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Resistance in field pea (*Pisum sativum*) to the black spot disease complex in Western Australia

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Abstract Black spot (also referred as Ascochyta blight, Ascochyta foot rot and black stem; Ascochyta leaf and pod spot) is a devastating disease of field pea (*Pisum sativum*) caused by one or more pathogenic fungi including *Didymella pinodes*, *Ascochyta pisi*, *Phoma pinodella* and *P. koolunga*. Development of resistant germplasm has been slow because of the low level of resistance found in the available germplasm, poor reliability of screening methods and the polygenic nature of inheritance. Field studies, undertaken to assess F8 and F9 derived lines for resistance against the black spot complex, confirmed that some lines developed in the Australian breeding program show improvement in resistance over commercial cultivars. Disease scores across test lines ranged from 5.33 to 7.82 (0–9 scale where 0=no disease symptoms, and 9≥90 % leaf area affected) and from 5.37 to 8 in 2012 and 2013, respectively. In 2012, the eight most resistant lines had scores 5.33 to 6, with OZP1207 the most resistant line. In 2012, forty three lines were significantly more resistant (disease score≤6.67) than the susceptible standard, Helena (7.82), 14 lines were not significantly different to the most resistant commercial cultivar, Parafield (6.33), and 27 lines were significantly more resistant than PBA Percy (7.67). In 2013, WAPEA2211 was clearly the most resistant line (5.37) followed by a group of seven lines with slightly less resistance scored at 6. Use of these lines in breeding will further enhance resistance in commercial cultivars, particularly by inter-crossing among the more genetically diverse lines to accumulate minor genes for resistance. While there was no overall relationship between disease scores in 2012 and 2013 (R^2=0.029), presumably due to the highly variable pathogen composition of the black spot complex at the screening site and across seasons in Western Australia, a few lines, such as WAPEA2211, 04H349P-05HO2005, 06H109P-9 and 06H459P-1, showed significant resistance in both years, appear to have resistance to multiple pathogens in the black spot complex, and are of particular significance.

Keywords Field pea · *Pisum sativum* · Black spot · *Didymella pinodes* · *Ascochyta* · *Phoma*

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Introduction

Field pea, *Pisum sativum*, is a valuable food and feed crop (Beeck 2006); cultivated across more than 60 countries. It is the second largest legume crop worldwide (FAOSTAT 2012 access); and the largest grain legume crop in Europe (FAOSTAT data, 2007; http://faostat.fao.org/). In Australia, field pea was probably the

first pulse crop to be grown; and became a major crop from mid-1980s (Siddique and Sykes 1997). Further, field pea is also an important species in the cropping sequence (Khan et al. 2013) and in 2013 was the second largest pulse crop in Australia with production of approximately 400,000 tonnes (Anonymous 2013).

Necrotrophic pathogens dominate regions with a winter-dominant rainfall, including those with a Mediterranean-type climate, because of the ease of survival in these soil and trash-borne pathogens on infested residues through the dry summer periods. The impoverished and nutrient-deficient soils across many parts of these regions predispose the plant host to these pathogens as there is often little microbial competition with the necrotrophic pathogens (Sivasithamparam 1993). Hence, it is not unexpected that necrotrophic fungal pathogens provide significant challenges to the field pea crop in such regions. As global warming leads to climate change, significant changes are expected in relation to the relative importance and impacts of necrotrophic diseases, particularly in regions of Australia with Mediterranean-type climates, such as south-west Western Australia and significant areas of South Australia and Victoria in relation to crop legumes (Chakraborty et al. 1998), forage legumes (Jones and Barbetti 2013) and other major crops such as oilseed brassicas (Barbetti et al. 2012).

The major constraint in field pea production is black spot (Bretag et al. 1995; Davidson and Ramsey 2000; Bretag et al. 2006), with yield losses from black spot ranging from 30 to 75 % in Australia, France, and Canada (Roger et al. 1999; Kaiser et al. 2000). Generally the minimum yield loss from black spot reported in Australia is >10 % (Bretag et al. 2006; McDonald and Peck 2009), especially in Western Australia where yield loss can easily be up to 50 % (Salam et al. 2011).

Historically the well-known causal agents in the black spot complex include three fungi belonging to the phylum Ascomycota, viz. *Didymella pinodes* (also known as *Ascochyta pinodes*), *Ascochyta pisi*, *Phoma pinodella* (*syn. Phoma medicaginis* var. *pinodella* and formerly known as *Ascochyta pinodella*) (Davidson and Ramsey 2000; Beeck 2006; Davidson and Kimber 2007; McDonald and Peck 2009). Recently, more pathogens associated with black spot have been found, such as *Phoma koolunga* (Davidson et al. 2009), *Phoma herbarum* (Li et al. 2011), *Boerema exigua* var. *exigua* (Li et al. 2012), and *Phoma glomerata* (Tran et al. 2014b). Among the pathogens in the black spot complex, *D. pinodes* is believed to be generally the most important (Moussart et al. 1998; Tivoli and Banniza 2007); as also reported in Canada (Gossen et al. 2011), southern Australia (Bretag 1991) and France (Tivoli et al. 1991).

Management strategies for black spot are well documented (Wallen et al. 1967; Warkentin et al. 1996; Warkentin et al. 2000; Bretag et al. 2006; Davidson and Kimber 2007). However, development and deployment of host resistance potentially offers the most cost-effective control. At present, available commercial varieties are susceptible and sources of host resistance are few and only partially effective (Khan et al. 2013). Despite there being only slow development of resistant germplasm due to low level of resistance found and the polygenic inheritance of the resistance (Wroth 1998; Bretag et al. 2006; Zhang et al. 2006; Khan et al. 2013), considerable progress in pea breeding has been made in the last decade in Western Australia (Adhikari et al. 2010; 2014). This is in part due to identification of a 'hot spot' for persistent natural epidemics of black spot identified at Medina, Western Australia, where field screenings for resistance are routinely undertaken. Even there, unreliable screening occurred before the early 1990's, as it was only after that time when field disease screeing protocols were utilised that defined optimum sowing time, crop rotation to minimise root diseases, and ensured adequate and reliable natural infection and differentiation of genotype susceptibilities/resistances. Adhikari et al. (2014) reported on a breeding program that combined the agronomically important traits with moderate levels of resistance in F4-F5 derived F6 and F7 lines. Subsequent selections have progressed to F8 and F9 stage. The Adhikari et al. (2014) study deals with transferring resistance from an agronomically undesirable gene pool into agronomically suitable backgrounds. This current paper, in contrast, shows the application to an operational pea breeding program in developing more resistant lines for potential release to farmers. We highlight significant improvement in resistance over commercial cultivars against the black spot complex in F8/F9 derived field pea lines. This will ensure enhanced resistance for new commercial cultivars, particularly by inter-crossing the more genetically diverse lines to accumulate minor genes for resistance. Lines identified showing resistance to multiple pathogens within the black spot complex are of particular significance to breeding programs.

Materials and methods

Germplasm

Original resistant parents came from a variety of sources (Adhikari et al. 2010; 2014). Development of germplasm each year of the breeding program involved about 50 crosses made by: i), intercrossing amongst best resistant breeding lines with different sources of resistance; ii), crossing the best resistant breeding lines each year with what could be considered new sources of resistance; and, iii), crossing the best resistant breeding lines each year with agronomically suitable, but susceptible, advanced breeding lines (Adhikari et al. 2014). This resulted in a recurring cycle every three years when new and improved parents would be added in the crossing program to enhance the resistance, as outlined in Adhikari et al. (2014). A single seed descent (SSD) method in breeding has been used, as has been reported by Adhikari et al. (2010, 2014).This investigation reports further progress in enhancing resistance since the last evaluation by Adhikari et al. (2014). Enhanced germplasm developed was regularly fed to the core national pea breeding program of the Pulse Breeding Australia (PBA) for incorporation through use as parents and through further selection. The germplasm reported in this paper is breeding material that is representative of this enhanced germplasm. To show the difference and/or improvement of the F8/F9 derived lines, some other commercial cultivars were also added into this black spot screening, i.e. Kaspa (commercialised by AWB Seeds Pty Ltd.), Parafield (commercialised by Plant Tech Pty Ltd) (Jenkins et al. 2005) PBA Gunyah, PBA Percy, PBA Oura, PBA Twilight (semi-dwarf dun seeded, developed by Australian Field Pea Breeding Program - AFPBP and marketed by AWB Seeds Pty Ltd.), Sturt (tall field pea, developed by AFPBP and marketed by Premier Seeds) (Stratford 2011) (Table 1).

Field resistance screening experiments

Germplasm evaluations were undertaken at Medina (32.2358°S; 115.8074°E) in Western Australia, a location where black spot occurs in epidemic proportions each year (Adhikari et al. 2010, 2014). Black spot at Medina is initiated by ascospores that travel long distances from pea stubbles and inoculum sources would include many different commercial market gardener and 'home' gardener field pea plantings across a range of different cultivars

Table 1 Black spot disease scores of field pea (*Pisum sativum*) lines assessed for resistance in two separate trials undertaken in 2012 and 2013 at Medina Research Station, Western Australia

Field pea line	Pedigree	Disease score 2012	Disease score 2013
OZP1207	PS2016/PS1677	5.33	7.00
WAPEA2211	PSL4/98107-62E	5.61	5.37
04H297P-05HO2018	PS1721/PS1718	5.83	7.00
06H291P-15	PS2985/PS2037	5.83	7.00
05H336-06HOS2003	PS2030/PS2039	6.00	7.00
06H033P-6	PS2019/PS2037	6.00	7.00
06H093P-8	PS2077/PS2030	6.00	7.00
06H384P-7	PS3006/PS2951	6.00	8.00
05H334-06HOS2003	PS2030/PS2037	6.17	7.00
05H161P-1	PS1727/PS2016	6.33	7.00
05H375-06HOS2004	PS2037/PS2039	6.33	7.00
05H400-06HOS2005	PS2039/PS2037	6.33	7.00
OZP1001	PS1594/PS1555	6.33	7.00
OZP1208	PS2032/PS2041	6.33	7.00
Parafield	SA343/SA1405	6.33	7.00
04H349P-05HO2005	PS1728/PS1752	6.50	6.00
04H356P-05HO2004	PS1729/PS1753	6.50	7.00
06H093P-6	PS2077/PS2030	6.50	8.00
06H109P-9	PS2097/PS2037	6.50	6.00
06H205P-2	PS2966/PS2091	6.50	7.00
06H213P-6	PS2967/PS2957	6.50	7.00
06H216P-15	PS2968/PS2967	6.50	7.00
06H310P-8	PS2989/PS2037	6.50	7.00
06H408P-1	PS3012/PS2091	6.50	7.00
06H432P-3	PS3019/PS2958	6.50	7.00
06H459P-1	PS3027/PS2037	6.50	6.00
06H461P-7	PS3027/PS2967	6.50	7.00
00P040-2-5	Not available (N/A)	6.67	8.00
04H049P-05HO2003	N/A	6.67	7.00
04H341P-05HO2010-1	N/A	6.67	6.00
05H141-06HOS2003	PS1714/PS1752	6.67	7.00
05H277-06HOS2002	PS2022/PS1755	6.67	7.00
05H371-06HOS2003	PS2037/PS2020	6.67	7.00
06H056P-4	PS2032/PS2951	6.67	7.00
06H181P-1	PS2957/PS2032	6.67	7.00
06H246P-4	PS2975/PS2967	6.67	7.00
06H329P-9	PS2993/PS2030	6.67	7.00
06H364P-12	PS3001/PS1156	6.67	7.00
OZP0903	PS1455/PS1619	6.67	7.00
OZP1002	N/A	6.67	7.00
OZP1202	PS1464/PS1156	6.67	7.00
OZP1203	PS1455/PS1304	6.67	7.00
Kaspa	PS772/PS770	6.68	7.07

Table 1 (continued)

Field pea line	Pedigree	Disease score 2012	Disease score 2013
04H341P-05HO2010-9	N/A	6.83	7.00
05H007-06HOS2004	N/A	6.83	7.00
06H033P-5	PS2019/PS2037	6.83	8.00
06H045P-6	PS2023/PS2951	6.83	7.00
06H168P-5	PS2954/PS1156	6.83	8.00
06H362P-1	PS3000/PS2091	6.83	7.00
06H438P-3	PS3021/PS2091	6.83	7.00
OZP1101	PS1420/PS1718	6.83	6.00
PBA Oura	PS485/PS998// PS1955/// PS1958	6.83	7.20
PBA Twilight	PS1156/PS1537	6.83	6.67
01H273-02HO2005-04HO6003-06NAS001	N/A	7.00	7.00
03H296-04HO2013-06NAS001	N/A	7.00	7.00
04H150P-05HO2006	N/A	7.00	8.00
04H374P-05HO2003	N/A	7.00	7.00
05H060-06HOS2001	N/A	7.00	7.00
05H161-06HOS2001	N/A	7.00	7.00
05H161-06HOS2004	N/A	7.00	7.00
05H248P-2	N/A	7.00	7.00
05H264-06HOS2004	N/A	7.00	7.00
06H012P-4	PS1715/PS2951	7.00	8.00
06H016P-7	PS1730/PS2030	7.00	8.00
06H036P-2	PS2019/PS2953	7.00	7.00
06H061P-4	PS2032/PS2967	7.00	7.00
06H064P-3	PS2036/PS2037	7.00	7.00
06H110P-5	PS2097/PS2951	7.00	8.00
06H200P-8	PS2965/PS2953	7.00	7.00
06H247P-8	PS2976/PS1528	7.00	7.00
06H254P-2	PS2977/PS2037	7.00	7.00
06H309P-9	PS2989/PS2030	7.00	7.00
06H349P-11	PS2997/PS2958	7.00	8.00
06H349P-12	PS2997/PS2958	7.00	7.00
06H413P-3	PS3013/PS2958	7.00	8.00
06H439P-1	PS3021/PS2958	7.00	7.00
06H445P-5	PS3023/PS2037	7.00	7.00
OZP1105	N/A	7.00	8.00
OZP1201	PS1677/PS1591	7.00	7.00
OZP1206	PS1677/PS1156	7.00	7.00
04H150P-05HO2007	N/A	7.17	7.00
04H341P-05HO2010	N/A	7.17	7.00
05H009-06HOS2005	N/A	7.17	7.00
05H029-06HOS2004	N/A	7.17	7.00
05H189-06HOS2001	N/A	7.17	6.00
05H269-06HOS2001	N/A	7.17	7.00
05H278-06HOS2003	N/A	7.17	8.00
05H365-06HOS2002	N/A	7.17	7.00
06H045P-3	N/A	7.17	7.00
06H052P-2	N/A	7.17	7.00
06H097P-9	PS2077/PS2958	7.17	6.00
06H204P-3	PS2966/PS2037	7.17	7.00
06H314P-3	PS2990/PS2951	7.17	7.00
06H405P-3	PS3011/PS2958	7.17	8.00
OZP1104	PS2016/PS1701	7.17	7.00
OZP1204	PS1723/PS1594	7.17	7.00
Sturt	PS726/PS864	7.17	7.00
05H128-06HOS2001	N/A	7.33	7.00
05H203-06HOS2002	N/A	7.33	7.00
05H347-06HOS2005	N/A	7.33	7.00
06H213P-14	PS2967/PS2957	7.33	7.00
06H266P-7	PS2979/PS2037	7.33	7.00
06H351P-1	PS2998/PS1528	7.33	7.00
06H357P-2	PS2999/PS2037	7.33	7.00
06H392P-1	PS3008/PS2091	7.33	7.00
06H422P-3	PS3017/PS1528	7.33	7.00
PBA Gunyah	PS1594/PS1535	7.33	7.00
06H379P-6	PS3004/PS2958	7.50	7.00
06H428P-2	PS3018/PS2953	7.50	7.00
05H363-06HOS2001	N/A	7.67	7.00
06H035P-1	N/A	7.67	7.00
06H269P-1	PS2980/PS2951	7.67	8.00
OZP1205	PS1752/PS1304	7.67	7.00
PBA Percy	N/A	7.67	7.00
Helena	Dundale/WA0001	7.82	7.38
Mean		6.86	
Standard error		0.57	
F-test (disease score in 2012)		3.09 ($P<0.01$)	
LSD		1.12	
R^2 (correlation of disease score in 2012 and 2013): 0.0289 ($P>0.05$)			

in the Medina region. Protocols followed have been developed by Adhikari et al. (2010, 2014). Briefly, this involves the very early sowing of a 'sick plot' about 100 m^2 created 5 m away from the disease nursery by planting a susceptible variety, Dundale, four weeks before

the nursery was sown. This ensures a severe natural black spot epidemic on the neighbouring test lines. The disease nurseries in both years were sown in the 2nd to 3rd week of May. Test lines were planted in 3 m long, single row plots. Susceptible controls (Dundale and Helena) were planted as 'sick plots' after every 20 plots to help spread the disease. Over the years we have noted that these two cultivars encourage predominance of *D. pinodes*, our primary pathogen target in these studies. Only Helena was included as a treatment comparison in the trial because Dundale is no longer used commercially. June to August are the wettest months at Medina and provide ideal conditions for black spot disease development. Overhead sprinkler irrigation was provided to maintain moist conditions if dry periods persisted for more than 2 days. Crop management followed standard practices for the region. Plants were allowed to be naturally infected. About 14–15 weeks after sowing, each plot was scored for the severity of black spot on a 0–9 scale (where 0 denotes no disease symptoms, and 9 denotes more than 90 % leaf area infected) (Adhikari et al. 2010, 2014). The experiment in 2012 had two replications. To examine the reliability of the natural occurrence of black spot at Medina and our assessment method, in 2013 further-derived F9 lines were tested in single replication (due to a lack of seed) with repeated checks as in 2012. WAPEA2211 was the first germplasm developed in an agronomically suitable background in Western Australia with a moderate level of resistance and this level of resistance was used as a benchmark in the study. WAPEA2211 is a line derived from the cross PSL4/98107-62E and the latter parent is the source of resistance. Scores of ≥7 were considered as susceptible; any line that showed scores of<7 was considered to have some improvement in resistance. This separation of susceptible *vs* resistance was used earlier by Adhikari et al. (2014) and is considered as the acceptable distinction between the two categories for field screening in Australia. In particular, we have viewed all improvements in resistance against the disease reaction of commercial varieties PBA Percy and Helena, where scores ranged from 7 to 7.82. Only field testing was undertaken in our study as at that time there was no reliable for screening against individual black spot pathogens under controlled conditions.

Data analysis

Data in this experiment was analysed using GenStat (14th Edition, Copyright 2011, VSN International Ltd.). One-way ANOVA function was employed to analyse the data of 2012 trial. Fisher's least significant differences ($P<0.05$) were used to compare line reactions to black spot. Due to lack of available seeds, there was no replication in the trial conducted in 2013, comparisons of differences in disease score of each line over two years were made using the regression function of GenStat.

Results

In 2012, the black spot disease score of field pea lines under test ranged from 5.33 (OZP1207) to 7.82 (Helena), with a mean score of 6.86. The eight most resistant lines in 2012 had scores in the narrow range of 5.33 to 6; with OZP1207 most resistant, followed by WAPEA2211, 04H297P-05HO2018, 06H291P-15, 05H336-06HOS2003, 06H033P-6, 06H093P-8 and 06H384P-7. Thirty five of the most susceptible lines had disease scores ranging from 7.17 to 7.82. Among the commercial cultivars screened, Sturt (7.17), PBA Percy (7.67) and PBA Gunyah (7.33) were the most susceptible, in which the disease score of the three cultivars were not significantly different to that of the susceptible standard line, Helena (7.82). Further, the most susceptible commercial cultivars, e.g., Helena and PBA Percy, were consistently so with disease scores across the two years ranging from 7 to 7.8. Parafield (6.33) was the most resistant commercial cultivar tested, but not significantly different to the most resistant genotypes, viz. OZP1207 (5.33) and WAPEA 2211 (5.61). Overall, there were 43 lines significantly more resistant (disease score≤6.67) than the susceptible standard, Helena (7.82), 14 lines were not significantly different to the most resistant commercial cultivar, Parafield (6.33), and 27 lines were significantly more resistant than PBA Percy (7.67), a susceptible commercial cultivar employed in the screening conducted in 2012.

In 2013, black spot scores ranged from 5.37 to 8, with a mean score of 7.06. WAPEA2211 was clearly the most resistant line in 2013, followed by a group with slightly lower resistance consisting of 04H349P-05HO2005, 06H109P-9, 06H459P-1, 04H341P-05HO2010-1, 06H097P-9, 05H189-06HOS2001 and OZP1101. There were 15 highly susceptible lines with a score of 8.

There was no significant correlation between black spot disease scores in 2012 with those across the same

lines in 2013 (R^2=0.029), reflecting fluctuations of relative rankings of lines in their disease scores between the two years of observation. Fifty six and 39 lines showed increased or decreased disease scores, respectively, between the two seasons. In particular, a large increase in the disease scores was observed across three of the more resistant lines in the 2012 test (e.g., OZP1207, 06H384P-7, and 06H291P-15), where their scores increased; from 5.33 (2012) to 7 (2013), from 6 (2012) to 8 (2013), and from 5.83 (2102) to 7 (2013), respectively. In contrast, for some other lines, e.g., OZP1101, 06H428P-2, and OZP1205, their scores reduced from 6.83 (2012) to 6 (2013), from 7.5 (2012) to 7 (2013) and from 7.67 (2012) to 7 (2013), respectively.

Discussion

This study highlights significant improvement in resistance in some breeding lines over commercial cultivars against the black spot complex in F8/F9 derived field pea lines. Amongst lines with lowest scores in 2012, i.e. 5.33 to 6, OZP1207 was the most resistant line followed by WAPEA2211, 04H297P-05HO2018, 06H291P-15, 05H336-06HOS2003, 06H033P-6, 06H093P-8 and 06H384P-7. In comparison with commercial cultivars employed in this screening, 14 lines had disease scores not significantly different to the most resistant commercial cultivar, and 27 lines had significantly lower disease score than PBA Percy (7.67). That OZP1207 ranked better than WAPEA2211 in 2012 is notable as WAPEA2211 was the most resistant line in 2013 when the black spot epidemic was more severe. In 2013, a group of slightly less resistant lines, consisting of 04H349P-05HO2005, 06H109P-9, 06H459P-1, 04H341P-05HO2010-1, 06H097P-9, 05H189-06HOS2001 and OZP1101, was also highlighted. It is clear that the level of resistance has increased significantly in the breeding gene pool since breeding started in Western Australia in the late 1990's (Adhikari et al. 2014). To start with, the resistance level was at the level of Kaspa and Dundale, i.e. marginal or no resistance. WAPEA211 was developed since then and it is currently used as a benchmark for resistance (Adhikari et al. 2014). However, WAPEA2211 was not released to commercial growers because of its susceptibility to downy mildew, a serious disease in some parts of Australia (Adhikari et al. 2014). Further, WAPEA2211 is also highly susceptible to powdery mildew (S.H. Tran, unpubl.) That OZP1207 had even a greater level of resistance than WAPEA2211, and that it is also less susceptible to powdery mildew (S.H. Tran et al., unpubl.), opens the way for further improvement not only in level of black spot resistance, but also in simultaneously improving overall agronomic suitability for commercial production. The most resistant lines we identified will now be used to enhance resistance for new commercial cultivars, particularly by inter-crossing the more genetically diverse lines to accumulate minor genes for resistance.

No major genes for resistance have been identified for resistance against black spot (Wroth 1998). It is generally accepted that the disease is polygenically controlled and historically there is no pathotype specificity in Western Australia (Wroth 1999; Zhang et al. 2006). What appeared to be continuous variation for resistance in our study, also confirms quantitative inheritance. This highlights the need to develop major black spot resistance QTLs to assist selection. Furthermore, involvement of more than one pathogen within the black spot complex adds to the complexity of the genetic resistance. Some QTLs have been described (Timmerman-Vaughan et al. 2004), but it is suggested that marker assisted selection might still be difficult to implement because of this genetic complexity which has been recently confirmed by Tran et al. (2014a) at genus, species and sub-specific levels for the pathogens involved in the black spot complex in Western Australia.

Fortunately, minor genes generally have an additive effect on the improvement of black spot resistance (Beeck et al. 2008), suggesting that inter-crossing of diverse genotypes could be a useful way to bring different alleles together to enhance the resistance. In our study, the most resistant lines, such as OZP1207 in particular, but also others such as 04H297P-05HO2018, 06H291P-15, 05H336-06HOS2003, 06H033P-6, 06H093P-8 and 06H384P-7, should provide valuable sources of resistance. It is noteworthy that the moderately resistant lines 05H375-06HOS2004 and 05H371-06HOS2003 have PS2037 as the source of resistance, whereas line 05H334-06HOS2003 has a similar level of expression of resistance but from a different source (PS2030). Significantly, both these parental resistance sources originate from the breeding program of the Department of Primary Industry, Victoria. Inter-crossing among these two genetically diverse genotype groups may further enhance resistance by allowing

accumulation of minor genes, and as continues to be undertaken in relation to a plethora of identified different sources of resistance in agronomically suitable backgrounds (Adhikari et al. 2014).

There was a degree of discrepancy between relative rankings of lines from one year to another in terms of their disease reaction, as highlighted by the lack of correlation between black spot disease scores in 2012 with those across the same lines in 2013. For example, while all lines were equally or more resistant than the variety Helena in 2012, a number of lines performed worse than Helena in 2013; and there were increases in disease scores for some lines and decreases for other lines across the two years. This remains a challenge, as the success of a resistance breeding program relies on the capacity to screen segregating populations reliably such that resistant lines can be identified accurately. While a reliable field screening method has been developed at Medina (Adhikari et al. 2014), the fluctuations of relative disease scores observed in two separate trials in 2012 and 2013 highlights the concerns first raised recently by Tran et al. (2014a). Tran et al. (2014a) highlighted the changing of relative proportions of the different pathogens in the black spot complex depending upon location and/or year across Western Australia Overall, *D. pinodes* is the most important pathogen in the complex in Western Australia, but at some locations and at certain periods of time, *P. pinodella*, *P. herbarum*, *B. exigua* var. *exigua*, or *P. koolunga*, alone or in combination with the other pathogens, have predominated (Tran et al. 2014a). For example, in commercial crops in Western Australia, *P. pinodella* was the dominant pathogen at Mount Baker in 1989 and at Medina in 2010, while *P. koolunga* and *D. pinodes* dominated at Northam and Esperance in 2012 (Tran et al. 2014a). It is noteworthy that at Medina, the same site where trials of this study were located, the relative proportions of the different pathogens changed markedly over time (Tran et al. 2014a). There, in 1989, *D. pinodes* was the predominant pathogen (87.7 %), while *P. pinodella* and *B. exigua* var. *exigua* accounted for 11 % and 1.4 % of total number of isolates, respectively. In 2010, *D. pinodes*, *P. pinodella* and *P. herbarum*, were all present and with 14 % of all isolates being *P. herbarum*. In contrast, in 2012, at the same location, *P. herbarum* was not isolated, but *P. glomerata*, a new species in terms of the black spot complex for Western Australia (Tran et al. 2014b), was isolated along with *D. pinodes* and *P. pinodella*. In 2012, at Medina *D. pinodes* was the predominant species, rather than *P. pinodella*, as had been the case in 2010, highlighting the challenges for breeders seeking to identify effective resistance under natural field epidemics. Further, it has recently been shown (H.S. Tran, unpubl.) that pea lines with resistance to one pathogen within this complex in Western Australia do not necessarily have resistance to other pathogens of this complex. Therefore, it is likely that the fluctuations of relative disease scores across the two years for some tested lines was a consequence of changes in the relative proportion of the different pathogens of the black spot complex at Medina. Such lines we identified showing resistance to multiple pathogens within the black spot complex are of particular significance for breeding programs.

Currently, despite an effort of Ali et al. (1978) who examined resistance of pea lines to *D. pinodes*, *A. pisi*, and *P. pinodella*, breeding programs not only in Australia but also elsewhere have supposedly targeted resistance of field pea to *D. pinodes* (Wroth 1998; Xue and Warkentin 2001; Prioul et al. 2004; Fondevilla et al. 2005; Zhang et al. 2006; Beeck et al. 2008; Khan et al. 2013). Further, there have recently been some additional pathogens reported associated with the black spot complex in Western Australia, including *P. koolunga*, (Tran et al. 2014a), *P. herbarum* (Li et al. 2011), *P. glomerata* (Tran et al. 2014b) and *B. exigua* var. *exigua* (Li et al. 2012). This is in addition to the well-recognised highly virulent pathogens *D. pinodes* (Bretag et al. 2006) and *P. pinodella* (Onfroy et al. 1999) in Western Australia, and *P. koolunga* elsewhere (Davidson et al. 2009; Davidson et al. 2011; McMurray et al. 2011). This pathogen diversity makes developing pea varieties resistant to the black spot complex challenging. Finally, if host resistance is developed and/or deployed against only one or two pathogens within the black spot complex, the composition of the pathogen population could shift towards pathogen species least challenged by that particular host resistance. It is clear that there is a major task ahead for breeders to develop effective combined resistance against the major pathogens within the black spot complex.

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