

Root Rot of Chickpeas and Lentils Caused by *Thielaviopsis basicola*

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ABSTRACT

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Surveys of pulse fields in northern Idaho and eastern Washington in 1984 showed that chickpeas (*Cicer arietinum*) often bore severe black root rot symptoms. Lentils (*Lens culinaris*) typically bore similar root rot symptoms, except they were less severe than on chickpeas. *Thielaviopsis basicola* was isolated from 58 and 13%, respectively, of chickpea and lentil root pieces. Both species were susceptible to infection by *T. basicola* in greenhouse tests, and the pathogen was successfully reisolated. Chickpeas were more susceptible to infection than lentils or peas (*Pisum sativum*). No differences in pathogenicity were detected among six cultures of *T. basicola* originally isolated from peas and chickpeas. Sources of partial resistance were found in breeding lines of chickpeas and lentils. This is the first report of root rot of chickpeas and lentils caused by *T. basicola* under field conditions.

Additional key words: *Fusarium avenaceum*, *F. oxysporum*, *F. solani*, *Phoma medicaginis* var. *pinodella*, *Pythium* spp.

In recent years, farmers in the Palouse region of eastern Washington and northern Idaho have been encouraged to include chickpeas (*Cicer arietinum* L.) and/or lentils (*Lens culinaris* Medik.) in rotations with winter wheat and peas (1,10). Chickpeas and lentils currently occupy about 2,200 and 44,000 ha, respectively, of the 0.8 million cultivated hectares in the Palouse. These alternate crops are compatible with farm machinery already used for small-grain production. Their inclusion in rotations increases periods between successive wheat crops and reduces the severity of diseases such as foot rot (*Pseudocercospora herpotrichoides*) and Cephalosporium stripe (*Cephalosporium gramineum*) in wheat. In addition, they reduce the requirement for nitrogen fertilizers and improve the control of grassy weeds. Markets for chickpeas and lentils are expected to be strong.

Thielaviopsis basicola (Berk. & Br.) Ferr. causes root rots of more than 90 plant species (3) including many legumes such as bean (*Phaseolus vulgaris* L.), alfalfa (*Medicago sativa* L.), soybean (*Glycine max* (L.) Merr.), and pea (*Pisum sativum* L.). Lentil was reported

susceptible in an uncontrolled test (3). Because *T. basicola* is commonly isolated from infected pea roots in the Palouse, we were interested in assessing whether it also posed a threat to the production of chickpeas and lentils. Our objectives were 1) to test the pathogenicity of Palouse isolates of *T. basicola* to chickpeas, lentils, and peas; 2) to compare the susceptibility of chickpeas, lentils, and peas at different inoculum concentrations; 3) to test breeding lines of chickpeas and lentils for resistance to *T. basicola*; and 4) to determine if *T. basicola* causes root rot of chickpeas and lentils in the field. A preliminary report of this research has been published (2).

MATERIALS AND METHODS

Pathogenicity of isolates. Five isolates of *T. basicola* were collected from field-grown pea roots and one was collected from chickpea. Monoconidial cultures were grown on Difco potato-dextrose agar (PDA) at 21–24 C for 2 wk. Endoconidia were rinsed from plates with sterile distilled water (SDW). Conidial concentrations were measured with a hemacytometer, then mixed with steam-sterilized soil mix (sand:peat, 1:1, v/v; bulk density = 0.83) at the rate of 10^5 conidia per milliliter of soil. A second portion of the same soil mix, used as a control, received an equivalent volume of SDW but no conidia.

Ten surface-disinfested (0.525% HOCl, 5 min) seeds of chickpea cultivar UC-5, lentil cultivar Chilean 78, and pea cultivar Alaska were sown in flats 25 × 25 × 7 cm containing soil mix that was uninfested or

infested with one of the isolates. After sowing, both inoculated and control flats were topped with 1 cm of steam-sterilized sand. After emergence, seedlings were thinned to four of each species per flat. Plants were grown in the greenhouse at 20 ± 5 C in natural light with photoperiods ranging from 12 to 17 hr. Flats were watered to saturation initially and whenever soil moisture tensions were less than -0.5 bar as measured with a tensiometer (Soil Moisture Equipment Corp., Santa Barbara, CA). After 4 wk, roots were removed, washed, and visually scored for percent necrosis of the root epidermis and cortex. Diseased 1-cm root sections of each host were plated on 2% water agar (WA) or APDA (3.9 g of PDA, 12 g of agar, and 1 L of distilled water, acidified with lactic acid to pH 5.0).

The experiment was run twice with two replicates and was analyzed as a split-split-plot design with time as main plot, isolate as subplot, and species as sub-subplot by the Statistical Analysis System (13). Root rot scores were given an arc sine square root transformation to improve homogeneity of variance and retransformed for presentation.

Effect of inoculum dose. Methods were as described, with the following exceptions: Inoculum of three isolates was bulked and added to steamed soil mix at the rate of 10^2 , 10^3 , and 10^4 endoconidia per milliliter of soil. Each treatment combination was grown in individual 10-cm pots. After 4 wk, plants were removed from pots, washed, scored for root necrosis, dried at 40 C for 24 hr, and weighed. The treatments were replicated three times and analyzed as a completely randomized factorial design. Means were separated with a protected LSD (8).

Varietal resistance. Twelve lines each of chickpeas and lentils (Tables 1 and 2) were screened for resistance to *T. basicola* by exposure to bulked inoculum of three isolates mixed with steamed soil mix at 3×10^4 conidia per milliliter. Plants were grown in flats 54 × 27 × 7 cm. Identical sets of plants were grown in uninfested soil as controls for calculating percent weight loss. The experiment was run twice with two replicates and was analyzed as a split plot with time as main plot and genotype as subplot.

Field survey. Between 10 and 30 July 1984, when plants were in the pod-filling

Table 1. Responses of chickpeas to *Thielaviopsis basicola*

Cultivar or line	Type ^w	Necrosis (%) ^x		Weight loss ^y (%)
		Epicotyl	Total ^z	
85-24	K	97.4 a	94.4 a	75.0 a
Lyons	K	96.4 a	94.1 a	72.6 ab
SVM1-G	K,G	90.9 a	92.3 ab	61.7 ab
Surutato	K,G	97.4 a	88.8 ab	62.2 ab
UC-5	K,G	98.0 a	88.1 ab	55.1 ab
Mission	K,G	93.2 a	85.8 ab	43.7 abcd
Beauregard	K,G	90.3 a	85.8 ab	51.1 abc
ICCC 4	D	56.2 b	80.7 bc	12.3 cde
PI 273879	D	40.0 b	70.2 cd	31.7 bcde
IC 7519	K	50.9 b	67.3 cd	11.7 cde
Aztec	D	29.9 b	66.2 cd	-2.4 e
CP 13	D	36.9 b	58.8 d	6.0 de

^wK = kabuli, D = desi, and G = garbanzo.

^xVisual estimate of percent necrosis of root epidermis and cortex. Means within columns followed by same letter are not significantly different ($P = 0.05$) according to protected LSD. Necrosis values were given arc sine square root transformations for analysis, then retransformed for presentation.

^yPlant dry weight loss as percent of uninoculated control.

^zRoot plus epicotyl.



Fig. 1. Chickpea cultivar Lyons (left) inoculated with *Thielaviopsis basicola* and (right) uninoculated.

stage, roots of five to 10 plants in each of 10 chickpea fields and five lentil fields were collected to a depth of 20–25 cm. Roots were washed and visually scored for percent necrosis. Three to five 1-cm pieces of diseased secondary roots from each field were examined microscopically for the presence of *T. basicola* chlamydospores (aleuriospores). Five to 20 1-cm root sections per field were plated on each of WA, APDA, *Fusarium*-selective medium (MPP) (12), and *Pythium*-selective medium (MPVM) (9). A weighted mean was calculated for isolation frequencies so that each location contributed equally.

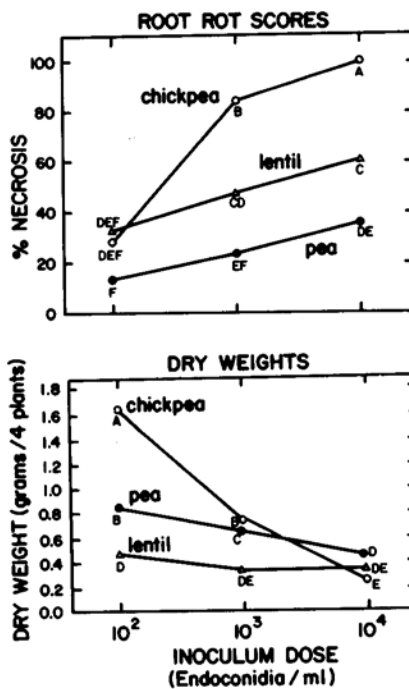


Fig. 2. Effects of *Thielaviopsis basicola* inoculum dose on chickpeas, lentils, and peas. Means followed by the same letter are not significantly different ($P = 0.05$) according to protected LSD.

RESULTS

Pathogenicity of isolates. After 4 wk in the greenhouse, chickpeas were stunted and/or wilted by all *T. basicola* isolates. The epicotyl was most severely affected and was black or gray and collapsed. Early symptoms on roots consisted of coalescing short black streaks. Eventually, roots were entirely blackened (Fig. 1). Peas were stunted; epicotyls were gray and roots had short linear black or gray lesions. In contrast, lentil roots had diffuse reddish brown streaks and top growth was not visibly affected. Unlike peas and chickpeas, lentil epicotyls were highly resistant to attack. Control plants

Table 2. Responses of lentils to *Thielaviopsis basicola*

Cultivar or line	Necrosis (%) ^x		Weight loss ^y (%)
	Epicotyl	Total ^z	
Brewer	1.7 a	73.5 a	44.9 a
Chilean 78	1.6 a	72.5 a	36.8 ab
Red Chief	1.9 a	68.8 a	43.6 a
Constitution	1.3 a	67.0 ab	26.3 ab
Eston	0.0 a	64.8 ab	14.2 ab
Araucarian 1223	0.0 a	62.3 ab	-9.9 bc
Ply	0.0 a	57.9 abc	28.2 ab
Tekoa	0.0 a	57.0 abc	-2.5 ab
Araucarian 1121	0.3 a	43.2 bcd	-11.4 bc
Laird	0.4 a	37.9 cd	-7.0 bc
Indian Head	0.0 a	34.5 cd	-53.7 c
Penn 14	0.1 a	26.0 d	24.2 ab

^xVisual estimate of percent necrosis of root epidermis and cortex. Means within columns followed by same letter are not significantly different ($P = 0.05$) according to protected LSD. Necrosis values were given arc sine square root transformations for analysis, then retransformed for presentation.

^yPlant dry weight loss as percent of uninoculated control.

^zRoot plus epicotyl.

remained symptomless and *T. basicola* was consistently reisolated from diseased tissue, thus completing Koch's postulates.

No significant differences were detected between the isolates tested, and there was no significant isolate \times species effect. A significant species effect, however, was found and investigated further in the inoculum dose experiment.

Effect of inoculum dose. There was a highly significant interaction between host species and inoculum dose for both root necrosis scores and dry weights. At 10^2 endoconidia per milliliter, root rot scores of the three species could not be differentiated (Fig. 2). At 10^3 or 10^4 endoconidia per milliliter, chickpeas had the highest scores, lentils were intermediate, and peas had the lowest scores. When inoculum dose increased from 10^2 to 10^4 , however, dry weight was significantly decreased in chickpeas and peas but not in lentils.

Varietal resistance. Significant differences in susceptibility to *T. basicola* were found among chickpea lines (Table 1). Single degree-of-freedom contrasts showed that epicotyls and roots of white-seeded kabuli-type chickpeas were significantly ($P < 0.0001$) more susceptible than those of dark-seeded desi types. An exception was line IC 7519, a small-seeded kabuli, which was as resistant as the desi types.

All lentil lines had highly resistant epicotyls, but none had highly resistant roots (Table 2). Among local commercial cultivars, Laird was less susceptible than Brewer or Chilean 78. The apparent increase in weight of infected Indian Head may have been an experimental error or may have been caused by the genetic variability in this line.

Field survey. Root rot severity (percent necrosis) in chickpea fields ranged from 1

to 50% and averaged 16.2%. Root rot symptoms were black, coalescing, narrow lesions in the epidermis and cortex that usually contained *T. basicola* chlamydospores. *T. basicola* was detected in roots from six of 10 chickpea fields. Fields where *T. basicola* was detected had average root rot scores of 24%, whereas fields without *T. basicola* had an average root rot score of 4.8%. The difference between means was significant at $P = 0.065$ (t test). Isolation frequencies (percentage of tissue pieces yielding fungus) from diseased chickpea roots were *T. basicola* (58%), *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyder & Hans. (42%), *Phoma medicaginis* var. *pinodella* (Jones) Boerema (36%), *F. oxysporum* Schlecht. emend. Snyder & Hans. (31%), *F. avenaceum* (Fr.) Sacc. (4%), and *Pythium* spp. (2%). Lesion nematodes (*Pratylenchus* sp.) were occasionally found in diseased roots.

Root rot severity in lentil fields ranged from 1 to 5% and averaged 3%. Root rot symptoms were diffuse reddish brown lesions that sometimes contained chlamydospores of *T. basicola*. *T. basicola* was found in lentil roots from three of five fields. Isolation frequencies from diseased lentil roots were *F. oxysporum* (24%), *F. solani* (14%), *T. basicola* (13%), *Fusarium* sp. (probably *F. avenaceum*) (6%), *P. medicaginis* var. *pinodella* (4%), and *Pythium* spp. (1%).

DISCUSSION

This is the first report of *T. basicola* on chickpeas (11). It is unclear why *T. basicola* previously has not been reported on chickpeas, especially in Asia, where more than 90% of the crop is grown. Perhaps the environmental requirements of chickpeas and *T. basicola* rarely overlap. It is also possible that *T. basicola* infections are masked by other pathogens. Johnson (3) reported that lentils were susceptible to *T. basicola* in greenhouse studies, but he did not fulfill Koch's postulates. As with chickpeas, there has been no previous report of *T. basicola* attacking lentils in the field.

In this study, root rot symptoms in chickpea fields were visible on as much as 50% of the recovered root mass. Root rot therefore appears to be a significant

disease of chickpea in the Palouse. This root rot is apparently caused primarily by *T. basicola* because most symptoms were coalescing black streaks characteristic of *T. basicola* infection. In addition, high root rot severities were related to the presence of *T. basicola* in surveyed fields. Finally, *T. basicola* was the most frequently isolated pathogen from diseased chickpea roots.

Damage caused by *T. basicola* might be reduced by seeding desi instead of kabuli types. Unfortunately, American consumers prefer the large-seeded kabulis (garbanzos). Germ plasm is available (Table 1) for improving the resistance of kabuli types.

Root rot was not a significant disease of lentils in the fields surveyed, though in no-till situations it may be important (6). Although *T. basicola* was detected in field-grown lentil roots, it apparently has a limited ability to cause disease in lentils under local field conditions.

All three major pulse crops grown in the Palouse are susceptible to the native population of *T. basicola* in Palouse soils. Although a degree of host specificity is known to occur in *T. basicola* (7,14), no differential reactions were found between the hosts and isolates used in this study.

Chickpeas were the most susceptible to root rot and were often killed at higher inoculum doses. In contrast, peas and lentils were never killed. This qualitative difference in reaction to *T. basicola* may be related to the high susceptibility of epicotyls in certain chickpea cultivars. On the basis of symptoms, the greater susceptibility of lentils compared with peas is in agreement with Johnson (3). However, weight data indicated that lentils sustained less damage than peas and therefore may exhibit tolerance to *T. basicola*.

In addition to *T. basicola*, root rot of chickpeas and lentils can be caused by *F. solani* f. sp. *pisi*, *Rhizoctonia solani*, and *Pythium ultimum* (4,5,15). *F. avenaceum* and *Phoma medicaginis* var. *pinodella* are also root rot pathogens of lentils (4,6) and chickpeas (W. J. Kaiser, *personal communication*). All of these fungi except *F. avenaceum* are known to attack peas.

One of the primary benefits of crop

rotation is reduction of disease by preventing inoculum buildup. Increased use of legumes may help reduce disease severity in wheat. However, the ability of the resident complex of pathogens to colonize all three pulse crops in the Palouse may result in higher disease severity and lower yields of chickpeas, peas, and lentils.

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