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Distribution of mating types and diversity in virulence of *Didymella rabiei* in Israel

J. Lichtenzveig[1], E. Gamliel[2], O. Frenkel[1,2], S. Michaelido[1], S. Abbo[1], A. Sherman[3] and D. Shtienberg[2,*]
[1]*Department of Field Crops, Vegetables and Genetics, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot, 76100, Israel;* [2]*Department of Plant Pathology, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan, 50250, Israel;* [3]*Department of Genomics, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan, 50250, Israel; *Author for correspondence (Phone: +972-3-9683688; E-mail: danish@volcanic.agri.gov.il)*

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Abstract

The distribution of mating types and diversity in virulence of *Didymella rabiei* populations were studied in Israel from 1997 to 1999. Forty-one monoconidial *D. rabiei* isolates from 18 commercial fields distributed among all the chickpea production areas of the country were paired with *MAT1-1* and *MAT1-2* mating type tester isolates of *D. rabiei*. Both mating types were found in all chickpea production areas of the country. Of the 18 fields sampled, *MAT1-1* was observed in 44%, and *MAT1-2* in 88% of the sites. In some sites both mating types were present in close proximity, suggesting that sexual reproduction of the pathogen was feasible. The contribution of sexual reproduction of the fungus to virulence diversity was tested on detached leaves of six differential chickpea cultivars. Nine isolates were derived from different well separated foci (derived from ascospores as inoculum) and eight isolates were derived from a single, well defined infection focus (derived from sister conidia). In the analyses of variance the *cultivar* × *isolate* interaction showed no significant (P of $F > 0.09$) effect on disease incidence; the chickpea cultivars differed significantly (P of $F < 0.0001$) in their response to *D. rabiei*; and the *isolate* effect was highly significant (P of $F = 0.0007$) for the conidial population, but not significant (P of $F > 0.1$) among isolates of the ascosporic population. Nevertheless, when comparing a cultivar at a time, the ascosporic and conidial populations did not differ significantly (P of $F > 0.1$) in their virulence diversity. Virulence of 41 isolates collected from the different chickpea fields was tested on detached leaves of four Israeli cultivars that differ in their field response to *D. rabiei*. The *cultivar* × *isolate* interaction showed no significant effect (P of $F = 0.95$) on disease incidence. The main effects of *cultivar* and *isolate* on disease incidence were highly significant (P of $F < 0.0001$). Accordingly, our data do not support the hypothesis that there is pathogenic specialization in the *D. rabiei*–*C. arietinum* pathosystem in Israel.

Introduction

Didymella rabiei (syn. *Mycosphaerella rabiei*; anamorph: *Ascochyta rabiei*), the causal agent of Ascochyta blight, is the most destructive foliar disease of chickpea (*Cicer arietinum* L.) worldwide. The pathogen is heterothallic, with a unifactorial (bipolar) homogenic mating incompatibility system; the two mating types have been referred to as *MAT1-1* and *MAT1-2* (Wilson and Kaiser, 1995). The teleomorph was first recorded by Kovachevski (1936) on overwintering chickpea debris in Bulgaria and was subsequently reported on chickpea residues in Russia (Gorlenko and Bushkova,

1958), Greece (Zachos et al., 1963), Hungary (Kövics et al., 1986), Spain (Jiménez-Díaz et al., 1987), Syria (Haware, 1987), Turkey (Kaiser and Küsmenoglu, 1997) and the United States (Kaiser and Hannan, 1987). In Israel, the perfect stage of the pathogen was identified for the first time in 1995 (Shtienberg et al., 1998).

Ascospores and conidia of the fungus are commonly multinucleate and most probably haploid (Wilson and Kaiser, 1995; Bruns and Barz, 2001; Lichtenzveig et al., 2002). The ascospores are dispersed long distances (>8 km) by wind and are thought to be a major source of initial inoculum in chickpea fields in Spain (Trapero-Casas et al., 1996) and the US (Kaiser, 1992). Conidia are disseminated short distances by rain splash, causing secondary disease cycles during the growing season. In some cases, conidia may also serve as primary inoculum. Primary infections that originate from conidia are likely to occur when seeds derived from Ascochyta blight-infected fields are used, or may arise from infested plant debris when there is a short or no rotation between consecutive chickpea crops. The pattern of Ascochyta blight distribution in infected fields may assist in differentiating between the possible sources of initial inoculum. Whenever many infections are observed on upper parts of the plant canopy, randomly scattered about the field, it is most likely that infections originated from ascospores. On the other hand, when a small number of foci is observed in the field, and plants located in the centre of each focus are infected at their lower parts, initial infections most probably originated from infected seeds (Milgroom and Peever, 2003). In Israel, only certified seeds produced from non-infected fields are used, and chickpea is grown in (at least) a 4-year rotation. However, severe Ascochyta blight epidemics occur regularly in all chickpea production areas of the country (even in fields cultivated for the first time), and the patterns of infection suggest that ascospores are often the source of initial inoculum. All these clues form circumstantial evidence that the perfect stage of the pathogen may play a significant role in Ascochyta blight epidemiology in Israel.

Evidence of diversity in virulence of *D. rabiei* populations, reports on physiological races, and claims for resistance breakdown because of the emergence of new pathotypes have accumulated over three decades (Vir and Grewal, 1974; Reddy and Kabbabeh, 1985; Navas-Cortés et al., 1998). Nevertheless, in Israel no signs of resistance breakdown were observed in cv. Bulgarit (a highly resistant cultivar introduced to Israel from Bulgaria during the 1950s) or in cv-Hadas (a locally bred cultivar introduced in the late 1980s). These cultivars retain their resistance despite the repeated occurrence of severe epidemics in adjacent fields sown with susceptible cultivars., and despite the presence of the pathogen throughout all chickpea production areas in Israel (Shtienberg et al., 2000).

In the light of such controversies, it is of great interest to understand the nature of virulence variability in *D. rabiei* populations in Israel. We hypothesize that the interaction in the *C. arietinum–D. rabiei* pathosystem in Israel is based on differences in virulence, rather than on pathogenic specificity. Our specific research aims were: (1) to determine the distribution of mating types of the pathogen in Israel, to assess its potential for sexual reproduction; (2) to estimate the relative contribution of sexual reproduction to diversity in virulence; and (3) to characterize the diversity in virulence of *D. rabiei* in Israel. A preliminary report on some of the results was published previously (Gamliel et al., 1999).

Materials and methods

Distribution of Didymella rabiei *mating types*

Chickpea stems bearing lesions with pycnidia of *D. rabiei* were sampled during 1997–1999, from 18 Israeli commercial fields naturally infected with Ascochyta blight (Figure 1). Stem segments (2 cm long) were air dried, surface disinfested in a domestic bleach solution (1% hypochlorite), washed with sterile distilled water (SDW), and plated on water agar (1.5%) to induce pycnidial formation. Single germinating conidia were transferred onto potato dextrose agar and incubated at 21–23 °C under alternating cycles of 12 h of fluorescent light and darkness. Cultures were maintained according to Wilson and Kaiser (1995). Forty-one monoconidial isolates were obtained and are referred to hereinafter as the 'local collection'; each isolate represented a separate well-defined disease focus in the fields. Ten of these isolates were derived from a single field in Kedma, located in the Lakhish area in central Israel (Figure 1). Accord-

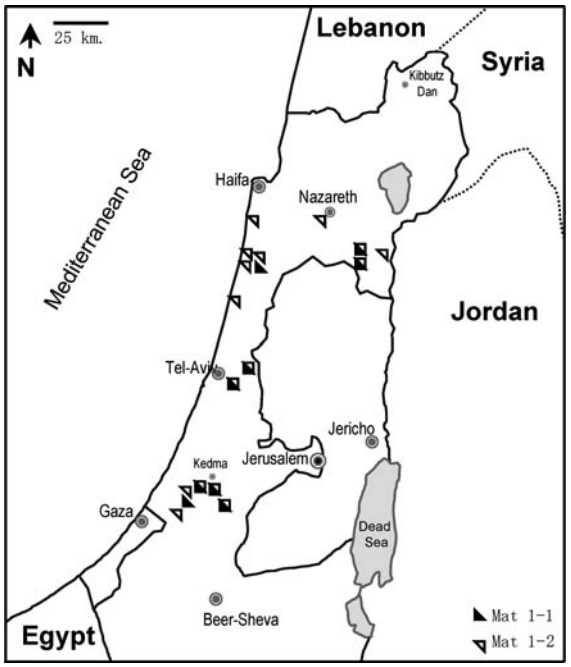

Figure 1. Distribution of mating types of *Didymella rabiei*, the causal agent of Ascochyta blight, in Israel. Forty-one monoconidial isolates were sampled from 18 commercial fields from 1997 to 1999.

ing to the Ascochyta blight infection pattern in that field, we presumed that the primary inoculum was derived from ascospores.

Monoconidial isolates from the *D. rabiei* local collection were paired with compatible mating-type tester isolates of *D. rabiei MAT1-1* (ATCC76501) and *MAT1-2* (ATCC76502) (Kaiser and Küsmenoglu, 1997). Uninfected stems from the local chickpea cv. Sfaradit were cut into 2 cm segments, sterilized by autoclaving and air dried. The stems were placed into 2 ml plastic tubes containing conidial suspensions (1×10^6 conidia ml^{-1}), either from a single isolate (serving as a control) or from mixtures of two isolates for each local-tester combination of isolates; a mixture of the two tester isolates served as an additional control. The stem segments (five per tube; two tubes per treatment) were soaked in the conidial suspensions for 30 min, drained on sterile paper towels for 10 min, and placed on sterile wet filter paper in 9 cm plastic Petri dishes. The stems were covered with filter paper and wetted with SDW containing chloramphenicol at 250 ppm. High humidity was maintained by adding the chloramphenicol solution every 4 days. Dishes were incubated for 24 h at 21–23 °C and then at 10 °C in darkness. Six weeks after inoculation, the pseudothecia that developed were stained with cotton blue and examined under a light microscope at 100 × and 200 × to distinguish between pycnidia and pseudothecia, according to characters described by Trapero-Casas and Kaiser (1992).

Relative contribution of sexual reproduction to diversity in virulence

Infected stems were collected in 2000 from a single field in Kibbutz Dan, located in the Hula Valley in northern Israel (Figure 1). The field infection pattern suggested that ascospores were the primary source of infection. There were nine isolates that originated from different foci (designated below as the 'ascosporic population') and eight isolates that originated from one infection focus of approximately 1 m^2, within the range of rain-splash dispersal (designated below as the 'conidial population'). To confirm the uniformity of the asexual isolates and variation among the sexual cultures, a set of PCR-based DNA markers was used. First, the specific *MAT* locus primers developed by Barve et al. (2003) showed that while all isolates of the conidial population were *MAT1-2*, the isolates of the presumed ascosporic population consisted of both *MAT1-1* and *MAT1-2*. In addition, the PCR markers reported by Lichtenzveig et al. (2002) were used and a similar pattern emerged. Therefore, we considered that the various disease foci originated from different ascospores, corresponding to the field primary inoculum, whereas infections within a single focus were derived from sister conidia originated from the primary infection by asexual multiplication of the fungus. Recently, Milgroom and Peever (2003) used a similar interpretation of the evidence in attempting to identify the possible sources of initial inoculum of Ascochyta blight (ascospores vs. conidia) in the northwestern US.

Six chickpea cultivars, ICC1903, ICC3996, ILC1929, ILC249, ILC3279 and ILC482, obtained from the germplasm collection of ICARDA (International Center for Agricultural Research in the Dry Areas, Aleppo, Syria) and ICRISAT (International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India), were used for the virulence tests of the ascosporic and

conidial populations. These cultivars were chosen because they are frequently used as differentials for classifying isolates of *D. rabiei* (Reddy and Kabbabeh, 1985; Jan and Wiese, 1991; Dolar et al., 1994; Udupa et al., 1998) and, therefore, their use would enable us to compare our data with the response of internationally characterized germplasm. For all experiments, seedlings were grown in 10 cm diam pots (five plants per pot) containing 0.6 l of peat:tuff mixture (3:7), in a greenhouse held at 23 ± 2 °C and illuminated for 12 h per day with high-intensity fluorescent light.

Virulence tests were done on detached leaves under controlled conditions, according to Dolar et al. (1994) with a few modifications. The youngest fully expanded leaves were detached from 2–3 week-old seedlings. Each leaf was placed, lower surface down, in a 5 cm plastic Petri dish containing water agar solution (0.25%) and 50 ppm of benzimidazole. The upper surface of each leaflet (at least 10 per plate) was inoculated with a 5 µl drop of conidial suspension (1×10^5 spores ml^{-1} SDW). The leaves were incubated in a growth chamber at 20 ± 2 °C with a 12 h photoperiod. Data were obtained from two identical experiments; the main effects tested were the six chickpea differential cultivars and the *D. rabiei* isolates derived from either the ascosporic or the conidial populations. Each *cultivar* × *isolate* combination (treatment) was repeated six times in each experiment. A leaf with SDW drops on each leaflet served as the control treatment. The dishes were examined daily until the first symptoms of necrosis and pycnidia appeared – 4 and 5 days after inoculation, respectively. Then, the disease incidence (the percentage of leaflets on each dish that showed symptoms) was assessed every 2 days according to two variables: the percentage of leaflets per dish showing necrosis and the percentage of leaflets per dish showing pycnidia. In addition, disease severity (the proportion of leaflet area exhibiting disease symptoms) was recorded for each leaflet. Disease incidence levels deduced from either the proportion of necrotic leaflets or the proportion of leaflets showing pycnidia were highly correlated ($r = 0.93$; $P < 0.0001$; $df = 102$, based on isolate means from both populations). Likewise, conclusions derived from analyses of disease incidence and disease severity records were similar. Thus, disease incidence data are presented herein. The first senescence symptoms among the control leaves were observed 12 days after inoculation and the experiments were terminated. The clearest differences among treatments were observed 9 days after inoculation; these data served for further evaluation.

Statistical analyses of the data were performed with the JMP-IN software, version 4, for Windows (SAS Institute, Cary, NC). To enable analyses of variance and multiple means comparisons, the disease incidence values were approximately normalized by arcsine square-root transformation prior to analysis. The following general linear model was used for analyses of variance $Y_{ijk} = \mu + \alpha_i + \beta_k + \gamma_j + \alpha_i \times \gamma_j + \epsilon_{ijk}$; where Y_{ijk} represents the expected disease incidence variable; μ represents the true mean; α_i symbolizes the effect of a given *cultivar*; β_k represents the *experiment* effect (analyzed as a random effect); γ_j symbolizes the *isolate* effect (analyzed as a random effect); $\alpha_i \times \gamma_j$ represents the effect of the *cultivar* × *isolate* interaction (also a random effect); ϵ_{ijk} symbolizes the residual. In this case, the datum obtained from a single plate served as the minimal experimental unit (y_{ijkl}). To test the hypothesis that the ascosporic and conidial populations differ in their diversity in virulence, Bartlett's tests for unequal variances were employed to compare the variances of the two populations. Multiple comparisons of means were made by the Tukey–Kramer Honestly Significant Difference (HSD) test ($P = 0.05$).

Characterization of the diversity in virulence of Didymella rabiei *in Israel*

Four common Israeli cultivars were used to characterize the virulence of the local *D. rabiei* isolates. These cultivars vary in their response to *D. rabiei*: 'Sfaradit' is highly susceptible; 'Ayala' is moderately susceptible; 'Hadas' is moderately resistant; and 'Bulgarit' is highly resistant (Shtienberg et al., 2000). Virulence tests were performed on detached leaves as described above. Each of the 41 isolates was tested in at least two independent experiments, and every *cultivar* × *isolate* treatment comprised four replicates per experiment. Disease incidence was scored 7 days after inoculation. Disease incidence data were treated and analyzed as described above, with a few modifications. The following general linear model was used for analyses of variance: $Y_{ijk} = \mu + \alpha_i + \gamma_j + \alpha_i \times \gamma_j + \epsilon_{ijk}$; where Y_{ijk} represents the expected disease incidence variable;

μ represents the true mean; α_i symbolizes the effect of a given *cultivar*; γ_j symbolizes the *isolate* effect (analyzed as a random effect); $\alpha_i \times \gamma_j$ represents the effect of the *cultivar* × *isolate* interaction (analyzed as a random effect); ϵ_{ijk} represents the residual. Disease incidences obtained from four replicates were averaged; thus the treatment mean per experiment served as the minimal experimental unit (y_{ijk}). The frequency distribution curves of the transformed disease incidence scores were tested for normality with the Shapiro–Wilk *W*-test.

Results

Didymella rabiei *mating-type distribution*

Pseudothecia of *D. rabiei* with mature asci developed exclusively in the compatible combinations of a local isolate with one of the testers, or of the two testers. Pycnidia were observed in all of the treatments, when a stem was inoculated either with a single isolate or with a mixed suspension of an isolate and *MAT1-1* or *MAT1-2*. In the control treatments (stem pieces inoculated with a single isolate, either local or tester), only pycnidia were observed indicating that these isolates were self-sterile. Data derived from the pairings of isolates indicated that both *D. rabiei* mating types prevail in all chickpea-production areas in Israel. *MAT1-1* was observed in 44% and *MAT1-2* in 88% of the 18 fields sampled. In some sites, both mating types were present (Figure 1). Such was the case with the isolates collected from the Kedma farm, where six of the isolates were *MAT1-1* and four were *MAT1-2*.

Relative contribution of sexual reproduction to diversity in virulence

All possible combinations of six chickpea differential cultivars with isolates from ascosporic and conidial populations of *D. rabiei* were tested in two similar experiments. Results of the analyses of variance of disease incidence data for each pathogen population are presented in Table 1. The R^2 values of the linear models were 40 and 39% for the ascosporic and conidial populations, respectively. In both cases, the estimates of the variance component of the *experiment* term were relatively low (5.4 and 4.5%), but with significant (*P* of $F < 0.003$) effects.

The *cultivar* × *isolate* interaction showed no significant (*P* of $F > 0.09$) effect on the disease incidence for either of the populations (Table 1). The chickpea cultivars differed significantly (*P* of $F < 0.0001$) in their response to *D. rabiei*; the *cultivar* term accounted for the largest estimated component of the variance: 88% and 79% of the non-residual variance for the ascosporic and

Table 1. Analysis of variance of disease incidence data obtained from inoculation of six differential chickpea lines with isolates derived from ascosporic and conidial populations of *Didymella rabiei*[a]

	Ascosporic population					Conidial population				
Source [b]	Df	Mean Square	*F* value	Probability	Variance component[c]	df	Mean Square	*F* value	Probability	Variance component[c]
Experiment	1	1.99	12.0	0.0006	5.4	1	1.48	8.8	0.0031	4.5
Cultivar	5	10.22	46.9	< 0.0001	88.2	5	7.88	34.8	< 0.0001	79.0
Isolate	8	0.39	1.8	0.1038	2.3	7	1.08	4.8	0.0007	11.7
C × I	40	0.22	1.3	0.0993	4.1	35	0.23	1.3	0.0935	4.8
Model	54	1.20	7.2	< 0.0001	100.0[d]	48	1.17	7.0	< 0.0001	100.0[d]
Residual	592	0.17				525	0.17			
Total	647					573				

[a] Disease incidence (proportion of leaflets per leaf showing necrosis) data were assessed with a detached-leaf assay 9 days after inoculation. Data were arcsine square root transformed before analysis. Each treatment had six replicates. The differential lines were ICC1903, ICC3996, ILC1929, ILC249, ILC3279 and ILC482.

[b] Source of variance: Experiment was defined as a block and analyzed as a random term. Isolate and Cultivar × Isolate were also analyzed as random terms.

[c] Expressed as percentages of the Model variance; these estimates were based on equating observed Mean Square values to expected values.

[d] The Model explains 40% and 39% of the total variance (R^2) for the ascosporic and conidial populations, respectively.

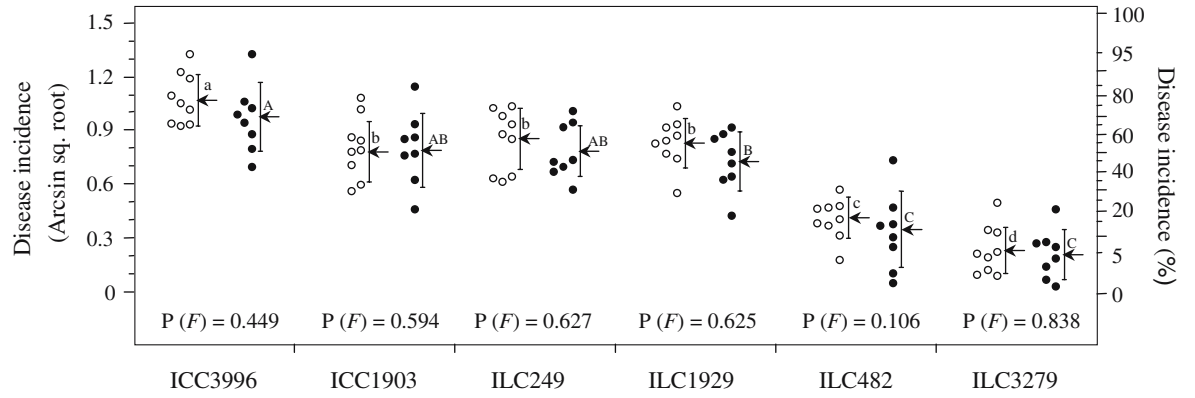

Figure 2. Diversity in virulence among *Didymella rabiei* ascosporic population (open circles) and conidial population (closed circles) for chickpea accessions that differed in their response to the pathogen. The points illustrate the isolate means estimated from transformed disease-incidence data and adjusted by the experiment effect (see Table 1); right *Y*-axis shows the equivalent untransformed scale. Bars show standard deviation estimates enclosing the population means indicated by arrows. Multiple comparisons of cultivar least square means were made with the Tukey–Kramer Honestly Significant Difference test. For each isolate population, cultivar means accompanied by different letters are significantly different ($P = 0.05$); (a–d) for the ascosporic population and (A–C) for the conidial population. The variances of ascosporic and conidial populations were evaluated by the Bartlett's test for unequal variances; estimated probabilities of *F* values are shown for each chickpea line.

conidial population, respectively (Table 1). The cultivars' least square means were ranked according to the Tukey–Kramer HSD test ($P = 0.05$) and are shown for each population in Figure 2. Similar results were obtained for the two pathogen populations: ICC3996 showed significantly higher disease incidence than ICC1903, ILC249 and ILC1929, which in turn were significantly more susceptible than ILC482 and ILC3279.

The *isolate* effect was highly significant (*P* of $F = 0.0007$) for the conidial population, accounting for 11.7% of the non-residual variance. However, the *isolate* factor was non-significant (*P* of $F > 0.1$) for the ascosporic population (Table 1). Nevertheless, comparison of the populations for each chickpea cultivar separately (by means of Bartlett's test for unequal variances) showed that the populations did not differ significantly (*P* of $F > 0.1$) in their virulence diversity.

Characterization of the diversity in virulence of Didymella rabiei *in Israel*

In the inoculation of four cultivars with the 41 collected isolates, the *cultivar* × *isolate* interaction showed no significant effect (*P* of $F = 0.95$; Table 2) on disease incidence. The main effects of *cultivar* and *isolate* on disease incidence were highly significant (*P* of $F < 0.0001$) explaining 60 and 40% of the total non-residual variance, respectively (Table 2). As expected, 'Sfaradit' and 'Ayala' were the most susceptible cultivars, 'Hadas' showed an intermediate response, which was significantly different from that of 'Bulgarit', the most resistant cultivar (Figure 3). The frequency-distribution curves of the transformed disease incidence scores were normal (*P* of $W > 0.14$) and

Table 2. Analysis of variance of disease incidence data obtained from inoculation of chickpea cultivars 'Ayala', 'Bulgarit', 'Hadas' and 'Sfaradit' with 41 *Didymella rabiei* isolates collected from 18 commercial fields in Israel[a]

Source[b]	df	Mean square	*F* value	Probability	Variance component[c]
Cultivars	3	2.064	27.35	< 0.0001	60.5
Isolate	40	0.259	3.78	< 0.0001	39.5
C × I	120	0.068	0.76	0.9492	0.0
Model	163	0.150	1.67	0.0002	100.0[d]
Residual	212	0.090			
Total	375				

[a] Disease incidence (proportion of leaflets per leaf showing necrosis) data are means of four replications assessed with a detached-leaf assay 7 days after inoculation. Data were arcsine square root transformed before analyses.
[b] Sources of variance; Isolate and *cultivar* × *isolate* (C × I) were analyzed as random terms.
[c] Expressed as percentages of the Model variance; these estimates were based on equating observed Mean Square values to expected values.
[d] The Model explains 56.3% of the total variance (R^2).

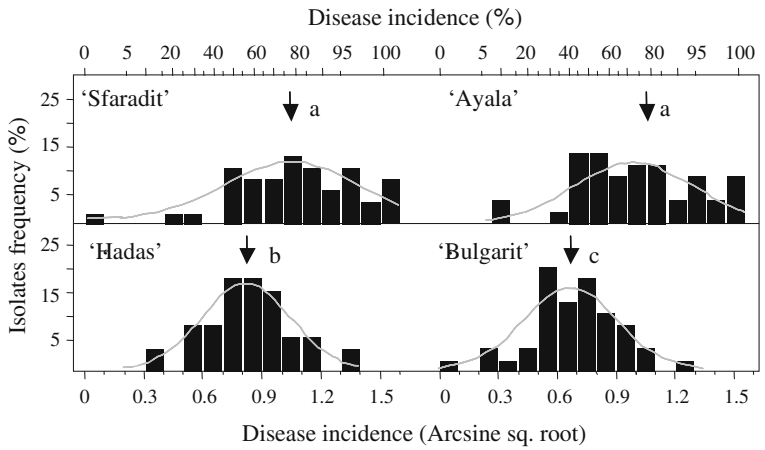

Figure 3. Virulence frequency distribution of the virulence of 41 *Didymella rabiei* isolates to Israeli chickpea cultivars that differ in susceptibility to the pathogen. The histograms are based on isolate means estimated from transformed disease incidence data; top *X*-axis shows the equivalent untransformed scale. The arrows indicate cultivar means obtained from all isolates tested; multiple means comparisons between cultivars that were evaluated with the Tukey–Kramer HSD test ($P = 0.05$) are indicated by letters (a–c).

nearly continuous (Figure 3). Together, the main effects accounted for 56.3% (R^2) of the total trait variance. Similar results were obtained when data derived from the single field in Kedma were excluded from the analysis. When analyzed separately, the isolates from the single field in Kedma did not differ significantly (P of $F = 0.62$) from each other, and the *cultivar* effect was the only significant effect (P of $F = 0.01$) of the linear model ($R^2 = 0.27$).

Discussion

Both mating types of *D. rabiei* were found in all chickpea production areas of Israel and occasionally both within the same field (Figure 1). The presence of both mating types in close proximity provides the potential for sexual reproduction of the pathogen. Several lines of evidence suggest that the teleomorph of *D. rabiei* plays a significant role in Ascochyta blight epidemiology in Israel. First, pseudothecia were observed on naturally infested chickpea straw and were also produced on artificially inoculated stems exposed to natural environmental conditions (Shtienberg et al., 1998). Second, potted chickpea plants placed for 3–4 days in a chickpea production area exhibited Ascochyta blight symptoms after being incubated in a greenhouse; a nearby Burkard spore trap revealed airborne *D. rabiei* ascospores in that area (Shtienberg et al., 1998). Finally, the spatial distribution of initial infections within commercial chickpea fields and the pattern of infection on diseased plants suggest that airborne ascospores, rather than conidia from infected seeds or crop residues, are the major source of initial inoculum (Shtienberg, unpublished data). Similarly, ascospores were reported to act as primary inoculum in Spain and the US (Kaiser, 1992; Trapero-Casas et al., 1996).

Isolates of mating type *MAT1-2* were encountered more frequently than *MAT1-1* in our study but the sampling size was too small to enable reliable conclusions regarding the relative frequency of the two mating types. In any case, our results corroborate those reported in neighbouring countries such as Egypt, Jordan, Syria and Lebanon, as well as in other Mediterranean countries, including Cyprus, Greece, Italy, Libya and Morocco (Kaiser and Küsmenoglu, 1997). Interestingly, in India, Iran, Pakistan and Turkey it seems that *MAT1-1* predominates over *MAT1-2* (Kaiser, 1992; Kaiser and Küsmenoglu, 1997). More data are needed to support any conclusion about a possible differential geographic distribution of the mating alleles.

Sexual reproduction most likely leads to higher genotypic variability, which allows the pathogen population to be dynamic in the face of climatic

changes, or other selective pressures, such as systemic fungicides and resistant host plants. However, the hypothesis that an increment in the genetic variability of a given pathogen population necessarily means a drastic increment in its virulence diversity does not apply to all pathosystems. This connection depends on the genetic basis of the virulence trait. To test this hypothesis in the chickpea-*D. rabiei* pathosystem, we compared the diversity in virulence of an ascosporic population with that of a conidial population and found that the populations did not differ significantly in their virulence diversity (Table 1). These results might indicate that either: (i) the ascospores originated from a cross between individuals with genetically similar virulence loci; or (ii) even though isolates originating from ascospores differing in their virulence, the differences are too small to be detected using the assay system. Further genetic analyses are needed to discriminate between these possibilities. In agreement, results reported by Milgroom and Peever (2003) did not support the standard assumption about more genetic variability in a presumably ascosporic population than in a presumably conidial population.

All the experiments in the present study were characterized by relatively high variance within treatments. Considering that the experiments were performed under uniform and controlled conditions, the residual variances encountered were surprisingly high. Thus far, this considerable intra-treatment variation in the *C. arietinum-D. rabiei* pathosystem has been regarded exclusively as a disadvantage of the rating scale used (Reddy et al., 1981) and it has often been eliminated by using discrete scales to describe the interaction. In our view, this variation might be an intrinsic feature of the *C. arietinum–D. rabiei* pathosystem, although its underlying factors are still unknown. Heterokaryosis (Allen, 1983) or polyploid heterozygous genomes (Bruns and Barz, 2001) had been suggested as possible reasons for the high phenotypic variability among *D. rabiei* isolates, but no heterozygosity was detected in an F_1 segregating population that was examined with co-dominant STMS markers, and almost all progeny alleles segregated 1:1, as expected for a haploid F_1 population (Lichtenzveig et al., 2002). These results are in accordance with the assumption that the numerous nuclei in ascospores are haploid and are derived from several mitotic divisions (Wilson and Kaiser, 1995) after meiosis.

In the light of the close proximity of the chickpea cropping areas in Israel to those in neighbouring countries and of the fact that sexual reproduction of the pathogen – which enables long-range spore dissemination – has been reported in the region, it is reasonable to assume that *D. rabiei* populations distributed across parts of the Middle East form a complex metapopulation. The similarities among the countries in mating-type occurrence, as discussed above, support this assumption. However, the virulence diversity seems to vary. Among the isolates reported in the present paper, none showed the pathotype II and III pathogenicity patterns described by Udupa et al. (1998). These are highly virulent types that overcame the resistance of accessions such as ILC482 and ILC3279, respectively. The associated differences in the host–pathogen interaction that occur under the differing climatic conditions in Israel (more than 40 years of winter sowing) and its neighbouring countries (mostly spring sowing) could be the reason for the differences between our present observations and those of Udupa et al. (1988). Indeed, Trapero-Casas et al. (1996) suggested that the different cropping cycles provide grounds for differing selection pressures in this pathosystem. Nevertheless, the short geographic distances between the Lebanese, Syrian, Jordanian and Israeli growing areas would suggest that any changes in the pathogen population in any of these countries would be detected sooner or later in adjacent areas in Israel.

Diversity in virulence can be described in terms of pathogenic specificity, which addresses the behaviour of a specific pathogen strain on a specific host or range of hosts, and in terms of virulence, which addresses the relative amount of damage caused to a host by a given pathogen (Shaner et al., 1992). Pathogenic specificity is characterized by specific interactions between pathogen isolates and host cultivars, and it is indicated by a statistically significant component of variance that arises from the interaction between cultivars and isolates (Agrios, 1997). The present data enabled us to examine the interactions in the *C. arietinum–D. rabiei* system. The cultivar/isolate test with detached leaves showed only main-effect variance. The *cultivar* effect was

the largest and the *isolate* effect was significant and showed continuous variance; the contribution of the statistical interaction to the variance was not significant (Table 2). Thus, we suggest that, in Israel, the magnitude of this interaction, if it exists at all, is very small and difficult to detect and, therefore, does not support the hypothesis of the existence of pathogenic specificity.

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