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Pathogenic variation within the 2009 Australian *Ascochyta rabiei* population and implications for future disease management strategy

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Abstract Twenty four *Ascochyta rabiei* isolates, collected from the 2009 eastern Australian chickpea growing regions, were assessed for their ability to infect 12 previously characterised chickpea genotypes. Comparison of means analysis and principal components analysis of area under the disease progress curve measures resulted in continua rather than discrete isolate groupings, suggesting that a broad diversity of pathogenicity exists in the current *A. rabiei* population. Breeders can now choose isolates that represent the variation found in the field to screen their germplasm, providing greater knowledge on the likely longevity of cultivars before they are commercially released.

Keywords Cicer arietinum · Aggressiveness · Population structure

Introduction

Ascochyta blight (causal agent *Ascochyta rabiei* (Pass.) Labrousse, teleomorph *Didymella rabiei* (Kovachevski) von Arx) is the most important foliar disease of chickpea (*Cicer arietinum*) worldwide (Nene and Reddy 1987). The disease affects all aerial parts of the plant, originating from seed, and spread by wind and rain splash (Pande et al. 2005). Complete yield losses have been reported (Kaiser and Muehlbaur 1988; Navas-Cortes et al. 1998) and seed quality is commonly affected, with seed sometimes becoming unmarketable. An epidemic of ascochyta blight across northern New South Wales during the 2010 growing season was conservatively estimated to reduce the yield of commercially grown chickpea crops by thirty percent, even when fungicide was repeatedly applied (Kevin Moore pers. comm.).

Knowledge of isolate population variation within and among chickpea growing regions is required in order to determine the risk for the pathogen to potentially overcome disease management and control strategies. In particular, it is important to determine if the pathogen is able to adapt, and if so, to estimate the rate of adaptation to cause disease on newly released and widely adopted cultivars from the national resistance breeding program, such as Genesis 090 (Pulse Australia 2009c) and PBA HatTrick (Pulse Breeding Australia 2009).

To date, relatively low genetic diversity has been detected in the Australian *A. rabiei* population compared to that observed elsewhere (Phan et al. 2003a; Pradhan 2006; Leo et al. 2011). The detection of a low genetic diversity was most likely due to relatively few introductions resulting in localised founder populations that have then spread through the movement of infected seed and other materials (Phan et al. 2003a). Also, although known to be heterothallic, only a single mating type (MAT1-2) has been detected in Australia using a molecular diagnosis tool among all of the Australian isolates collected to date (Phan et al. 2003b; Pradhan 2006). Each of these factors contributed to the different pathosystem observed in Australia compared to the situation in other countries where both mating types have been identified and molecular diversity was high (Udupa et al. 1998; Navas-Cortes et al. 1998; Jamil et al. 2000; Chongo et al. 2004; Vail and Banniza 2009).

Whilst informative in determining levels of neutral genetic diversity among individuals within and between

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populations and in tracing genetic movement and exchange; molecular tools such as microsatellite markers do not provide an indication of phenotypic diversity among isolates. In order to determine differences in the ability to infect and cause disease on host genotypes, actual pathogenic interactions must be assessed. Aggressiveness is the natural variation in the severity of disease caused by isolates within the pathogen population towards a host genotype (Taylor and Ford 2007). Several previous studies have demonstrated the variation in pathogenicity and aggressiveness among *A. rabiei* isolates worldwide (Navas-Cortes et al. 1998; Chen et al. 2004).

Pathogenicity groups of *A. rabiei* have been identified in many countries (Vir and Grewal 1974; Udupa et al. 1998; Jamil et al. 2000; Chen et al. 2004; Pradhan 2006) based on measurements of aggressiveness of infection by isolates over a relatively small number of host genotypes. Whether all of these groups are distinct pathotypes, or part of a continuum of aggressiveness within the population is unknown. Vail and Banniza (2008) were unable to categorise 99 isolates collected from the Canadian prairies into discrete pathotypes and concluded that there was a continuous distribution of pathogenicity.

In order to produce chickpea cultivars with robust Ascochyta resistance, breeders need to screen germplasm against *A. rabiei* isolates that represent the diversity of pathogenicity within the current pathogen population. If pathogenic differences among isolates are present in Australia, isolates representing the breadth of these differences should be selected for screening purposes. Cultivars that are selected as resistant to all of the possible pathotypes will then stand the best chance of longevity in the Australian chickpea industry.

Two newly released Australian cultivars, Genesis 090 and PBA HatTrick, have moderately resistant Ascochyta blight ratings. PBA HatTrick is a cross between the Australian cultivar Jimbour with a susceptible ascochyta blight rating, and a resistant Iranian landrace (Pulse Breeding Australia 2009). Genesis 090 is an introduction from ICARDA Syria, selected and released by the National Chickpea Breeding Program in 2005 (Pulse Australia 2009c). Although cultivars Genesis 090 and PBA HatTrick have a resistant disease rating they are not immune to *A. rabiei*. Therefore, it is possible that in further large scale field plantings of these varieties, the pathogen will adapt to overcome these resistance sources. To date, neither PBA HatTrick or Genesis 090 have experienced a significant yield loss due to Ascochyta blight (Pulse Breeding Australia 2009; Pulse Australia 2008), although widespread adoption of these varieties by Australian chickpea growers is likely to increase selection pressure on the pathogen.

Understanding the risk to the durability of current resistance sources used in the Australian chickpea industry will better inform and prepare breeders and farmers for the disease management strategies that will be necessary to maintain good crop yields and quality. Therefore, the aims of this study were to 1) identify if differences in aggressiveness among Australian *A. rabiei* isolates exist from a range of locations and host genotypes, in order to assess the current risk from the most aggressive isolates to the newly released resistant chickpea cultivars, and 2) identify a representative group of isolates that may be used as a selection tool in future Australian chickpea resistance breeding programs.

Methodology

Isolate and host material

A representation of the population present during the 2009 season was collected and consisted of 24 *A. rabiei* isolates from six locations in Victoria, New South Wales and South Australia (Table 1). Two methods were used to collect isolates depending on the presence or absence of pycnidia. When pycnidia were present in lesions, they were picked off using a needle and placed into 1 mL of sterile water. The solution was then vortexed for 2 min before one loop of the solution was streaked over a V8 agar plate [containing 20% V8 vegetable juice, (Campbell's Grocery Ltd), 2% technical agar (DifcoTM), 0.375% calcium carbonate (Merck) in distilled water]. Three days later a single colony was taken off the plate using a sterile needle under a dissecting microscope and placed onto V8 agar. When a lesion was present without visible pycnidia, the leaf tissue was surface sterilised using a 2.6% sodium hypochlorite solution for two minutes and plated onto V8 agar. Growth from the sterilised leaf material which was identified as *A. rabiei* was then subcultured onto a V8 agar plate. All isolates were single spore derived and maintained at 20°C with a 12-h photoperiod until required.

Twelve chickpea genotypes were selected as representative of a potential differential range in disease reaction to *A. rabiei* in consultation with Australian chickpea breeders and pathologists. They included current commercial cultivars as well as lines used as parents in the Australian breeding program, and susceptible commercial cultivars (Table 2). Chickpea plants were grown in 20 cm diameter pots containing commercial potting mix (BioGro), four plants per pot. Three replicates were sown for each of the genotype x isolate combinations, as well as three control pots containing the cultivar Howzat. All plants were grown in a shade house facility at the Grains Innovation Park, Department of Primary Industries, Horsham, Victoria.

Inoculation and bioassay

Four weeks prior to inoculation, the 24 *A. rabiei* isolates were plated onto water agar containing autoclaved chickpea

Table 1 Chickpea genotypes from which isolates were recovered, location of collection and corresponding isolate label of the 24 *Ascochyta rabiei* isolates for Fig. 1

	Horsham, Victoria	Kalkee, Victoria	Kaniva, Victoria	Kingsford, South Australia	Melton, South Australia	Tamworth, New South Wales
Almaz	V5			S3	S11	
CICA 0503	V3			S5		
CICA 0512	V4		V10	S8	S9	N1
Genesis 079				S1		
Genesis 090		V7	V9	S7	S10	
Genesis 114	V2			S2		
Genesis 509	V1			S6		
Howzat	V6		V8	S4	S12	N2

tissue. Inoculum was produced four weeks later by flooding the surface of the plate containing pycnidia with sterile water for 10 min. A glass rod was then used to gently scrape the surface and dislodge spores. A haemocytometer was used to adjust the concentration of spores to 1×10^5 spores/ml and Tween 20 (BDH) was added (0.05%) as a surfactant. The inoculum was sprayed onto four-week-old chickpea plants using a handheld pressurised sprayer (Preval® Power Unit). At the same time, the control plants of Howzat were sprayed with sterile water and Tween 20 only. Each plant was then covered with an individual minidome (MaxValu cup 6) (Chen et al. 2005) for 24 h to maintain humidity.

Each plant was scored for disease symptoms using the following rating scale, adapted from Singh et al. (1981), at 7, 14, 21 and 28 days post inoculation: Leaf lesions; 1 = No leaf symptoms, 3 = Pin prick lesions, 5 = Small, inconspicuous necrotic lesions without pycnidia, 7 = Individual lesions with darker margins and some pycnidia and 9 = Lesions with dark margin coalesced containing pycnidia.

The area under the disease progress curve (AUDPC) was then calculated using the following equation:

$$\text{AUDPC} = \sum [(X_i + X_{i+1})/2](t_{i+1} - t_i)$$

where, X_i is the blight score of the *i*th evaluation, X_{i+1} is the blight score of the $i + 1$th evaluation and $(t_{i+1} - t_i)$ is the number of days between the evaluations.

Minitab® Statistical Software (release 15.1.1.0) was used for Analysis of Variance and NTSYSpc (version 2.21) was used for Principal Component Analysis (PCA) of means.

Results

Analysis of variance showed highly significant differences in mean disease scores ($P<0.0001$) for both hosts and isolates as was the isolate by host interaction ($P<0.0001$). There was no isolate by genotype combination that had a mean disease score value of one, where no leaf symptoms were present. The variation in the ability of individual isolates to cause disease across the host genotypes assessed was substantial and mean comparison of AUDP of isolates on each host genotype represented continua rather than identifying distinct groups (Table 3).

Similarly, principal components analysis of area under the disease progress curve measures resulted in a continuum rather than discrete isolate groupings (Fig. 1). The first axis (Dim-1) accounted for 72.5% of the variation, the second

Table 2 Chickpea genotypes used to screen *Ascochyta rabiei* isolates and their assigned ascochyta blight resistance ratings

Chickpea genotype	Resistance	Reference
90102-5Q-1103	Resistant	K. Hobson (pers. comm.)
94-121*99V4006	Resistant	K. Hobson (pers. comm.)
Genesis 090	Resistant	Pulse Australia (2009c)
ICC3996	Resistant	Nasir et al. (2000)
PBA HatTrick	Resistant	Pulse Breeding Australia (2009)
Flipper	Moderately resistant	Pulse Australia (2009b)
Genesis 114	Moderately resistant	Pulse Australia (2009d)
Almaz	Moderately resistant – moderately susceptible	Pulse Australia (2009a)
Howzat	Moderately susceptible	Pulse Australia (2009e)
Yorker	Moderately susceptible	Pulse Australia (2009f)
Kaniva	Susceptible	Carter (1999)
Lasseter	Susceptible	Carter (1999)

Table 3 Area under the disease progress curve for 24 *Ascochyta rabiei* isolates screened over 12 chickpea genotypes. LSD represents Fisher's least significant difference at 5%

	Chickpea differentials													
	PCA Label	90102-5Q-1103	94-121*99V4006	Genesis 090	ICC3996	PBA HatTrick	Flipper	Genesis 114	Almaz	Howzat	Yorker	Kaniva	Lasseter	MEAN	LSD
Isolate															
09HOR03	V1	75	65	67	83	86	98	86	92	93	74	109	118	87	47
09HOR04	V2	97	109	89	100	109	125	123	128	122	123	129	129	115	27
09HOR05	V3	93	95	74	99	102	104	113	117	111	123	124	123	107	40
09HOR06	V4	87	74	81	94	95	127	115	125	109	116	120	123	106	34
09HOR07	V5	96	75	49	86	81	97	79	108	114	107	104	119	93	48
09HOR08	V6	90	91	80	92	101	123	95	127	116	109	130	125	107	34
09KAL09	V7	112	109	85	116	111	116	109	132	115	116	122	135	115	28
09KAN08	V8	81	75	67	75	84	97	84	102	95	88	116	117	90	41
09KAN19	V9	111	97	78	112	109	121	106	127	121	115	129	134	113	29
09KAN32	V10	97	76	90	101	112	98	116	126	114	108	126	120	107	38
09KIN01	S1	90	107	74	91	105	119	123	128	112	113	126	123	109	36
09KIN02	S2	110	88	57	109	105	109	107	130	115	104	122	124	107	40
09KIN03	S3	102	88	75	96	106	104	95	109	121	110	116	123	104	37
09KIN06	S4	94	71	62	87	88	102	95	115	102	96	100	109	93	40
09KIN11	S5	69	58	43	96	96	67	79	100	104	96	114	112	86	53
09KIN13	S6	101	88	53	106	112	112	101	117	116	103	120	129	105	42
09KIN34	S7	91	70	80	82	88	109	85	104	100	91	123	118	95	41
09KIN43	S8	101	102	50	102	91	116	103	132	113	108	134	120	106	33
09MEL01	S9	81	84	74	102	88	103	103	126	104	116	128	123	103	39
09MEL03	S10	95	103	78	100	102	92	114	127	112	120	134	121	108	34
09MEL04	S11	83	47	46	74	90	90	72	97	106	85	117	93	83	61
09MEL35	S12	104	106	88	107	93	107	114	114	112	118	124	123	109	41
09TAM02	N1	81	81	64	83	84	88	100	104	114	94	111	116	93	58
09TAM05	N2	114	116	95	95	103	116	123	130	125	118	130	127	116	31
MEAN		94	86	71	95	98	106	102	117	111	106	121	121		

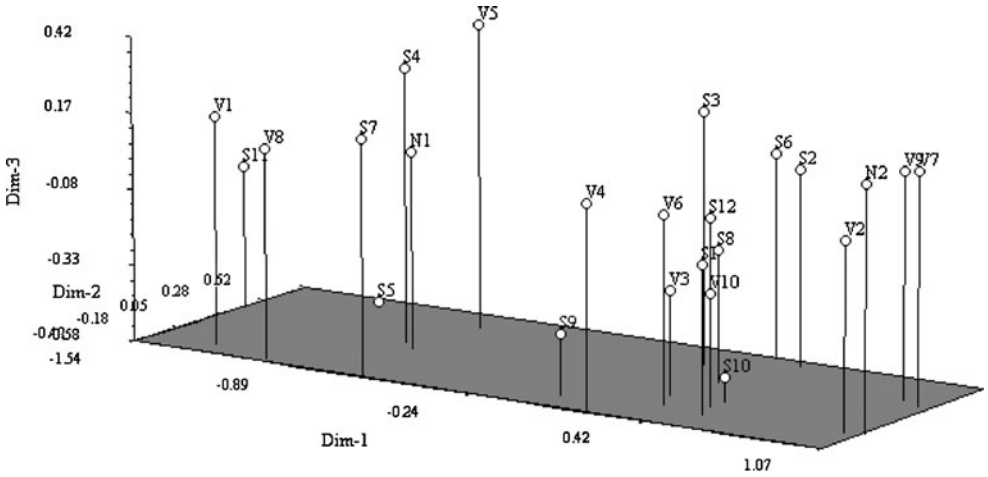

Fig. 1 Principal Components Analysis (PCA) of *Ascochyta rabiei* isolates based on their ability to cause disease (measured as area under the disease progress curve) on twelve chickpea differentials. Axis Dim-1 accounts for 72.48% of the variation, Dim-2 9.81%, and Dim-3 7.12%. See Table 1 for isolate information. Isolates labelled with a 'V' were collected in Victoria, an 'S' were collected in South Australia and an 'N' were collected in New South Wales

(Dim-2) accounted for 9.8%, and the third (Dim-3) accounted for 7.1%. A cumulative total of 89.41% of the variation was explained by these first three axes.

Isolates did not form any clear groupings but were spread over the PCA analysis. The only observable division in the isolates occurred in the middle of the graph. The isolates on the right hand side of the PCA were the most aggressive with decreasing aggressiveness towards the left of the graph. The four most aggressive isolates were from Victoria (09HOR04, 09KAL09 and 09KAN19) and Tamworth New South Wales (09TAM05). The two least aggressive isolates (09KIN11 and 09MEL04) were both collected from South Australian locations.

Discussion

A continuum of aggressiveness within the Australian A. rabiei population with no clear groupings was evident. In pathosystems where resistance are quantitative (Flandez-Galvez et al. 2003), it is unlikely that isolates will group in discrete pathotypes (Caten 1987). The *A. rabiei* – *C. arietinum* pathosystem was thought to be based on a quantitative interaction (Gowen et al. 1989; Vail and Banniza 2008), which suggested that a continuum of pathogenicity was more likely than true pathotypes. Although many other investigations have grouped isolates into pathotypes, in most cases few host genotypes have been used to differentiate them (Udupa et al. 1998; Jamil et al. 2000), and further investigation may find that the isolates tested did not represent the pathogen population. The statistical interaction between host genotypes and *A. rabiei* isolates also indicated that the pathosystem was based on quantitative resistance (Parlevliet 1979).

Of the four most aggressive isolates identified, three originated from locations in Victoria and one from a New South Wales location, over a thousand kilometres apart. It is likely that seed movement was responsible for the spread of isolates, the same method which allowed *A. rabiei* to enter Australia (Cother 1977). The location from which each isolate was collected was random in relation to aggressiveness among the isolates (Fig. 1), which also indicates that related *A. rabiei* isolates had been dispersed throughout the chickpea growing regions, most likely through infected seed. Molecular evidence has also shown that the origin of isolates did not explain any variation seen in genetic diversity (A. Leo pers. comm.). As Western Australia has a more strict process for regulating the importation of seed from other states (Quarantine 2009), it was possible that the isolates collected from this state may have been distinct from the eastern Australian isolates but this is yet to be determined.

A range of pathogenicity within the *A. rabiei* population was identified, and breeders can now select from these isolates to ensure that germplasm is screened against a representation of field populations. The use of these isolates in breeding programs will only be applicable for germplasm that is expected to be commercialised in the same areas that the isolates were collected from. For this reason the isolate selection used in screening breeding material should represent the future growing region of any specific cultivar. Therefore, it will be important to screen isolates from chickpea growing regions that were not covered in this study, such as Western Australia, and increase the number of isolates screened from regions such as Tamworth, NSW, where only two isolates were examined.

If recombination of the pathogen was occurring in Australia it would be possible for a wider range of aggressiveness to occur in the future. In order to ensure the reliability of this selection tool in breeding programs, continued sampling of isolates from all Australian chickpea growing regions should occur to identify any new isolates. Also, as this research is based on a single biological experiment and environmental replication, multiple environments testing will be required to validate the findings prior to implementation. As well as screening with single

spored isolates, disease nurseries should also be utilised to assess resistance over a wide range of locations and against a wide range of isolates. This will help to ensure that breeding material is screened against the widest range of isolate aggressiveness even if the most aggressive isolates have yet to be collected.

Mean AUDPC values of each cultivar used in this study (Table 3) indicated that the published resistance ratings are mostly accurate with respect to the 2009 *A. rabiei* population (Table 2). The only exception is the cultivar 'Almaz' which had a much higher mean AUDPC than the moderately resistant and moderately susceptible cultivars.

As isolates have been found that can cause substantial disease on the most resistant genotypes it will be very important to emphasise both cultural and chemical control measures, and that growers do not rely on host resistance alone. The resistant genotypes such as Genesis 090 and PBA HatTrick are recommended to receive a foliar fungicide spray at early podding, as well as monitoring before this point, although further fungicide sprays are unlikely to be required (Pulse Australia 2009c; Pulse Breeding Australia 2009). It is important that this early monitoring occurs, so that if pathogenicity or aggressiveness has increased within the pathogen population, early disease symptoms can be observed and disease management procedures put into place.

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References

Carter JM (1999) Chickpea growers guide: a guide for the production of chickpeas. Agriculture Victoria, Department of Natural Resources and Environment

Caten C (1987) The concept of race in plant pathology. In: Wolfe M, Caten C (eds) Populations of Plant Pathogens: their dynamics genetics. 24 Blackwell Scientific Publications, Oxford, pp 21–37

Chen W, Coyne CJ, Peever TL, Muehlbauer FJ (2004) Characterisation of chickpea differentials for pathogenicity assay of ascochyta blight and identification of chickpea accessions resistant to *Didymella rabiei*. Plant Pathol 53:759–769

Chen W, McPhee KE, Muehlbauer FJ (2005) Use of a mini-dome bioassay and grafting to study resistance of chickpea to Ascochyta blight. J Phytopathol 153:579–587

Chongo G, Gossen BD, Buchwaldt L, Adhikari T, Rimmer SR (2004) Genetic diversity of *Ascochyta rabiei* in Canada. Plant Dis 88:4–10

Cother EJ (1977) Identification and control of root-rot fungi in *Cicer arietinurn* (chickpea). Plant Dis Rep 61:736–740

Flandez-Galvez H, Ford R, Ades PK, Pang ECK, Taylor PWJ (2003) QTL analysis for ascochyta blight resistance in an intraspecific population of chickpea (*Cicer arietinum* L.). Theor Appl Genet 107:1257–1265

Gowen SR, Orton M, Thurley B, White A (1989) Variation in pathogenicity of *Ascochyta rabiei* on chickpeas. Trop Pest Manag 35:180–186

Jamil FF, Sarwar N, Sarwar M (2000) Genetic and pathogenic diversity within *Ascochyta rabiei* (Pass.) Laboratory populations in Pakistan causing blight of chickpea (*Cicer arietinum* L). Physiol Mol Plant Pathol 57:243–254

Kaiser WJ, Muehlbaur FJ (1988) An outblight of chickpea in the outbreak of Ascochyta blight of chickpea in the Pacific Northwest, USA, in 1987. International Chickpea Newsletter 18:16–17

Leo AE, Ford R, Linde CC, Shah RM, Oliver R, Taylor PWJ, Lichtenzveig J (2011) Characterization of fifteen newly developed microsatellite loci for the chickpea fungal pathogen *Ascochyta rabiei*. Molecular Ecology Resources, In press

Nasir M, Bretag TW, Kaiser WJ, Meredith KA, Brouwer JB (2000) Screening chickpea germplasm for ascochyta blight resistance. Australas Plant Pathol 29:102–107

Navas-Cortés JA, Pérez-Artés E, Jiménez-Diaz RM, Llobell A, Bainbridge BW, Heale JB (1998) Mating type, pathotype, and RAPDs analysis in *Didymella rabiei*, the agent of Ascochyta blight of chickpea. Phytoparasitica 26:199–212

Nene YL, Reddy MV (1987) Chickpea diseases and their control. In: Saxena MC, Singh KB (eds) The Chickpea. CAB International, Oxon, pp 233–270

Pande S, Siddique KHM, Kishore GK, Bayaa B, Gaur PM, Gowda CLL, Bretag TW, Crouch JH (2005) Ascochyta blight of chickpea (*Cicer arietinum* L.): a review of biology, pathogenicity, and disease management. Aust J Agric Res 56:317–332

Parlevliet JE (1979) Components of resistance that reduce the rate of epidemic development. Annu Rev Phytopathol 17:203–222

Phan HTT, Ford R, Taylor PWJ (2003a) Population structure of *Ascochyta rabiei* in Australia based on STMS fingerprints. Fungal Divers 13:111–129

Phan HTT, Ford R, Taylor PWJ (2003b) Mapping the mating type locus of *Ascochyta rabiei*, the causal agent of ascochyta blight of chickpea. Mol Plant Pathol 4:373–381

Pradhan P (2006) Studies of *Ascochyta rabiei* in Australia. M Agr Sci thesis, University of Melbourne

Pulse Australia (2008) Genesis 425. Pulse variety management package. http://www.pulseaus.com.au/pdf/Genesis%20425%20VMP.pdf

Pulse Australia (2009a) Almaz. Pulse variety management package. http://www.pulseaus.com.au/pdf/Almaz%20VMP.pdf

Pulse Australia (2009b) Flipper. Pulse variety management package. http://www.pulseaus.com.au/pdf/Flipper%20VMP.pdf

Pulse Australia (2009c) Genesis 090. Pulse variety management package. http://www.pulseaus.com.au/pdf/GENESIS%20090%20VMP.pdf

Pulse Australia (2009d) Genesis 114. Pulse variety management package. http://www.pulseaus.com.au/pdf/GENESIS%20114%20VMP.pdf

Pulse Australia (2009e) Genesis 509. Pulse variety management package. http://www.pulseaus.com.au/pdf/GENESIS%20509%20VMP.pdf

Pulse Australia (2009f) Yorker. Pulse variety management package. http://www.pulseaus.com.au/pdf/Yorker%20VMP.pdf

Pulse Breeding Australia (2009) PBA HatTrick. Variety management package. http://www.pulseaus.com.au/pdf/Genesis%20425%20VMP.pdf

Quarantine WA (2009) Western Australia's seed import requirements. Department of Agriculture and Food, Government of Western Australia. http://www.agric.wa.gov.au/objtwr/imported_assets/content/pw/q/seed_manual.pdf

Singh KB, Hawtin GC, Nene YL, Reddy MV (1981) Resistance in chickpea to ascochyta blight. Plant Dis 65:586–587

Taylor PWJ, Ford R (2007) Diagnostics, genetic diversity and pathogenic variation of ascochyta blight of cool season food and feed legumes. Eur J Plant Pathol 119:127–133

Udupa SM, Weigand F, Saxena MC, Kahl G (1998) Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the ascochyta blight of chickpea. Theor Appl Genet 97:299–307

Vail S, Banniza S (2008) Structure and pathogenic variability in *Ascochyta rabiei* populations on chickpea in the Canadian prairies. Plant Pathol 57:665–673

Vail S, Banniza S (2009) Molecular variability and mating-type frequency of *Ascochyta rabiei* of chickpea from Saskatchewan, Canada. Australas Plant Pathol 38:392–398

Vir S, Grewal JS (1974) Physiologic specialization in *Ascochyta rabiei*, the causal organism of gram blight. Indian Phytopathology 27:355–360