Development of *Didymella rabiei*, the Teleomorph of *Ascochyta rabiei*, on Chickpea Straw

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ABSTRACT

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The teleomorph of *Ascochyta rabiei* was induced to develop and mature on artificially infested chickpea straw. High moisture and low temperature (5–10 C) were the major factors required for pseudothecial development and maturation. At 8 C, pseudothecia developed and matured in about 8 wk. Morphological characteristics of pseudothecia, asci, and ascospores confirmed the identification as *Didymella rabiei* rather than *Mycosphaerella rabiei*. The fungus is heterothallic, and the two mating types have been referred to as MAT1-1 and MAT1-2. Under field conditions in the Palouse region of the Pacific Northwest, the teleomorph developed extensively on overwintered chickpea crop residues that remained on the soil surface. Pseudothecia appeared in early fall, matured during the fall and winter, produced ascospores during the spring, and were almost completely exhausted of ascospores by the beginning of the summer. The estimate of ascospore production by highly infested chickpea crop residues was about 15,000 ascospores per square millimeter of tissue surface. Most ascospores were discharged from the beginning of March to the end of May. The discharge period overlapped with the vegetative stage of the chickpea crop, indicating that ascospores may serve as primary inoculum for epidemics of Ascochyta blight in the Palouse region.

Additional keywords: Cicer arietinum, epidemiology.

Ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Labrousse, is a devastating disease of chickpea (*Cicer arietinum* L.) (15). The fungus survives in infested chickpea crop residues and in seed (15). Previously, pycnidiospores produced on these tissues have been considered the only primary inoculum (15). However, the teleomorph, *Didymella rabiei* (Kovachevski) v. Arx (syn. *Mycosphaerella rabiei* Kovachevski), may also develop on chickpea residues that overwinter on the soil surface (10,15). The teleomorph was first described by Kovachevski (10) in Bulgaria in 1936, and its occurrence on overwintered chickpea residues has subsequently been reported from the former USSR (3) and Greece (25) and, more recently, from Hungary (11), the United States (9), Spain (7), and Syria (5). No studies have been carried out on development of the teleomorphic stage, even though the teleomorph may be important in long-distance dissemination of airborne ascospores and in greater genetic diversity of the pathogen population. The lack of studies on the teleomorph and the scarcity of reports on its occurrence have been attributed to special requirements for its development (8,15). In the Palouse region of eastern Washington and northern Idaho, the teleomorph occurred extensively in 1986 and appeared to play an important role in the epidemiology of Ascochyta blight (9). This study was undertaken to determine the factors affecting teleomorph development and the epidemiological consequences of that development. Preliminary results of this work have been reported (20).

MATERIALS AND METHODS

Chickpea straw and fungal isolates. Chickpea stems and pods naturally infested with *D. rabiei* were collected from fields in Genesee, ID, at harvest time (September) in 1985 and 1986. They were air-dried and maintained at 4 C and 30–40% relative humidity until used. Stems were cut in pieces (6–12 cm) that contained at least two Ascochyta blight lesions. Pods were cut in pieces (2–4 cm) with several lesions. Most lesions contained characteristic pycnidia of *A. rabiei*. Pseudothecia were never observed in lesions on chickpea stems and pods collected at harvest time. Chickpea stems and pods from a field in Central Ferry, WA, where no Ascochyta blight occurred in 1986, were sterilized with propylene oxide (4) and used as an *Ascochyta*-free control.

Single-spore isolates of *D. rabiei* were used in all plant inoculations. Ascospores were discharged onto 2% water agar (WA) and transferred individually to fresh chickpea meal-dextrose agar (CDA) medium (21). To discharge ascospores, a small strip of dried stem or pod tissue containing mature pseudothecia was placed on a WA block that was attached to the inner surface of the cover of a petri dish. The dish cover with adhering tissue was placed over the bottom plate containing WA. Dishes were incubated in the dark at 20 C for 2 h for ascospore discharge onto WA. Single conidiospore isolates were obtained by streaking a conidial suspension of each isolate on WA and transferring single germinating conidia to CDA.

Induction of pseudothecial development in vitro. After some preliminary trials, three moisture sources (perlite, sand, and paper towels) and two moisture treatments (continuously wet and 1 wk wet alternated with 1 wk dry) were selected to produce pseudothecia of *D. rabiei* on naturally infested chickpea straw. Chickpea stem pieces were surface-disinfested in 1% NaOCl for 5 min. Then they were placed on the surface of an 8-cm layer of perlite or sand in a plastic tray and covered with a loose-fitting lid, or, in the blotter method, they were placed between two double layers of paper towels in a unsealed plastic bag. The perlite or sand was kept continuously wet by placing the plastic trays, which were perforated on the bottom, in a water bath with a constant level of water. In the blotter method, the paper towels were kept

continuously wet by submerging one end of the towels in a water bath with a constant level of water. For the wet/dry treatment, paper towels were submerged in the water bath every other week. The dry check consisted of placing disinfested and air-dried stem pieces in paper bags. Trays and bags were incubated at room temperature (20–24 C) for 96 h to allow the fungus to become well established on the surface of the straw pieces. They were then placed in growth chambers (no lights) with constant temperatures of 5, 10, 15, 20, and 25 ± 1 C and an alternating temperature of 15/5 ± 1 C (12 h at 15 C alternated with 12 h at 5 C). Twelve stem pieces were included in each treatment. At 2-wk intervals, two stem pieces were sampled and examined for mature pseudothecia. The experiment was repeated once using only the blotter method.

At 8 wk, when most pseudothecia had matured in some treatments, four stem pieces were assessed for surface colonization, relative proportion of pseudothecia versus pycnidia in the colonized tissue, and pseudothecial maturity. The percentage of tissue surface covered with pycnidia and pseudothecia was assessed using a 0–4 rating scale (0 = 0%, only original lesions with pycnidia, 1 = 1–25% covered, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%). The proportion of pseudothecia versus pycnidia in the colonized tissue was assessed using a 0–4 rating scale (0 = no pseudothecia, 1 = 1–25% pseudothecia, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%). The percentage of mature pseudothecia was calculated using 20 pseudothecia from each of four stem pieces. Pseudothecia were dissected from the tissue, squashed in lactophenol-cotton blue, and assessed as immature (no ascospores formed and lumina of pseudothecia filled with only pseudoparaphyses or with pseudoparaphyses and immature asci), mature (pseudothecia with asci and ascospores), or aborted.

Teleomorph identification. Pseudothecia were tentatively identified using a stereoscopic microscope. Individual pseudothecia were then dissected, squashed in lactophenol-cotton blue, and observed at ×200 and 400 to confirm their identification and to assess their stage of maturity. Developmental stages of pseudothecia also were observed in pseudothecial sections made with a microtome on small strips of infested tissue imbedded in paraffin. In mature pseudothecia, measurements of pseudothecial diameter were taken on the moistened chickpea straw under the stereoscopic microscope; measurements of asci and ascospores in the mounted pseudothecia were made under the compound microscope at higher magnification. Measurements also were taken of ascospores discharged onto slides and stained with lactophenol-cotton blue.

Ascospores were discharged onto WA and transferred individually to CDA. Cultures on CDA were incubated at 20–24 C to confirm the identity of the anamorph. Two inoculation methods were used to test the pathogenicity of ascospores. In the first method, ascospores were discharged from infested chickpea straw into distilled water, using the method described above to obtain single ascospores. Chickpea plants of a susceptible cultivar (PI 458870) were sprayed to runoff with a suspension of ascospores (2×10^4 ascospores per milliliter).

In the second method, chickpea plants were inoculated with eight single ascospore isolates by spraying the plants with suspensions of conidia (2×10^4 conidia per milliliter plus 0.05% Tween 80) obtained from the respective cultures on CDA. Inoculated plants were incubated in a humid atmosphere at 20 C for 48 h and then placed in the greenhouse (18–25 C) for symptom development.

Mating type. Artificially inoculated chickpea plants or inoculated chickpea straw from healthy plants were used to test the homo- or heterothallic nature of *D. rabiei*. In the first experiment, plants of chickpea PI 458870 were inoculated separately with six single-ascospore isolates and two mixtures of three different isolates. Chickpea plants were sprayed to runoff with a suspension of conidia of each isolate or mixture (about 1×10^6 conidia per milliliter), incubated in a humid atmosphere at 20–24 C for 48 h, and placed in the greenhouse for symptom development. Stem pieces from these plants with lesions of Ascochyta blight were placed in nylon mesh bags (500-μm openings) on the soil surface in two fields in Pullman, WA. In addition to the eight combinations mentioned above, stem pieces from chickpea plants naturally infected by ascospores in the field, from plants inoculated by ascospores in the greenhouse, and from healthy plants were also placed in nylon mesh bags in the two fields. Care was taken to isolate each plant-isolate combination in all manipulations, and bags were separated by at least 3 m in each field.

After several negative attempts to promote the formation of pseudothecia of *D. rabiei* on different media (20), the following method was used to test sexual compatibility among isolates on sterile chickpea straw. Stem pieces from healthy chickpea plants were sterilized with propylene oxide and placed in a conidial suspension (1×10^5 conidia per milliliter) of the single isolate or mixtures of isolates for about 1 h. Stem pieces were drained and placed between two double paper towels in a unsealed plastic bag. The paper towels were moistened with sterile distilled water, and the bags were incubated in the dark at 20 C for 2–7 days and then at 8 C for about 7–8 wk. Petri dishes (10 cm in diameter) were also used to contain inoculated stem pieces placed on 10 sterile blotter paper disks moistened with sterile distilled water and incubated at 8 C for about 8 wk. At weekly intervals, sterile distilled water at 8 C was added to the paper towels in the bags or to blotter paper disks in petri dishes to maintain a high moisture.

With the blotter method, the three isolates that produced fertile pseudothecia in the field experiment were paired in the three possible combinations and also inoculated alone to test sexual compatibility on chickpea stem pieces. Two compatible isolates, referred to as 8627 and 8629 from Genesee, ID, have been deposited with the American Type Culture Collection (ATCC), Rockville, MD, as ATCC 76501 and 76502, respectively. Both isolates were selected as testers for mating-type studies with 18 new single-spore isolates of *D. rabiei*. Three isolates of each mating type were also tested individually and paired in all possible combinations by the blotter method. All inoculations were repeated at least once.

Pseudothecial development in the field. Dried chickpea stem pieces (about 9 cm in length) and half pods, both with at least one blight lesion, and stem pieces from healthy chickpea plants were placed in nylon mesh bags, each containing 12, six, and four pieces, respectively. Bags were placed on the soil surface in fields at Genesee, ID, and Pullman, WA. Infested stem pieces from the same field also were placed on the soil surface near the bags, and they were separated by about 4 m with a 2-m central strip of stems from healthy chickpeas between them. Experimental plots were covered with a large mesh screen to prevent dispersal of the bags and stem pieces by wind. Additionally, infested and healthy stem and pod pieces were incubated on the surface of field soil in metal trays (30 cm wide × 50 cm long × 7 cm deep) placed inside large sheds located near the field plots. At about 2-wk intervals, one nylon mesh bag and some infested and healthy stem pieces were collected to observe the saprophytic growth of *D. rabiei* and formation and maturation of pseudothecia. At this time, trays in the two sheds were watered to keep the straw pieces moist.

Saprophytic growth was assessed in each piece of tissue by using the 0–4 rating scale described earlier. Each tissue piece sampled was observed under a stereoscopic microscope to distinguish between pycnidia and pseudothecia of *D. rabiei*. For each sample, at least 50 pseudothecia were dissected from the tissue pieces, squashed in lactophenol-cotton blue, and assessed for maturity. On the basis of previous experiments, new observations, and published studies of related fungi (6,12), pseudothecia were rated according to internal stage of development as follows: stage 1, stromatic pseudothecial initial; stage 2, pseudoparaphyses filling the lumen of the pseudothecium; stage 3, appearance of asci arising among pseudoparaphyses; stage 4, asci formed but contents not differentiated; stage 5, asci with ascospores being formed or completely formed and mature, very few pseudoparaphyses remaining; stage 6, empty or half-empty asci and released ascospores; stage 7, empty pseudothecium, all ascospores have been discharged and some asci walls can be detected. For

each sample, a maturity index (MI) of the pseudothecia was calculated as the weighted average of the observed stages by the equation:

$$MI = \sum_{i=1}^{7} n_i (st_i)/N$$

in which n_i = number of pseudothecia in the developmental stage i, st_i = value of the pseudothecial development stage (1–7), and N = total number of pseudothecia assessed.

For each sample, 4 cm^2 of tissue densely covered with pseudothecia and pycnidia were discharged onto WA or into distilled water for 24 h by the method described to obtain single ascospores. When the expected number of ascospores was low, ascospores were discharged onto WA and counted directly on the WA surface. When the expected number of ascospores was high, ascospores were discharged into distilled water in petri dishes. The dishes were gently agitated to obtain a homogeneous distribution of ascospores and then allowed to stand for about 1 h. Counts of ascospores deposited on the bottom of the petri dish were made in 50 microscopic fields (×200) per each petri dish (5 cm in diameter). Finally, when the number of discharged ascospores was less than 10/cm^2, more pieces of tissue were discharged onto WA.

To estimate the potential for ascospore production in highly infested chickpea debris, the number of pseudothecia per square millimeter of tissue and the number of ascospores per pseudothecium were counted. Pseudothecial counts were made directly under the stereoscopic microscope using tissue pieces completely colonized (100%) by the fungus. The number of asci per pseudothecium was calculated using 120 pseudothecia at development stage 5. At this stage, most asci were mature, but very few ascospores had been discharged.

To determine the relation of pseudothecial development to meteorological variables of temperature and precipitation (rain or melted snow), MI was correlated with the following accumulated values for each 2-wk sampling period: number of days, degree days using a 0 C base, amount of precipitation, number of days with precipitation and average temperature above 0 C (PD), degree days in PD, PD plus 1 day for each period of precipitation (PD1), and degree days in PD1. Linear regressions were performed on the data from the two fields using the variables listed above as the independent variable and MI as the dependent variable.

TABLE 1. Influence of temperature on formation and maturation of pseudothecia of *Didymella rabiei* in naturally infested chickpea straw[a]

Temperature[b] (C)	Straw colonization[c] (0–4)	Proportion of pseudothecia[d] (0–4)	Percent pseudothecia[e]		
			Immature	Mature	Aborted
5	2.3	2.0	34.0	66.0	0
10	2.3	2.1	1.5	78.7	19.8
15	2.5	2.0	0	12.1	87.9
20	2.0	2.3	0	0	100
25	2.0	2.0	0	0	100
15/5	2.4	2.1	0	20.2	79.8
LSD[f]	0.6	0.4	...	...	...

[a] Chickpea stems with lesions of blight were disinfested and incubated in the dark on various moist surfaces for 8 wk.

[b] Five growth chamber temperatures were constants, and one alternated (15 C for 12 h, 5 C for 12 h).

[c] The percentage of tissue surface covered with pycnidia and pseudothecia was assessed in each stem piece using a 0–4 rating scale (0 = 0%, only original lesions with pycnidia, 1 = 1–25% covered, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%).

[d] Relative frequency of pseudothecia in the colonized tissue was assessed using a 0–4 rating scale (0 = no pseudothecia, 1 = 1–25% pseudothecia, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%).

[e] For each temperature, 480 pseudothecia were assessed as immature (ascospores formed, but lumen of pseudothecium filled with pseudoparaphyses or immature asci), mature (pseudothecium with asci and ascospores), or aborted (lumen of pseudothecium granular, sometimes with a few globose cells or malformed spores).

[f] Fisher's protected least significant difference ($P = 0.05$).

In addition to the above experiment, fields that had been cropped with chickpea the previous season were sampled several times during 1986 and 1987 to assess pseudothecial development and ascospore production on crop residues. Each year, the crop residues were sampled in one field of a farm in Genesee, ID, that had a severe attack of Ascochyta blight during the previous growing season.

RESULTS

Induction of pseudothecial development. In the moistened tissue, *D. rabiei* grew rapidly from lesions, colonizing the stem and pod pieces and forming pycnidia and pseudothecia. After 4 wk, colonization of the tissue ceased, and no differences were observed among the various moisture regimes and temperatures tested. The average colonization for each temperature at 8 wk is presented in Table 1. In the colonized tissue, the proportion of pseudothecia formed was slightly lower than that of pycnidia (Table 1). Pycnidia formed at all treatments and produced viable conidia during the entire experiment. Although pseudothecia formed at all treatments, mature asci and ascospores developed after 8 wk only at 5 and 10 C. At 15 and 15/5 C, the contents of most pseudothecia had become granular, without asci or ascospores or sometimes with a few globose cells and malformed ascospores. These pseudothecialike structures have been referred to as aborted pseudothecia (Table 1). At 20 and 25 C, all pseudothecia were aborted, and no ascosporelike structures were observed.

Saprophytic colonization of tissue by fungi or bacteria increased with increasing temperature. It was scarce at 5 or 10 C but abundant at 20 or 25 C. Stem pieces kept between paper towels that were periodically rewetted had the lowest contamination. Because a few pseudothecia aborted at 10 C (Table 1), the blotter method and 8 C were selected for further experiments. No saprophytic growth occurred on the stem pieces maintained continuously dry, and preformed pycnidia in the lesions contained viable conidia when they were sampled periodically.

Teleomorph identification. Pseudothecia formed at 5–10 C were similar to those that developed under field conditions or those that formed on artificially infested chickpea tissue. Initially pseudothecia were not easy to distinguish from pycnidia under the stereoscopic microscope (×50). However, the darker wall, larger size, markedly subglobose and more erumpent shape, lack of a conspicuous ostiole, and presence of a white exudate that formed under moist conditions of the mature pseudothecia allowed the distinction to be made with little error. Some pseudothecia split open, perhaps in response to excessive moisture.

Pseudothecia of *D. rabiei* were usually arranged in rows on chickpea straw and initially immersed in host tissue. When mature, they were erumpent, dark brown to black, subglobose, and 120–270 μm in diameter, with an inconspicuous ostiole. In immature pseudothecia, pseudoparaphyses were conspicuous, filamentous, hyaline, and septate; they disappeared at maturity. Asci were cylindrical to subclavate, eight-spored, 50–80 × 10–12 μm. The ascus wall was bitunicate (Fig. 1). Ascospores were irregularly distichous, hyaline, ellipsoidal to biconic, two-celled (with the upper cell broader than the lower cell), strongly constricted at the septum (septum below the middle), and 9.5–16 × 4.5–7 μm in size (Fig. 1).

Typical cultures of *A. rabiei* developed from single ascospores on CDA, but only pycnidia were formed on this medium. Chickpea plants inoculated with ascospores or with conidia showed typical Ascochyta blight lesions 2 wk after inoculation (21). Disease severity was slightly higher in plants inoculated with ascospores.

Mating type. Pseudothecia of *D. rabiei* were never observed in blight lesions on living plants. However, pseudothecia developed on straw from healthy chickpea plants when this tissue was inoculated with ascospores or with some mixtures of single ascospore or conidial isolates, but they did not develop on tissue inoculated with isolates obtained from a single ascospore or conidium or with other mixtures of certain single-spore isolates. No pseudothecia or pycnidia of *D. rabiei* developed on healthy

tissue. In the compatible combinations, immature pseudothecia were observed within 14 days after inoculation of the straw or incubation of the infested stems in the field, and they reached maturation in about 8 wk at 8 C or 5 mo under field conditions.

All 20 single-spore isolates tested by the blotter test were self-sterile, and only pycnidia formed on the inoculated chickpea straw. Twelve isolates produced pseudothecia with tester ATCC 76502; the other eight isolates produced pseudothecia with tester ATCC 76501.

When three selected isolates of each group were paired in all possible combinations and were also inoculated alone, pseudothecia formation on chickpea straw confirmed the previous division into two groups or mating types designated MAT1-1 and MAT1-2, with sexual reproduction only occurring in crosses between isolates in different groups (Table 2).

Pseudothecial development in the field. D. rabiei grew saprophytically and covered entire pieces of infested stems and pods with pycnidia and pseudothecia in 4–6 wk on the chickpea straw incubated in the nylon mesh bags. Saprophytic colonization of healthy straw by *D. rabiei* was delayed. However, after 8 wk, 5–100% of the tissue surface of infested and healthy chickpea tissues was similarly covered with pycnidia and pseudothecia. Pycnidia developed within and around the original necrotic lesions, although most were devoid of conidia in 4–6 mo after placing the bags in the field.

Pseudothecial initials (stage 1) were observed on the first sample date in mid-October, 14 days after placement in the field, and they continued to develop until the pseudothecia were completely devoid of asci and ascospores by the end of June (Table 3 and Fig. 2). Although a few pseudothecia matured by mid-December, most of them were mature (stage 5) by the beginning of March, and they released a majority of ascospores in a 2-mo period (Table 3 and Fig. 2). Maximum discharge of ascospores occurred by mid-April (Table 3 and Fig. 2). After the exhaustion of pseudothecia by the end of June, no more ascospores were produced and pseudothecial walls degenerated. New pseudothecia did not develop on the infested chickpea debris during the next crop season.

The average numbers of pseudothecia per square millimeter of highly infested tissue and asci per pseudothecium were 12 and 156, respectively. Therefore, the estimated potential for ascospore production was 14,976 ascospores per square millimeter of infested tissue.

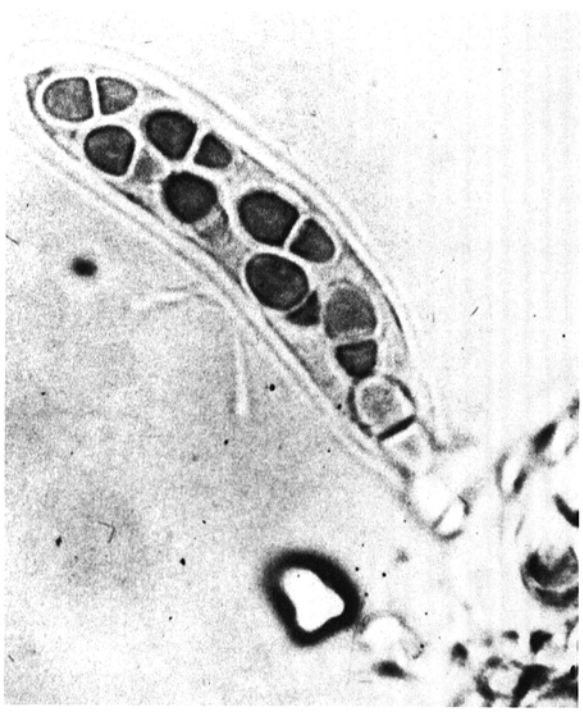

Fig. 1. Bitunicate ascus and ascospores of *Didymella rabiei.*

No differences in pseudothecial development were observed between the samples from the two fields (Fig. 2). Additionally, preliminary linear regression analyses of each field gave the same regression equation. Therefore, average data from the two fields were used for regression analyses. From various independent variables analyzed, PD had the best agreement with MI data. The regression formula was MI = 0.089 PD. The coefficient of determination (R^2) for this regression was 0.994; R^2 adjusted for degrees of freedom (R_a^2) was 0.993. There was no clear pattern in the distribution of standardized residuals over adjusted MI or PD.

Results similar to those indicated above for pseudothecial development were obtained when the infested straw was placed on the soil surface without nylon mesh bags in the two fields or inside of sheds near the two fields. However, more saprophytic organisms were observed on these tissues. Pycnidia or pseudothecia of *D. rabiei* were not detected on stems from healthy chickpeas that were incubated about 1 m from the infested straw. The fungi most frequently observed that formed structures resembling those of *D. rabiei* on the infested or healthy straw were *M. tassiana* (De Not.) Johans., *Phoma medicaginis* Malbr. & Roum. in Roum. var. *pinodella* (L. K. Jones) Boerema, *Leptosphaeria* spp., and *Pleospora* spp.

In the farmer fields sampled in 1986 and 1987, pseudothecial development in the second year was similar to that observed in the nylon mesh bag experiments. Values for MI per ascospore discharge by the middle of March, April, May, and June were 5.18/104, 5.62/754, 6.69/12, and 6.97/0.1, respectively. Values of MI and ascospore discharge corresponding to the 1986 season are presented in Figure 2. In this season, the regression analysis of the incomplete MI data that began on 18 March over PD showed a significant ($P = 0.05$) linear relationship: MI = 0.084 PD ($R^2 = 0.995$, $R_a^2 = 0.994$).

DISCUSSION

The teleomorph of *A. rabiei* was induced to develop and mature on naturally and artificially infested chickpea stem and pod pieces. High moisture was essential for saprophytic growth of the fungus and pseudothecial development. No tissue colonization occurred and no pseudothecia formed in air-dried chickpea stems and pods. Also, pseudothecial development ceased when infested straw containing pseudothecia in various stages of development was air-dried. The methods of supplying moisture were adapted from the technique used by Ross and Hamlin (19) for producing pseudothecia of *Venturia inaequalis* on apple leaf disks. However, they (19) found considerable variation in the number and maturity of pseudothecia produced with different moisture sources. In our study, paper towels were considered to be the most consistent moisture supplier, and the blotter method was used for routine work.

Equivalent numbers of pseudothecialike structures formed at all temperatures (Table 1), indicating that temperature had a limited role in the induction of pseudothecia. However, temperature had a major influence on their maturation. Abundant

TABLE 2. Production of pseudothecia by *Didymella rabiei* in crosses between three single-spore isolates of each mating type

Isolate[a]	Origin	Mating type[b]	Isolate					
			8627	8101	8701	8629	8302	8609
8627	Genesee, ID	1	–[c]	–	–	+	+	+
8101	Córdoba, Spain	1		–	–	+	+	+
8701	Turkey	1			–	+	+	+
8629	Genesee, ID	2				–	–	–
8302	Córdoba, Spain	2					–	–
8609	Aleppo, Syria	2						–

[a]Isolates 8627 and 8629 (ATCC 76501 and 76502, respectively) from Genesee, ID, were used as testers in these experiments.
[b]Tested previously by crossing with the two testers, 8627 and 8629.
[c]– = Pseudothecia did not form, + = mature pseudothecia formed.

TABLE 3. Pseudothecial development and ascospore release by *Didymella rabiei* on chickpea crop residues[a]

Date	Percent pseudothecia in developmental stage[b]							Pseudothecial maturity index (1–7)	Ascospores discharged in 24 h[c]
	1	2	3	4	5	6	7		
10/7/86	86.7	13.3	0	0	0	0	0	1.13	0
11/6/86	0.8	92.3	6.9	0	0	0	0	1.89	0
12/7/86	0	70.5	26.4	2.3	0.8	0	0	2.33	0.4
2/2/87	0	5.9	46.1	32.3	15.7	0	0	3.58	3.1
3/2/87	0	0	0	4.4	77.8	17.8	0	5.13	47.0
4/1/87	0	0	0	0	20.7	79.3	0	5.79	506.2
5/1/87	0	0	0	0	3.7	48.5	47.8	6.44	18.2
6/1/87	0	0	0	0	0	10.5	89.5	6.90	0.8
7/1/87	0	0	0	0	0	0	100	7.00	0

[a]Chickpea stems and pods with lesions of Ascochyta blight were placed in nylon mesh bags on the soil surface in the field at Genesee, ID, and Pullman, WA, on 24 September 1986. At 2-wk intervals, one bag from each field was sampled. Data presented are the average of the two fields at about 4-wk intervals.

[b]For each field and date, at least 50 pseudothecia were assessed according to seven developmental stages (1 = pseudothecial initials, 2 = pseudoparaphyses filling the lumen of the pseudothecium, 3 = asci arising among pseudoparaphyses, 4 = immature asci, 5 = asci with ascospores, 6 = half-empty asci and released ascospores, 7 = empty pseudothecium).

[c]Number of ascospores discharged onto water agar or into water per square millimeter of chickpea tissue. For each date, 4 cm^2 of highly infested tissue was used.

asci and ascospores only developed at 5 and 10 C, whereas most pseudothecia aborted at 15 or 15/5 C, and all of them failed to produce asci and ascospores at 20 and 25 C. Low temperature and a relatively long incubation period are requirements for sexual reproduction in many plant-pathogenic members of Ascomycotina (13). The genus *Didymella* is not an exception (2), although some very well-known plant pathogenic species, such as *D. bryoniae, D. ligulicola,* and *D. pinodes,* do not share these requirements (1,18,23).

Pseudothecial development on chickpea debris indicated that nutrients, apart from those supplied by the stems or pods, are not needed for induction and maturation of the teleomorph. However, pseudothecia failed to develop on normal and low nutrient agar media in petri dishes (20); some nutrients from the chickpea straw or factors related to the incubation on agar media are required for teleomorph development.

Our observations and measurements of pseudothecia, asci, and ascospores agree closely with those of *M. rabiei* reported by Kovachevski (10), although the ascospores were somewhat smaller. In their revision of the genera of didymospored Pyrenomycetes, Müller and von Arx (14) transferred the names of several species from *Mycosphaerella* to *Didymella,* including *M. rabiei,* which they renamed *D. rabiei* (Kovachevski) v. Arx. This distinction has been maintained by Corbaz (2) and von Arx (22). In our study, the pattern of development of pseudothecia was typical of that which characterizes the Pleosporales (12)—nonfasciculate asci arising among preformed pseudoparaphyses and ascospores strongly constricted at the septum. With this information, we feel that the teleomorph of *A. rabiei* should be designated *D. rabiei* (Kovachevski) v. Arx rather than *M. rabiei* Kovachevski. Punithalingam and Holliday (17) preferred to retain the name of *A. rabiei* in their description of this pathogen because the correlation between the asexual and sexual stages indicated by Kovachevski (10) needed to be confirmed. Our research has confirmed the anamorph-teleomorph correlation of Kovachevski, and we suggest that the name of the teleomorph, *D. rabiei,* now be used when reference is made to this chickpea pathogen.

The studies on sexual compatibility using single-spore isolates demonstrated that *D. rabiei* is heterothallic. On the basis of pairing experiments, 20 isolates could be divided into two groups or mating types that were represented by isolates ATCC 76501 and 76502. Recent work (Trapero-Casas, Nũnez Cañete, Kaiser, and Jímenez Díaz, *unpublished data*) with more than 100 isolates of *D. rabiei* has confirmed these results. According to the conventions proposed by Yoder et al (24), we propose MAT1-1 for isolates belonging to the mating type represented by isolate ATCC 76501 and MAT1-2 for the mating type represented by isolate ATCC 76502. Heterothallism is common in the Ascomycotina (13), although homothallism appeared to be the rule in the genus *Didymella* (1,2,18,23).

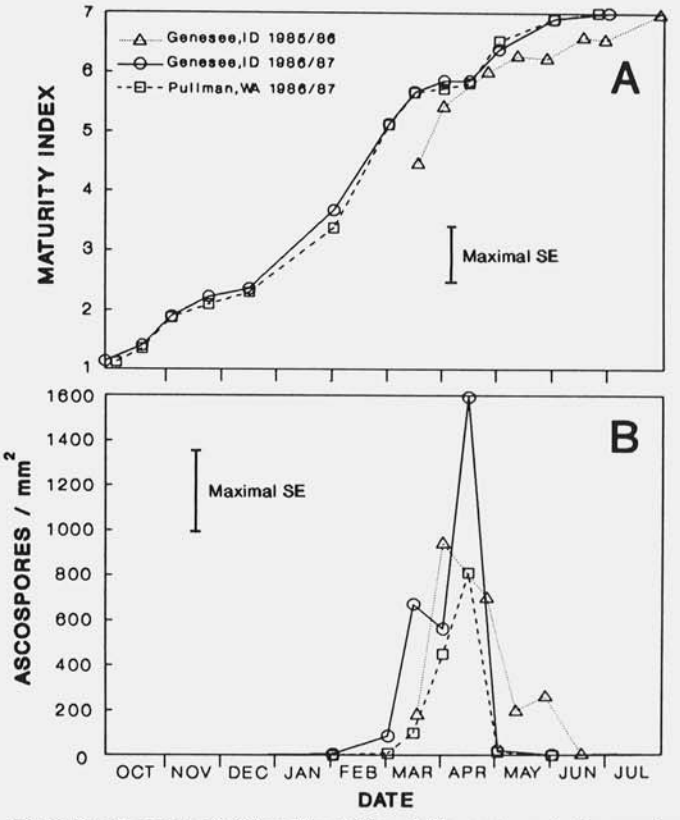

Fig. 2. Development of *Didymella rabiei* on chickpea straw. **A,** Progression on time of pseudothecial maturity index on chickpea crop residues placed on the soil surface in three fields. **B,** Ascospore discharge over time from the same chickpea residues. For each sampling date and location, at least 50 pseudothecia and 4 cm^2 of highly infested tissue were used to estimate the pseudothecial maturity index and the discharge of ascospores, respectively. SE = Standard error.

Pseudothecial development under field conditions in the Palouse region of eastern Washington and northern Idaho indicated the important role that the teleomorph may play in the epidemics of Ascochyta blight in this region. Pseudothecia developed extensively on chickpea crop residues placed on the soil surface after harvest and produced large numbers of ascospores that were released during the spring. In this region, chickpeas are sown from late March to the beginning of May. Therefore, in the 2 yr of study, the second half of the ascospore release period overlapped with the vegetative stage of the chickpea crop (Fig. 2) and indicated that airborne ascospores may serve

as primary inoculum for the disease. Additional evidence has been provided by Kaiser (unpublished data) that ascospores can appear in fields where pathogen-free chickpea seed has been planted.

The traditional disease cycle, which considered conidia released from pycnidia on seeds, crop debris, and volunteer plants as the only inoculum sources, now needs to be modified for the Palouse region to consider ascospore production on chickpea straw. Under present conditions in the Palouse, the sexual phase of the pathogen cycle is particularly relevant, because many farmers are practicing minimum tillage to conserve moisture and reduce soil erosion. As a result, most of the infested chickpea debris remains on the soil surface, thus favoring development of pseudothecia of *D. rabiei* during the fall and winter months. New control measures are needed to reduce or prevent ascospore production on chickpea crop residues.

PD was the environmental parameter best correlated with the MI of pseudothecia. The regression equation predicted 56 precipitation days to reach a MI of 5. This period of time, which corresponded to a 5-mo period where the average temperature above 0 C during each sampling period varied between 0.9 and 12 C, coincided with the 8 wk needed for pseudothecial maturation under controlled conditions at 8 C. However, the regression analyses were only used for comparison between regression lines and should not be used to make predictions.

Although development of pseudothecia of *D. rabiei* had not been studied before, all field reports indicated that mature pseudothecia occurred only on overwintered chickpea debris (3,5,7,10,11,25). In Greece (25), pseudothecia were immature by the end of January and they matured during February. In Bulgaria (10) and Hungary (11), mature pseudothecia appeared in the spring. Our results on maturation of pseudothecia and production and release of ascospores from infested chickpea debris differed from those reported by Zachos et al (25) in Greece. Zachos et al (25) indicated that mature pseudothecia with ascospores remained functional on the chickpea debris for 1 yr, and pycnidia continued to produce conidia for a longer period. They recommended a 2-yr crop rotation to prevent infection of the chickpea crop by the primary inoculum produced on chickpea debris. The mild fall and winter temperatures and low rainfall in this region of Greece in 1961 (25) may help to explain these differences. Recent work in Spain (Trapero-Casas, Nũnez Cañete, Kaiser, and Jímenez Díaz, *unpublished data*) has demonstrated that temperature and rainfall determine the time of maturation of pseudothecia and the duration of the ascospore release period. In these studies, pseudothecia only produced ascospores during one season, as in the Palouse. The differences observed in pseudothecial maturation and the duration of the ascospore discharge period between the 2 yr in our study (Fig. 2) may be explained by these environmental factors. In 1985–1986, a colder fall and winter and a drier spring in the Palouse region may have accounted for the slightly later maturation of pseudothecia and the longer duration of the ascospore discharge period, respectively.

The various fungal and environmental factors influencing teleomorph development on chickpea debris in our study may account for the unsuccessful attempts to produce the sexual state under controlled conditions (8,10,16) and for the rare occurrence of the teleomorph in many chickpea-growing regions of the world (8, 15). However, the development of *D. rabiei* on chickpea debris indicated its adaptation, shown by other members of Ascomycotina (13), to winter conditions of low temperature, possibly low light, and sporadic humidity. Therefore, the teleomorph may be important in other regions of the world where the climate between chickpea crops is cool and moist.

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