Effects of Inoculum Density and Temperature on Root Rot and Wilt of Chickpea

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ABSTRACT

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A large-seeded kabuli chickpea (cv. Burpee 5043) and a small-seeded desi chickpea (cv. JG-62) were grown at different temperatures (10, 15, 20, 25, and 30 C) with a range of inoculum densities of four root pathogens. Root rot or wilt increased with increased inoculum levels of *Fusarium solani* f. sp. *pisi*, *F. oxysporum* f. sp. *ciceris*, *Pythium ultimum*, and *Thielaviopsis basicola*. *F. o. ciceris* was equally pathogenic to both cultivars, and wilt severity did not increase with increased inoculum levels of 10^4 or 10^5 microconidia and macroconidia per milliliter. However, wilt symptoms were less severe at 10, 15, and 20 C than at 25 and 30 C. Similarly, *F. s. pisi* caused the most root and hypocotyl necrosis on both cultivars at 30 C. At 10, 15, or 20 C, however, the pathogen caused very little disease on cultivar JG-62. Thielaviopsis and Fusarium root rots were most severe when plants were grown at 30 C and exposed to 5,000 cfu/g of *T. basicola* and *F. s. pisi*.

Farmers in the Palouse region of eastern Washington and northern Idaho have recently included chickpeas (*Cicer arietinum* L.) in rotations with peas (*Pisum sativum* L.) and winter cereals (10,11). Numerous soilborne fungal pathogens infect pea roots in the Palouse, including *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (F.R. Jones) W.C. Snyder & H.N. Hans., *Pythium ultimum* Trow, and *Thielaviopsis basicola* (Berk. & Broome) Ferraris (1,3,10,14,15). Previous research has shown that these same pathogens infect chickpea roots (2,3,10, 11,13,18,19,27). Fusarium wilt of chickpea, caused by *F. oxysporum* Schlechtend.:Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato, occurs in California (25,28) but has not been reported in the Pacific Northwest.

The severity of root rot and wilt of peas is directly related to inoculum density and environmental stress (1,4,14,15). The annual variation in root disease severity of chickpeas is often attributed to differences in temperature and inoculum density (8,28). Yield losses in chickpeas due to soilborne diseases are severe in Pakistan and India when moisture is scarce and day temperatures are high (8,21,27). Chickpea culture in the western United States is profitable, but production is threatened by the pathogens named above. Our objectives were to study the effects of inoculum levels at varying temperatures on severity of root rot and wilt in chickpea. A portion of this research was reported earlier (2).

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MATERIALS AND METHODS

Virulent isolates of *F. s. pisi* (F54) and *P. ultimum* (P-8) and an isolate of *T. basicola* recovered from Palouse soil were used in all tests and are maintained by the second author. *F. o. ciceris* was isolated from roots of wilted chickpea plants collected by W. J. Kaiser from California. All four fungal pathogens used in these experiments were maintained in autoclaved dry soil stored at 4 C.

To produce primary inoculum, 20 mg of infested stock soil of each pathogen was sprinkled on selective medium. Agar plugs (6 mm in diameter) were removed from the margins of each colony, aseptically transferred to a multiplication medium, and incubated with the appropriate temperature and light requirements (Table 1). Microconidia and macroconidia of each *Fusarium* spp. were incorporated into soil and converted to chlamydospores as described previously (15). Chlamydospores of *T. basicola* were prepared (24) and endoconidia were removed by centrifugation; residual mycelium was removed by straining through four layers of cheesecloth. Chlamydospore inoculum was then added to soil. Oospores and sporangia of *P. ultimum* were prepared and separated from mycelium according to previous reports (5,9), then added to soil.

Test soil was passed through a 3-mm screen, air-dried, and stored at room temperature. Before infestation with each pathogen, test and control soil was treated with methyl bromide (1.0 kg/m^3 of soil). Numbers of colony-forming units per gram of soil of each pathogen were determined using appropriate selective media and dilution plate procedures (20,22,26). The inoculum levels of each pathogen were adjusted by adding noninfested soil. For all infested soil studies, a Moxee silt loam soil (39% sand, 57% silt, and 3.6% clay) with a pH of 6.9 was used. All infested soil was air-dried again, then stored at 4 C.

All test seed was stored at 5 C and 45% RH. All tests were conducted in a controlled environment chamber programmed with a 14-hr light period with 350 $\mu E \cdot m^{-2} \cdot s^{-1}$ of illumination. For all tests, seed was surface-disinfested with a 0.53% NaOCl solution before being planted in soil or coarse-grade perlite. During all tests, pots were watered as necessary with filtered tap water (0.45-μm filter) to avoid contamination.

Effect of pruning and inoculum concentration. Conidial inoculum of *F. o. ciceris* and *F. s. pisi*, chlamydospore inoculum of *T. basicola*, and oospore and sporangia inoculum of *P. ultimum* were adjusted to each test concentration by means of hemacytometer counts.

Seeds of JG-62 (small-seeded desi) and Burpee 5043 (large-seeded kabuli) were surace-disinfested and planted in coarse-grade perlite in 11-cm-diameter plastic pots. Ten-day-old seedlings were removed from the perlite, and the distal one-third of each root was pruned or left as a control while immersed in a 0, 10^2, 10^3, 10^4, or 10^5 conidial suspension of *F. oxysporum* or *F. solani* or a chlamydospore suspension of *T. basicola*. An inoculum concentration of 0, 100, 500, 750, and 1,000 oospores per milliliter was used for *P. ultimum*. Control seedlings were dipped and pruned in sterile water for 5 min. Seedlings were then transplanted into perlite in 11-cm-diameter plastic pots, then incubated for 25 days at 20 ± 3 C. This experiment had three replications of five seedlings per replication and was repeated. The experimental design was a split-split block.

Soil inoculum levels and varying soil temperatures. Fumigated soil was mixed with artificially infested soil to produce

Table 1. Media and environmental conditions used for isolation, enumeration, and multiplication of four chickpea root pathogens

Pathogen	Isolation and enumeration		Primary inoculum production	
	Medium (ref.)	Environmental conditions	Medium (ref.)	Environmental conditions
Fusarium solani f. sp. *pisi*	PCNB (22)	5-day incubation, room temperature, 24-hr fluorescent light	Kerr (12)	6-day rotary shaker (120 cycles/min), 25 C
F. oxysporum f. sp. *ciceris*	PCNB (22)	5-day incubation, room temperature, 24-hr fluorescent light	Kerr (12)	6-day rotary shaker (120 cycles/min), 24-hr fluorescent light
Pythium ultimum	Mircetich (20)	2-day incubation, room temperature	Oatmeal (9)	3- to 4-wk incubation, room temperature, 24-hr dark
Thielaviopsis basicola	TB-CEN (26)	10-day incubation, room temperature, 12-hr light/12-hr dark	Carrot PDA (17)	21-day incubation, room temperature, 12-hr light/12-hr dark

inoculum levels of 0, 100, 500, 1,000, and 5,000 propagules per gram of air-dried soil for each pathogen. Each inoculum level and pathogen treatment consisted of four replications with five plants per replication. Each 11-cm-diameter pot was filled with 650 g of test soil and placed in a controlled-environment room set at 10, 15, 20, 25, or 30 ± 2 C. There were five temperatures × five inoculum levels × four pathogens in a randomized complete block design. The experiment was repeated with three temperatures (20, 25, and 30 ± 2 C), four inoculum levels (100, 500, 1,000, and 5,000 cfu per gram of air-dried soil), and three pathogens (*F. o. ciceris, F. s. pisi*, and *T. basicola*). *P. ultimum* was not included in this study. All test plants were grown for 30 days after emergence (when 90% of test plants had emerged).

At the conclusion of each test, severity of disease caused by each pathogen was determined according to a 1–9 scale (Table 2) in which 1 = a healthy plant and 9 = a dead plant.

Table 2. Disease severity rating scale

Rating[a]	Wilt	Root rot
1	No visible symptoms	No visible symptoms
3	Very few discolored leaves (less than 10%); limited discoloration of root tissue	Small lesions on taproot or secondary roots; less than 10% of root and hypocotyl tissue covered with lesions
5	Approximately 11–25% of leaves and branches show chlorosis; small lesions on roots with slight vascular discoloration	Moderate discoloration of crown or taproot; approximately 11–25% of root and hypocotyl tissue covered with lesions
7	Approximately 26–50% of leaves and branches show wilting, chlorosis, and limited necrosis; plants stunted; vascular discoloration more prominent	Lesions coalesce to form large lesions; approximately 26–50% of root and hypocotyl tissue covered with lesions; considerable softening and rotting of root system
9	More than 51% of leaves and branches show wilting, chlorosis, and necrosis; extensive vascular discoloration; plant death	Approximately 51–100% of crown and root tissue discolored; rotting and reduction of root system

[a] Numbers 2, 4, 6, and 8 were assigned to plants showing symptoms between the appropriate odd number ratings.

Statistical analysis. All experiments were repeated twice. Because the results among the trials were similar as determined by a test of homogeneity of variances (7), the data were combined and averages were analyzed using factorial analysis of variance (MSTAT Statistical Package). Linear and multiple linear regression analyses for the fungal pathogens were also computed to determine the effects of soil inoculum level and temperature on the severity of root rot and wilt. Homogeneity of regression coefficients was tested to compare the slopes using a previously described procedure (7). In all regressions, lack of fit was tested to ensure that the postulated model reasonably approximated the functional form of data.

RESULTS

Effects of root pruning and inoculum levels. Root pruning before inoculation did not increase disease severity with any of the four pathogens (*data not presented*). Root rot and wilt severity increased, and plant height and biomass decreased, with an increase in inoculum density of all four pathogens. There was a significant ($P = 0.05$) interaction between cultivars and fungal pathogens. Disease caused by *P. ultimum* was more severe on cv. Burpee 5043 than on cv. JG-62 (Fig. 1A). Seedlings of JG-62 inoculated with a suspension of 1×10^3 oospores of *P. ultimum* showed moderate root rot (disease index = 4) but no visible aboveground symptoms. In contrast, stunted growth and severe root rot (disease index = 7) occurred when Burpee 5043 was exposed to the same inoculum concentration of *P. ultimum*. In addition, root rot severity increased significantly ($P = 0.05$) on Burpee 5043 plants with each increase in level of *P. ultimum* inoculum. The relationship between inoculum concentration of *P. ultimum* and disease incidence was linear. However, the slopes of regression for cultivars JG-62 and Burpee 5043 were significantly ($P > 0.05$) different.

Severity of root rot disease due to *T. basicola* leveled off at an inoculum concentration of 10^5 chlamydospores per milliliter for Burpee 5043 and continued to increase for JG-62 (Fig. 1B). In contrast, severity of root rot caused by *F. s. pisi* increased for both cultivars when they were exposed to inoculum concentrations of 10^4 and 10^5 microconidia and macroconidia per milliliter (Fig. 1C). Nonetheless, Burpee 5043 showed more root rot symptoms (disease index = 3) at an inoculum concentration of 10^2 microconidia and macroconidia per milliliter than did JG-62 (disease index = 2.3). The severity of root rot at all inoculum levels was greater on Burpee 5043 than on JG-62. The relationship between disease severity and inoculum concentration of *T. basicola* and *F. s. pisi* was best described by the known (7) logarithmic model ($Y = a + blnx$). The tests for homogeneity of regression coefficients indicated that the slopes of the two pathogens for Burpee 5043 and JG-62 did not differ.

The wilt pathogen, *F. o. ciceris*, was equally pathogenic to both chickpea cultivars, and severity of wilt increased as inoculum level increased (Fig. 1D). The slopes and intercepts of regressions did not differ for the cultivars, and the logarithmic model best explained the relationship between wilt severity and inoculum level.

Soil temperatures and inoculum levels. The incidence of root rot and wilt in-

creased in a linear ($P < 0.05$) manner with increasing inoculum level and temperature. In addition, there was a significant interaction ($P < 0.05$) between inoculum level and temperature.

At 10 C, no disease symptoms were obvious on plants grown in soil infested with *F. o. ciceris* and *F. s. pisi*, even at the highest inoculum density (Fig. 2). However, each pathogen was isolated from surface-disinfested roots at harvest. Disease was expressed as small black lesions on taproots of plants grown at 10 C with 500–5,000 propagules per gram of *T. basicola*.

At 25 or 30 C, *F. o. ciceris* caused severe wilt symptoms (disease index = 4–9) at all inoculum levels (Fig. 2A). Partial regression coefficients determined by multiple regression analysis showed that temperature accounted for 60% of the variability in wilt severity and inoculum level accounted for only 14% ($R^2 = 0.71$). Slope values of disease progress for each level of inoculum were significantly ($P > 0.05$) different.

F. s. pisi caused very little root rot and hypocotyl necrosis (disease index = 3.1–4.3) at 15 or 20 C over an inoculum range of 100–5,000 propagules per gram. However, disease indices were significantly higher (5.4–8.7) at 25 or 30 C. In fact, there was a linear relationship for disease severity between inoculum density of *F. s. pisi* and temperature (Fig. 2B). Partial coefficients of determination revealed that temperature accounted for 92% of the variability in root rot severity and inoculum density accounted for only 2% ($R^2 = 0.94$). The slopes of regression lines for the four inoculum levels of *F. s. pisi* did not differ ($P = 0.05$).

At 15 C, plants grown in soil infested with 100–5,000 propagules of *T. basicola* per gram of soil showed only mild symptoms (disease index = 3.0–3.8) on roots. At 20 C, *T. basicola* caused small black lesions on the roots at 100 and 500 propagules per gram (disease index = 3.7 and 4.3, respectively). At 1,000 and 5,000 propagules per gram, these lesions coalesced, with a corresponding increase in disease severity (disease index = 5.4 and 5.7, respectively). Aboveground symptoms of Thielaviopsis root rot were not evident at 15 and 20 C. However, at 30 C and with an inoculum level of 5,000 propagules per gram, the pathogen caused severe root rot (disease index = 6.0–7.5) and stunted growth of chickpea plants (Fig. 2C). Slope values of disease progress for the four inoculum levels differed significantly ($P > 0.05$). Temperature accounted for 60% of the variability in disease severity and inoculum density accounted for only 9%, as determined by multiple regression analysis ($R^2 = 0.70$).

DISCUSSION

Both chickpea cultivars were susceptible to wilt caused by *F. o. ciceris*. However, the large-seeded kabuli (Burpee 5043) was more susceptible to Fusarium, Thielaviopsis, and Pythium root rot than the small, dark-seeded desi (JG-62) (Fig. 1A, B, and C). This is in agreement with previous reports (3,10,11). Desi types predominate in semiarid countries, where 97% of the world's total chickpeas are produced. Pythium and Thielaviopsis root rot have not been reported as a production problem in these countries. In our studies, root rot and wilt increased in both cultivars with increased inoculum concentrations of each pathogen.

Temperature and inoculum levels had a direct effect on the incidence of root rot and wilt of chickpea. Partial coefficients of determination (R^2) of 0.57, 0.60, and 0.92 for *T. basicola, F. o. ciceris*, and *F. s. pisi*, respectively, indicate that temperature accounted for a moderately high proportion of the variability of disease incidence.

Severity of root rot caused by *F. s. pisi* was positively correlated with increasing temperatures and inoculum densities. At 20 C, which is near optimum for chickpea growth, *F. s. pisi* infected the cotyledonary attachment area but not the root system (disease index = 3.5–4.3) over the range of inoculum levels tested.

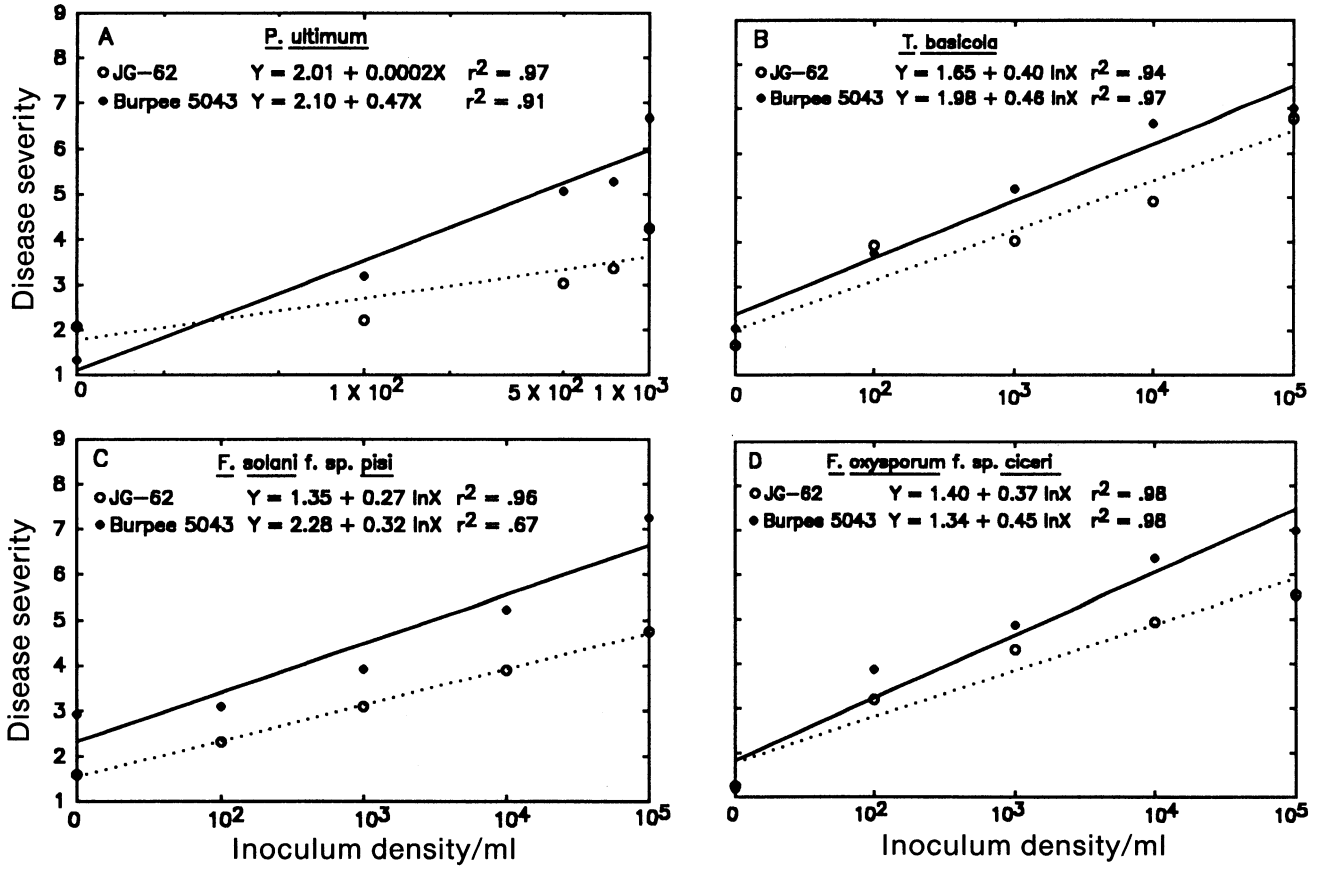

Fig. 1. Effects of different inoculum densities of **(A)** *Pythium ultimum*, **(B)** *Thielaviopsis basicola*, **(C)** *Fusarium solani* f. sp. *pisi*, and **(D)** *F. oxysporum* f. sp. *ciceris* on severity of root rot or wilt of a kabuli (Burpee 5043) or desi (JG-62) chickpea cultivar.

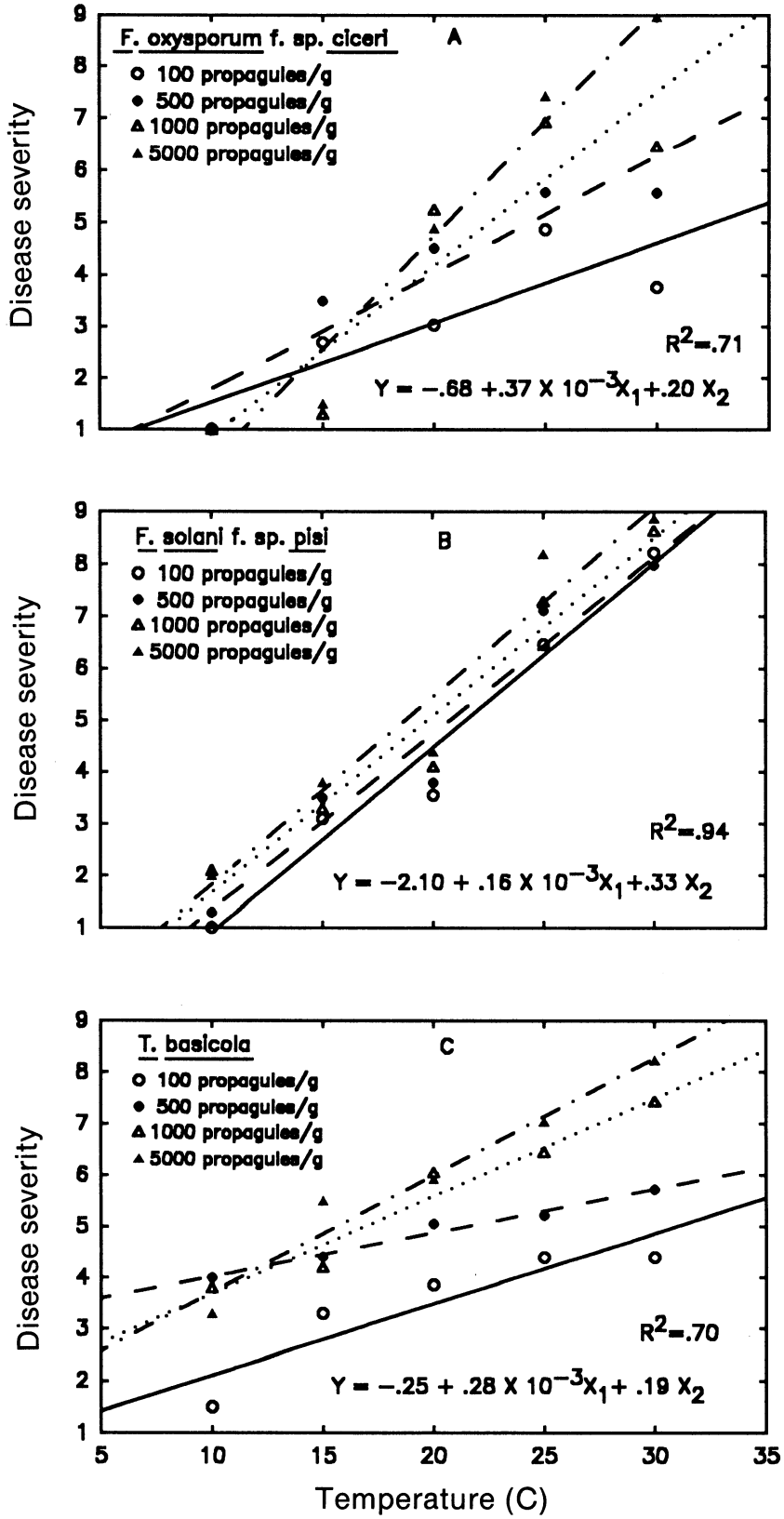

Fig. 2. Effects of different temperatures and inoculum densities on severity of disease in chickpea caused by **(A)** *Fusarium oxysporum* f. sp. *ciceris*, **(B)** *F. solani* f. sp. *pisi*, and **(C)** *Thielaviopsis basicola*.

However, this pathogen caused severe root rot (disease index = 8.1) at 30 C even at the lowest inoculum density. These findings support earlier work that the severity of disease caused by *F. s. pisi* depends on the environment in which the host plant is grown (1,2,14,15). Previous reports from India (18,27) and Chile (19) did not define which forma specialis was the cause of Fusarium root rot of chickpea. It is important to determine if the pathogen was *F. s. pisi*, since peas are grown in these countries and *F. s. pisi* may be present (14).

Our finding that the most severe (disease index = 9) wilt of chickpea occurred at 30 C with an inoculum level of 5,000 propagules per gram differs from the report of Gupta et al (8). They reported that wilt severity was maximum at 25 C and significantly decreased at 30 C, but they did not clearly define the level of *F. o. ciceris*. Our results agree with those of Nyvall and Haglund (23), who found that pea wilt, caused by *F. o. pisi* race 5, increased with increasing inoculum levels.

Black root rot, caused by *T. basicola*, was most severe at 30 C, which is above the optimum for chickpea growth (2). Lloyd and Lockwood (17) reported similar results with Thielaviopsis root rot of peas. Earlier reports (6,24) stated that *T. basicola* is most virulent on plants grown under adverse conditions, and our results confirm this.

The results of our study indicate that pathogenicity of *F. s. pisi, F. o. ciceris*, and *T. basicola* depends on temperature and inoculum levels. Leach (16) reported that disease severity is influenced by temperature effects on the relative growth rates of the host and pathogen. Our findings agree with those of Leach (16).

In the Palouse region of eastern Washington and northern Idaho, chickpeas are now grown in areas that have a long history of short rotations with peas and corresponding pea root diseases. These pathogens, with the exception of Fusarium wilt, also attack chickpeas. Growers who wish to produce chickpeas in the Palouse should be aware of this and should avoid fields where pea root rot has been severe.

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