

# Changes in Foliar Host Reaction to *Ascochyta Rabiei* With Plant Maturity

Vicki L. Elliott<sup>1</sup>, Paul W. J. Taylor<sup>2</sup> & Rebecca Ford<sup>2</sup>

<sup>1</sup> Melbourne School of Land and Environment, The University of Melbourne, Grains Innovation Park, Horsham, Victoria, Australia

<sup>2</sup> Melbourne School of Land and Environment, The University of Melbourne, Parkville, Victoria, Australia

Correspondence: Vicki Elliott, Melbourne School of Land and Environment, The University of Melbourne, Grains Innovation Park, Horsham, Melbourne, Australia. Tel: 61-3-5381-2294. E-mail: velliott@unimelb.edu.au

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## Abstract

*Ascochyta rabiei* is the most significant foliar disease of chickpea (*Cicer arietinum*) worldwide, and can cause both yield loss and reduced seed quality. Significant mean disease score differences were observed from the reactions among a collection of *Ascochyta rabiei* isolates and chickpea hosts at both seedling and maturity growth stages ( $P < 0.000$ ). Although isolates ranked similarly within their range of pathogenicity at either growth stage, the ranking of host disease reaction was different. This suggests that genotype-specific expression of resistance genes to *A. rabiei* may be related to growth stage. Hence, durable selection methods for resistance will require screening of isolates representative of the pathogenicity within the population on target genotypes at growth stages representative of the type of resistance being sought, for example seedling resistance or pod resistance.

**Keywords:** *Ascochyta rabiei*, *Cicer arietinum*, host plant resistance, plant maturity

## 1. Introduction

The most significant foliar disease of chickpea (*Cicer arietinum*) worldwide is Ascochyta blight (causal agent *Ascochyta rabiei* (Pass.) Labrousse, teleomorph *Didymella rabiei* (Kovachevski) von Arx. All aerial parts of the host are affected (Nene & Reddy, 1987; Pande et al., 2005) as are both yield and seed quality, and complete yield losses have been reported (Kaiser & Muehlbauer, 1988; Navas-Cortes et al., 1998). The inoculum originates from infected seed and previous season stubble, and secondary infections are spread throughout the crop by rain splash (Pande et al., 2005). Therefore, plants may become infected at a number of stages in their life cycle, dependent on inoculum level and epidemiological conditions. Although marketed as 'resistant', recently implemented Australian cultivars are susceptible to the disease at maturity (podding). Although cultivars such as Genesis 090 demonstrated strong resistance at the seedling stage, infection later in the season resulted in pod abortion, or reduced seed quality (Pulse Australia 2011), hence fungicide application at maturity is prescribed as part of the best practice for disease management along with stubble removal, minimum four year rotations and seed dressing (Pulse Australia 2011).

Resistance to *A. rabiei* is quantitatively governed with multiple genes contributing to the overall resistance trait (Santra, Tekeoglu, Ratnaparkhe, Kaiser, & Muehlbauer, 2000; Lichtenzveig, Shtienberg, Zhang, Bonfil, & Abbo, 2002; Flandez-Galvez, Ford, Ades, Pang, & Taylor, 2003; Flandez-Galvez, Ford, Pang, & Taylor, 2003; Udupa & Baum, 2003). It is likely that expression of individual resistance genes is triggered by external (environmental/pathogen) and internal (plant age/pathogen) stimuli. Expression of each of these genes can differ at different times in the plants life (Trapero-Casas & Kaiser, 1992; Chongo & Gossen, 2001; Sharma, Pande, & Rathore, 2010), and hence cultivars which show good seedling resistance may not have the same highly effective resistance at maturity. The resistance level to *A. rabiei* has previously been correlated with chickpea plant age (Nene & Reddy, 1987; Chongo & Gossen, 2001; Basandrai et al., 2007; Sharma et al., 2010). Chongo & Gossen, (2001) and Basandrai et al. (2007) found reduced resistance at flowering and podding compared to earlier growth stages except in susceptible cultivars where plant growth stage had no effect on disease severity. Sharma et al. (2010) also found more disease on resistant cultivars at flowering and podding, which did not correlate well with the resistance observed during vegetative growth.

Control methods in Australia recommend fungicide application at podding even in the more resistant cultivars. If breeding material can be found that contains effective resistance at the flowering and podding stages chickpea growers may be able to reduce their reliance on fungicides, and reduce the likelihood of fungicide tolerance becoming a problem in the future. To ensure a stable Australian chickpea industry, genotypes must be identified that contain resistance effective throughout the cropping season and on exposure to the diversity of isolate pathogenicities that exist in the population.

This study aimed to 1) assess and compare the levels of resistance that Australian bred genotypes have at seedling versus maturity to isolates representative of the Australian *A. rabiei* pathogenic population; and 2) identify isolates that are pathogenic at both host growth stages assessed and that should be used in future breeding programs.

## 2. Methodology

### 2.1 Isolate and Host Material

Six isolates representative of the variation in pathogenicity within the *A. rabiei* populations in Victoria and South Australia in the 2009 season were selected based on their pathogenicity over 12 chickpea genotypes (Table 1; Elliott et al., 2011). The isolates were collected using the two methods outlined in Elliott, Taylor, and Ford (2011).

Table 1. *Ascochyta rabiei* isolates used, their location of collection and cultivar from which they were recovered

Isolate ID	Location of collection	Cultivar from which isolate was recovered
09HOR05	Horsham, Victoria	PBA Slasher
09KAN08	Kaniva, Victoria	Howzat
09KAN19	Kaniva, Victoria	Genesis 090
09KIN06	Kingsford, South Australia	Howzat
09KIN13	Kingsford, South Australia	Genesis 509
09MEL04	Melton, South Australia	Almaz

Ten chickpea genotypes were selected as representative of a range of genotypes used in current cropping and breeding programs, together with two highly susceptible genotypes (Table 2). Chickpea plants were grown in 20 cm diameter pots containing commercial potting mix (BioGro, Australia), four plants per pot. Three replicates were sown for each of the genotype x isolate combinations for each of the seedling and maturity experiments. The cultivar Howzat was used as a susceptible control. Plants used for the seedling experiment were grown in a shade house facility, and plants used for the detached leaf assay at maturity were grown in a glasshouse, at the Grains Innovation Park, Department of Primary Industries, Horsham, Victoria.

Table 2. Chickpea genotypes and their assigned *Ascochyta* blight resistance ratings

Chickpea genotype	Resistance	Reference
90102-5Q-1103	Resistant	K. Hobson (pers. comm.)
Genesis 090	Resistant	Pulse Australia (2011)
ICC3996	Resistant	Nasir et al., (2000)
PBA HatTrick	Resistant	Pulse Breeding Australia (2009)
94-121*99V4006	Moderately resistant	K. Hobson (pers. comm.)
Flipper	Moderately resistant	Pulse Australia (2009b)
Genesis 114	Moderately resistant	Pulse Australia (2009c)
Almaz	Moderately resistant – moderately susceptible	Pulse Australia (2009a)
Yorker	Moderately resistant – moderately susceptible	Pulse Australia (2009d)
Howzat	Moderately susceptible	Pulse Australia (2009e)
Kaniva	Susceptible	Carter (1999)
Lasseter	Susceptible	Carter (1999)

## 2.2 Inoculation and Bioassay

The seedling experiment was as described in Elliott et al. (2011), and involved inoculation of 2-week-old seedlings followed by scoring of leaf symptoms every 7 days for 4 weeks. For the maturity experiment, leaves were taken from plants when flowering had begun for all varieties (9-14 weeks after sowing). Leaves were detached using a sterile scalpel and surface sterilised by placing in a 2.6% sodium hypochlorite solution for two minutes, and then rinsed in sterile water. Leaves were subsequently placed on filter paper in a laminar flow and allowed to air dry. Once dry, they were transferred to 0.5% water agar plates, eight leaves per plate, three replicate plates of each isolate x cultivar combination. Previously, the disease responses of detached chickpea leaves to *Ascochyta rabiei* were correlated with those of field experiments. Dolar, Tenuta, and Higgins (1994) found this to be the case as long as recently fully expanded leaves were used, and not the oldest leaflets, which may be influenced by senescence effects.

Inoculum was produced as described in Elliott et al. (2011) without the use of Tween 20. A single 15 µL drop of inoculum ( $1 \times 10^5$  spores/ml) was placed onto the upper surface of each leaf and plates were incubated at  $20 \pm 3^\circ\text{C}$  under a continuous cycle of 14 h of light and 10 h of dark. Leaves were assessed under the microscope for percentage area of the leaf affected each day for seven days.

The area under the disease progress curve (AUDPC) was calculated for both seedling and maturity experiments 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 (percentage leaf area affected) 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<sup>®</sup> Statistical Software (release 15.1.1.0) was used for Analysis of Variance.

Simultaneously, a viability check was completed of each spore solution. For this, a 2 µL drop of each solution was placed onto the surface of a V8 agar plate. Using a sterile glass rod, the drop was gently spread out, and the placement of the solution was marked on the underneath of the plate with a permanent marker. Plates were incubated under continuous light at  $20 \pm 3^\circ\text{C}$  for 48 h and examined under a microscope to assess spore viability. An inoculum solution would have been considered unviable if a constant spread of germinated spores was not observed.

## 3. Results

Significant differences ( $P < 0.000$ ) were observed in the mean isolate disease progressions among host genotypes at both seedling (Figure 1) and maturity (Figure 2) growth stages. Also the isolates had significantly different ( $P < 0.000$ ) disease progressions in mean reactions among the host genotypes (Figure 3). The independent isolate x host disease progress interactions were significantly different at maturity ( $P < 0.000$ ) but not at seedling ( $P < 0.072$ ) stage.

Mean AUDPC values at the seedling stage generally ranked host genotypes in accordance with their publicised resistance rating (Table 2). Genesis 090 (resistant) possessed the lowest, and Lasseter and Kaniva (susceptible) possessed the highest AUDPC values. However, several of these expected phenotypic ratings were not retained at maturity; in particular the “resistant” landrace ICC3996 appeared significantly more susceptible, as did the derived breeding line 94-121\*99V4006. In contrast, the cultivar Yorker, known to be moderately resistant to moderately susceptible (Pulse Australia 2009e), ranked significantly more resistant at maturity than at seedling stage compared to other chickpea genotypes. Meanwhile, Genesis 090 was stably resistant at both assessed growth stages.

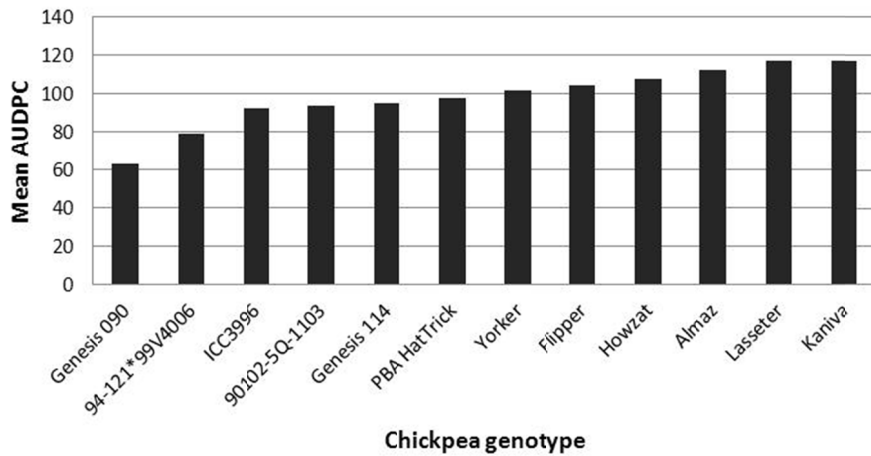


Figure 1. Mean area under the disease progress curve for each host genotype at the seedling stage (LSD = 19.43)

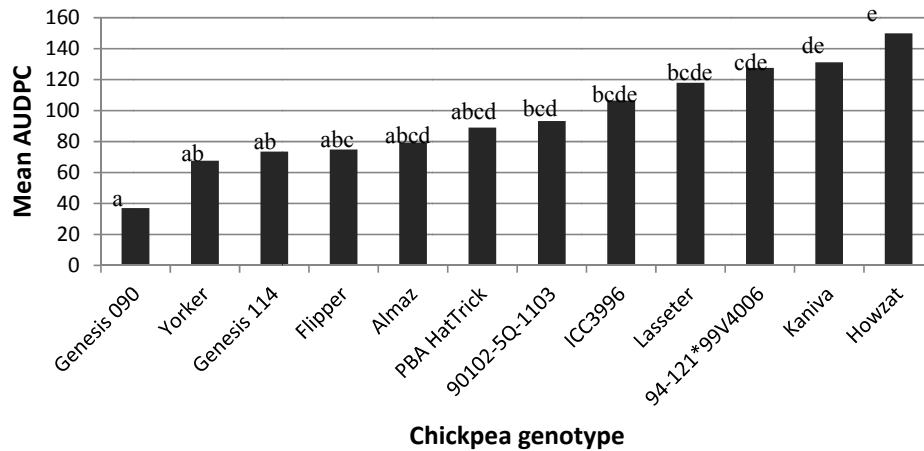


Figure 2. Mean area under the disease progress curve for each host genotype at maturity (LSD = 52.86)

Among the six isolates assessed, 09MEL04 was the least aggressive, and 09KAN19 was the most aggressive at both host growth stages.

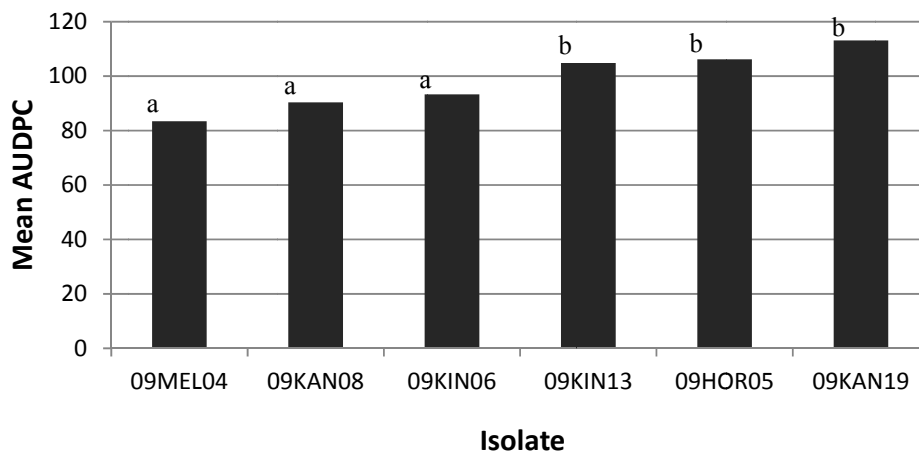


Figure 3. Mean area under the disease progress curve for each isolate at the seedling stage (LSD = 10.66)

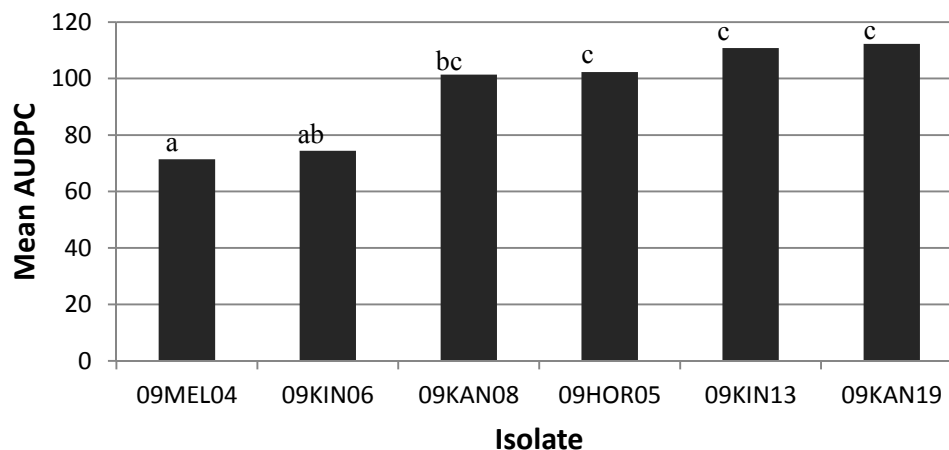


Figure 4. Mean area under the disease progress curve for each isolate at maturity (LSD = 27.39)

#### 4. Discussion

Significant differences in disease reaction to *A. rabiei* due to plant age among parental and breeding lines were detected in the current study. For example, the line 94-121\*99V4006 was resistant at seedling stage, but had one of the highest mean AUDPC values at maturity. A similar reduction in resistance at plant maturity compared to the seedling stage had previously been demonstrated for genotypes classified as moderate or highly resistant in the USA and India (Chongo & Gossen, 2001; Basandrai et al., 2007; Sharma et al., 2010). This highlights potential inefficiencies in resistance breeding based on selections made at a single growth stage (particularly at seedling stage only). This may subsequently result in genotypes that do not maintain resistance throughout the cropping season and an increased dependence on fungicides.

Meanwhile, the “moderately resistant to moderately susceptible” Yorker had the second lowest mean AUDPC value at maturity, indicating comparatively good resistance. Flipper and Genesis 114 also displayed greater resistance at maturity than other cultivars that are rated as more resistant. Since resistance is conditioned by multiple quantitative genetic effects (Gowen, Orton, Thurley, & White, 1989; Vail & Banniza, 2008; Flandez-Galves et al., 2003a) with a relatively large environmental influence (Trapero-Casas & Kaiser, 1992), different genes and possibly pathways may be in operation at different growth stages. This has previously been shown for *Ascochyta* blight of pea (Prioul, Frankewitz, Deniot, Morin, & Baranger, 2004). Selection and integration of the major genetic components of both seedling and maturity resistance would likely increase the durability of resistance and lead to potential for less fungicide applications.

Of the genotypes assessed, Genesis 090 contained the highest level of resistance at both growth stages. This supports the claim that Genesis 090 is the benchmark *Ascochyta* resistant chickpea for southern Australia (Pulse Australia 2011). Nevertheless, application of a fungicide at podding remains a part of the prescribed disease management program for this genotype since highly aggressive isolates are present in the Australian isolate population with potential to reduce quality and yield (Elliott et al., 2011).

Finally, the significant differences detected in disease severity due to isolate choice again demonstrate that isolate selection will be critical when screening future breeding material (Elliott et al., 2011). In particular, two isolates in the current study (09KAN08 and 09KIN13) caused significantly high levels of disease on Genesis 090. Hence, they should be included in future controlled environment screening and their prevalence should be monitored for using screening and possible DNA marker techniques (Leo et al., 2011) in the Genesis 090 growing locations as a pre-emptive warning for possible breakdown.

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