reproductive protein, and actin II, one of the many genes responsible for proper wing function or formation.

Current targets are general house-keeping genes like heat shock proteins and heat shock cognates, molecular chaperones that influence essentially all cellular pathways by trafficking proteins to their proper locations in the cell and fold or refold proteins into their enzymatically active configuration. Presently, we are attempting to use a cocktail of 3 dsRNA constructs against the heat shock protein 70 (HSP 70) gene and 3 dsRNA constructs against the heat shock cognate 70 (HSC 70) gene to limit the viability of potato psyllid cells in culture. In response to the dsRNA cocktail, the cells began to shrivel, disconnect from the monolayer and disperse into the media, indications of overall poor health. We plan to investigate the gene knock down efficiency of the cocktail by quantifying the amount of mRNA specific to the target genes in non-treated, buffer treated and dsRNA treated cells. If this cocktail proves successful we plan to scale up the process and work with whole insects on potato plants to test the ability of dsRNA to limit psyllid populations via the digestive tract. In a separate study we have developed a novel delivery method for the potato psyllid based on saturation of the host plant’s roots with dsRNA (Figure 1). The constructs are efficiently drawn up the roots, into the stems and delivered via the leaf veins to the insect.

Acknowledgements

Financial support for this research was provided by the USDA-SCRI (Project #2009 51181-20176).

References

Abad JA, Bandla M, French-Monar RD, Liefting LW, Clover GRG (2009) First Report of the Detection of 'Candidatus Liberibacter' Species in Zebra Chip Disease-Infected Potato Plants in the United States. *Plant Diseases* 93:108.

Borovsky, D., (2005) Insect peptide hormones and RNA-mediated interference (RNAi): promising technologies for future plant protection. *Phytoparasitica* 33, 109–112.

Gao F, Jifon J, Yang X, Liu TX (2009) Zebra chip disease incidence on potato is influenced by timing of potato psyllid infestation, but not by the host plants on which they were reared. *Ins Sci* 16:399–408.

Gordon, KJ, Waterhouse, P.M. (2007) RNAi for insect-proof plants. *Nature Biotechnology* 25: 1231–1232.

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

Liefting LW, Ward LI, Shiller JB, Clover GRG (2008) A New 'Candidatus Liberibacter' Species in Solanum betaceum (Tamarillo) and Physalis peruviana (Cape Gooseberry) in New Zealand. *Plant Diseases* 92:1588.

Hansen AK, Trumble JT, Stouthamer R, Paine TD (2008) 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.

C.N.A.S. (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

Secor GA, Rivera-Varas VV (2004) Emerging diseases of cultivated potato and their impact on Latin America. Rev Latino Americana de la Papa (Suppl.) 1:1-8.

Salas-Marina MA, Flores-Olivas A, Sanchez-Arizpe A, Garcia-Martinez O, Almeida-Leon IH, et al (2006) Eficiencia de insectos vectores en la transmission de fitoplasmas de la punta

morada de la papa, pp. O-1. In Memoria de XXII Congreso de la Asociacion Latinoamericana de la Papa, ALAP, Toluca, Mexico.

Fire A, Xu SQ, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 391:806–811.

Flores OA, Gallegos MG, Garcia MO (2004) Memorias del simposio punta morada de la papa. Universidad Auto´noma Agraria Antonia Narro, Saltillo, Coahuila, Mexico.

Hernandez-Garcia V, Sanchez-Arizpe A, Frias-Trevino GA, Padron-Corral E (2006) Factores abioticos y su relacion con el sindrome de punta morada de la papa, p. C-17. In Memoria de XXII Congreso de la Asociacion Latino Americana de la Papa, ALAP, Toluca, Mexico.

Munyaneza JE, Crosslin JM, Upton JE (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.

Dowd SE, Zaragoza J, Rodriguez JR, Oliver MJ and Payton PR. (2005) Windows.NET Network Distributed Basic Local Alignment Search Toolkit (W.ND-BLAST). *BMC.Bioinformatics* 6: 93.

Huang DW, Sherman BT and Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nature Protoc*. 4: 44-57.

Price DG and Gatehouse JA (2008) RNAi-mediated crop protection against insects. Trends in Biotechnology 26, 393–400.

Huvenne H and Smagghe G (2010) Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: A review. *Journal of Insect Physiology* 56:227–235.