Stomata as an Infection Court for *Phytophthora megasperma* f. sp. *medicaginis* in Chickpea and a Histological Study of Infection

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ABSTRACT

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Stomata located beneath the cotyledons of 7-day-old chickpea seedlings were the preferential infection court for zoospores of *Phytophthora megasperma* f. sp. *medicaginis*. Zoospores also accumulated preferentially in the root-hair zone. Penetration usually was intercellular between anticlinal epidermal cell walls, but intracellular penetration did occur via infection pegs produced from swollen germ-tube tips. Although no differences in prepenetration or penetration events were found between a field resistant (CPI56564) and a susceptible cultivar (ICC6334) of chickpea, hyphal growth rate of the fungus was greater through wounded roots of the susceptible cultivar than in the resistant cultivar. There was no significant difference between hyphal growth rates through wounded epicotyl tissue of the resistant and susceptible cultivars. Stomata located at or below the soil line could act as an infection court for zoospores present in surface water, and cultivars that express resistance in the root tissue would appear susceptible if the resistance was not also expressed in the hypocotyl and the lower epicotyl. A soil-line infection court also could be involved in spreading the disease via zoospores present in surface water in the field.

Additional keywords: *Cicer arietinum*.

Root rot of chickpea (*Cicer arietinum* L.), caused by *Phytophthora megasperma* Drechs. f. sp. *medicaginis* Kuan & Erwin, is the most important disease of chickpea in Queensland, Australia (6). The same organism also causes root rot of alfalfa (10). Current research is directed toward controlling root rot of chickpea through the use of resistant cultivars. So far, only one cultivar (CPI56564) with useful levels of resistance has been found, and even its resistance is overcome at high inoculum levels in the field and greenhouse (2,6). Because no genetical investigation of this host-parasite interaction has been reported previously, and because such terms as "partial resistance" and "general resistance" have caused confusion, we have used the term "field resistance" to indicate resistance that is expressed in the field but that is not readily apparent in the glasshouse or laboratory (22). In this interaction, resistance of CPI56564 is rate limiting under field conditions (M. L. Dale, *unpublished data*).

Histopathology of compatible and incompatible host-pathogen interactions can be useful in determining the sequence, location, and timing of resistance mechanisms. Beagle-Ristaino and Rissler (1), studying the histopathology of near-isogenic resistant and susceptible soybean roots inoculated with zoospores of *Phytophthora megasperma* f. sp. *glycinea*, reported that the race-specific resistance under study was effective during the colonization stage but that there was little difference in the pre- and early post-penetration events between cultivars. As far as we are aware, there are no such reports describing similar quantitative histological studies on field resistance to diseases of field crop legumes incited by species of *Phytophthora*. The process whereby zoospores of *P. m. medicaginis* infect chickpea roots has not been described previously.

Our objectives were to qualitatively and quantitatively describe the sequence of events involved in the pathogenesis of chickpea roots by zoospores of *P. m. medicaginis* and to examine the expression of field resistance through the quantification of pre- and postpenetration events in the infection process.

MATERIALS AND METHODS

To observe the infection process, both whole mounts and thin sections (2 μm) of chickpea seedlings infected by zoospores of *P. m. medicaginis* were examined and photographed. Quantitative studies of the prepenetration and penetration events were made on whole mounts only.

Inoculum production and inoculation. Zoospores of an isolate of *P. m. medicaginis* (UQ125) obtained from alfalfa, which was pathogenic to chickpea, were produced as described by Irwin (9). Chickpea seed of both a field resistant (CPI56564) and a highly susceptible (ICC 6334) cultivar (2) were germinated, and seedlings were grown for 5 days on moist paper toweling in covered trays. The seedlings then were placed upright in plastic mesh (16 mm^2) so that the taproots were suspended through the mesh into 500 ml of a zoospore suspension (5×10^3 zoospores/ml). Care was taken to ensure that the cotyledons came into contact with inoculum.

Sections and whole mounts. At each sampling time (2 and 4 hr for sectioning and 1, 1.5, and 2 hr for the whole mounts), three seedlings of each cultivar were removed, and the taproots were excised immediately below the cotyledons. Excised roots used for sectioning were fixed for a period of 24 hr in 50% FAA (90%, 50% ethanol; 5% formaldehyde; 5% acetic acid; v/v/v). For each seedling, a sample of the hypocotyl and a sample of the root hair region were taken. The tissue was washed and dehydrated through a graded ethanol series: 50% ethanol (1 hr), 70% ethanol (1 hr), 85% ethanol (2 hr), 95% ethanol (2 hr), 100% ethanol (2 hr), and a 50:50 ratio of 100% ethanol and LKB Historesin (LKB-Produkter AB, Bromma, Sweden) (overnight). The tissue was infiltrated for 5 days with LKB Historesin, with daily changes of infiltrating solution preceded by a short vacuum period. The tissue then was polymerized at room temperature in heat-sink molds. Resultant blocks were sectioned at 2 μm on an LKB Historange Microtome (LKB-Produkter AB), floated onto gelatin-coated slides, and placed on a 60 C hot plate for 30 min to firmly adhere sections to the glass slide.

Sections were stained for 1 min with 0.05% toluidine blue in benzoate buffer (benzoic acid, 0.25 g; sodium benzoate, 0.29 g;

water, 200 ml) at pH 4.4 (25), then rinsed in running water (7). A coverslip was applied, with water as the mounting medium. After the section was examined under the light microscope, the coverslip was removed, and the section was rinsed and then left to dry before being stored in a dust-free container.

Roots were stained and cleared by the technique of Shipton and Brown (24) for preparing whole leaves. Briefly, whole roots were boiled in alcoholic lactophenol cotton blue (one part lactophenol cotton blue to two parts 95% alcohol) for 2 min, allowed to cool, and boiled again for 1 min. Roots then were cleared in chloral hydrate (5 g of chloral hydrate to 2 ml of water) and examined microscopically after being split in half longitudinally, mounted in clear lactophenol, and squashed.

Quantitative data collection. Quantitative data were recorded from whole mounts. Three zones were designated: the region immediately behind the root cap (elongation zone), the region where root hairs were present (root-hair zone), and the region above the root-hair zone but below the cotyledons (hypocotyl). During preliminary observations, stomata were noted in the region extending from the aboveground shoot to the hypocotyl region below the soil line.

For three seedlings of each cultivar removed 2 hr after inoculation, three random fields of view under the ×40 objective of the microscope were examined for each root zone, and the number of encysted zoospores was counted. Data were converted to cysts per square millimeter; an eyepiece micrometer was used to determine the area of the field of view.

Data recorded from random observations of at least 50 encysted zoospores per section in the root-hair zone at each sampling time included number of germinated cysts, length of germ tube, direction of germ-tube growth relative to the longitudinal axis of the root (parallel or perpendicular), presence or absence of a swollen germ-tube tip, number of cysts that had penetrated the host, and number of inter- and intracellular penetrations.

These data were used to calculate the proportion of cysts germinated, mean lengths of germ tubes, the proportion of germ tubes growing either parallel or perpendicular to the long axis of the root, the proportion of germinated cysts that had penetrated the host, the proportion of germinated cysts that had swollen germ-tube tips, the proportion of penetrations from swollen germ-tube tips, and the proportion of inter- to intracellular penetrations (both overall and from swollen germ-tube tips).

For five seedlings of the cultivar CPI56564 sampled 2 hr after inoculation, the number of cysts penetrating stomatal guard cells, cells adjacent to guard cells, and epidermal cells in the hypocotyl region was recorded in two ×40 fields of view per section. One section was examined per seedling. The relative area of these three types of cells in the hypocotyl region was determined with an eyepiece grid on two sections, each from a seedling of the cultivar CPI56564 (five fields of view per section).

Fungal growth rate in epicotyl and root tissue. The growth rate of the fungus was measured in both the epicotyl and the root of the host. For both CPI56564 and ICC 6334, epicotyls of 7-day-old seedlings growing in peat-sand mix (three pots per cultivar, each containing 10 seedlings) were cut immediately below the first leaf, and a 5-mm-diameter agar plug taken from the edge of an 8-day-old V-8 agar culture of *P. m. medicaginis* was placed on each seedling and completely covered its cut surface (21). Pots were kept at 25 C in a closed container on moist paper toweling, and the length of the visible lesion was measured at 1, 2, and 3 days after inoculation.

To determine the growth of the fungus in the taproot, 7-day-old seedlings of cultivars CPI56564 and ICC 6334 grown in peat-sand mix were removed, washed, and arranged in trays on moist paper toweling. After the lateral roots were removed, root tips were excised to leave approximately 5 cm of taproot below the cotyledons, and the seedlings were inoculated on the cut surface with a block of agar containing the fungus, as for the epicotyl inoculation. The trays were covered, and the seedlings were incubated at 25 C. At 1, 2, 3, and 4 days after inoculation, three plants were removed, and roots were excised, stained, cleared, mounted as described for the whole mounts, and then examined microscopically. The distance from the point of inoculation to the farthest hyphal tip was determined in millimeters.

Statistical analyses. Data from the whole mounts were analyzed by simple linear regression, except for data describing the direction of germ-tube growth and the proportion of inter- to intracellular penetration, which were subjected to heterogeneity chi-square analyses. Relative proportions of cell types penetrated by encysted zoospores were analyzed by analysis of variance. Data were transformed to arcsine square roots where appropriate (30).

Results from both the epicotyl and root inoculation experiments were analyzed by simple linear regression, and the growth rates of the fungus in the two cultivars were compared by a t test.

A significance level of $P = 0.05$ was used for all statistical tests. All statistical analyses were performed with the GLIM computer program (Royal Statistical Society, London, England). All experiments were repeated at least once, with similar results.

RESULTS

Observations of the infection process. Zoospores of *P. m. medicaginis* accumulated and encysted on all parts of the roots except the root cap but mainly were attracted to the root-hair zone (Fig. 1). Cysts germinated to produce a single, usually simple, germ tube from a conical structure in the base of the cyst (Fig. 2). Entry into the host occurred after growth of the germ tube, which commonly was perpendicular to the longitudinal axis of the root. Penetration usually was intercellular, occurring between the anticlinal walls of two epidermal cells. Where penetration was intracellular, the germ tube generally became swollen and produced a penetration peg (Figs. 3–5).

After penetration, the infection hypha developed inter- and intracellularly within the cortex of the root and both parallel and perpendicular to the longitudinal axis of the root. Ramification hyphae appeared to swell before penetrating cell walls, constricting as they passed through the wall and regaining their normal diameter after they emerged. Once penetration had occurred, the cyst and germ tube became vacuolate.

In the hypocotyl region, zoospores generally accumulated and encysted around stomata. These stomata generally were similar to those observed on the aerial parts of the plant, although the shape and arrangement of cells surrounding the guard cells were different. The germ tube grew into the substomatal chamber, a septum then formed, and primary hyphae developed intercellulary (Fig. 6). The cyst and germ tube then became vacuolate. Each stomate usually was penetrated by germ tubes of several cysts; germ tubes of more than 15 cysts were observed entering one stomate.

Quantitative data. No statistically significant differences between the field resistant and susceptible cultivars were found for any of the prepenetration or penetration processes studied. Thus, data were averaged across cultivars.

Most zoospores accumulated and encysted in the root-hair region (55.7 cysts per square millimeter), with a few encysting in the elongation zone (8.3 cysts per square millimeter) and in the zone immediately above the root-hair zone but below the cotyledons (5.1 cysts per square millimeter). In the hypocotyl region, germ tubes from encysted zoospores penetrated through 73% of guard cells, compared with only 2% of cells adjacent to guard cells and 1% of other epidermal cells. Guard cells occupied less than 1% of the total surface area in the hypocotyl region, whereas cells adjacent to guard cells occupied 2.8% of the total surface area. In the root-hair zone and region of elongation, penetration of cells appeared random.

There was no consistent increase in germination of encysted zoospores in the root-hair zone detected beyond 1 hr after inoculation, when more than 90% of cysts had germinated. Growth of the germ tube was perpendicular to the long axis of the root in more than 90% of the cases. The average length of the germ tube increased significantly over the study period, from an average of 2.5 μm at 1 hr to more than 4.5 μm after 2 hr. The percentage of germ tubes that penetrated the host significantly increased during the study period to 81%, although by 1 hr after inoculation,

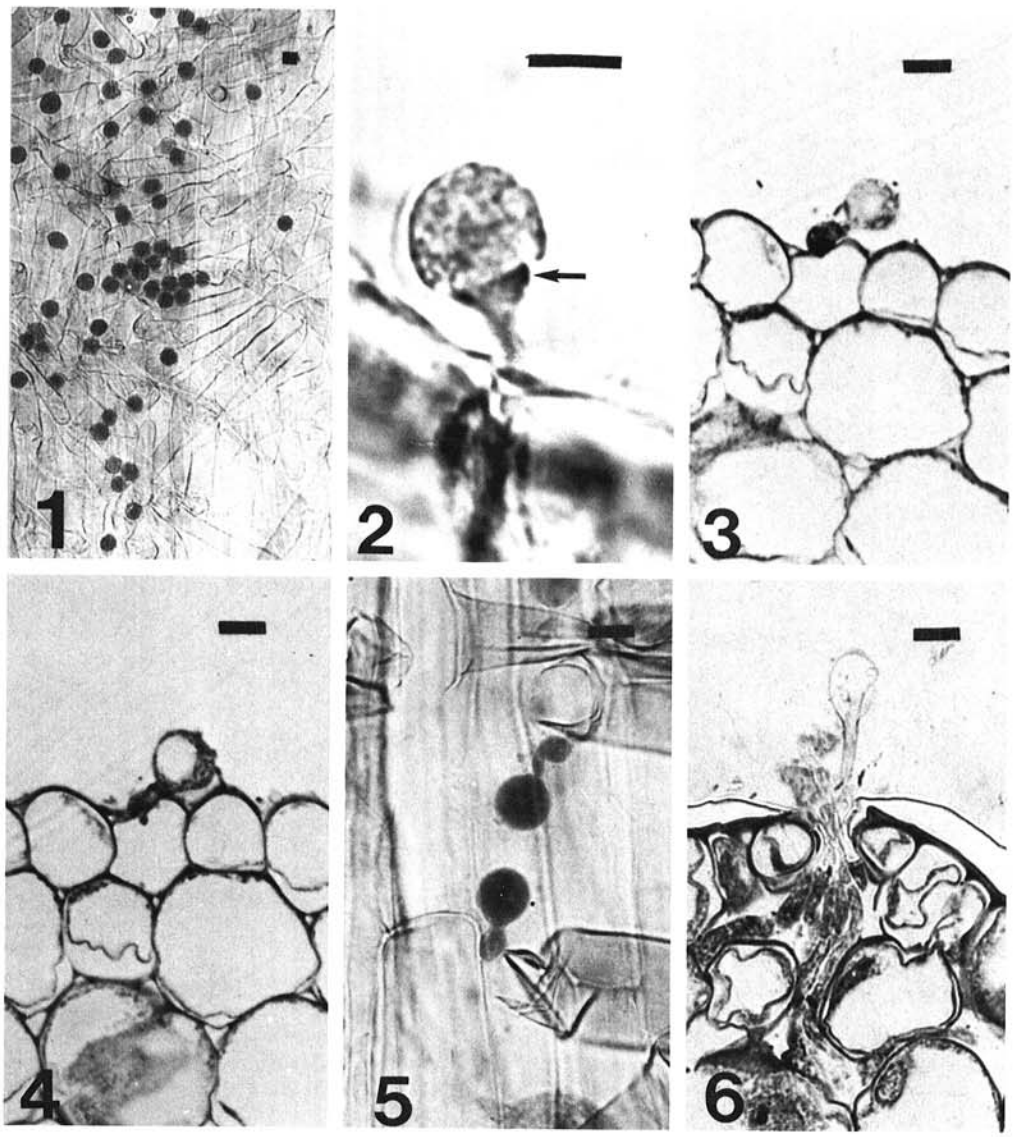
60% already had penetrated the host. There was a small but significant decline in the proportion of penetrations that were intercellular up to 2 hr after inoculation, although intercellular penetration remained the dominant mode of entry (>70%). This coincided with a significant increase in the percentage of penetrations via a penetration peg from swollen germ-tube tips, from 6.5 to 66.5% over the 2-hr period; most of the penetrations were intracellular (>70%). The proportion of encysted zoospores with swollen germ-tube tips significantly increased during the study period from 1 to just over 30% 2 hr after inoculation.

Fungal growth rate in epicotyl and root tissue. The growth rate of hyphae of *P. m. medicaginis* in the root tissue of cultivar CPI56564 (3.6 mm/day) was significantly lower than in cultivar ICC6334 (9.5 mm/day) (Fig. 7). There was no significant difference between the growth rates of *P. m. medicaginis* in the epicotyls of the two cultivars. The equation for the combined data of both cultivars from the regression of hyphal growth in the epicotyl on time was: lesion length (mm) = 5.8 × time (days) − 2.8 ($r^2 = 0.99$, significant at $P < 0.05$).

DISCUSSION

This appears to be the first report of stomata located beneath the soil line acting as preferential infection courts for a soilborne pythiaceous fungus. Zoospores of *P. m. medicaginis* accumulated around and their germ tubes successfully penetrated stomatal pores situated below the cotyledons of chickpea seedlings. Germination of chickpea seedlings is hypogeal, with the cotyledons remaining underground. Stomata on the aerial parts of potato plants have long been known to be infection courts for *Phytophthora infestans*, the cause of late blight (29).

Previous studies of infection courts for zoospores of *P. m. medicaginis* on alfalfa have included the region of elongation on the root (9,13), the junction of lateral and taproots (9), and wounds (5). The discovery of a soil-line infection court for *P. m. medicaginis* may have implications for the development of greenhouse resistance assays. Attempts to develop assays involving conditions where there is a continual supply of free water at or near the soil line could lead to zoospores being present in the



Figs. 1–6. Light micrographs of the infection process of chickpea roots and hypocotyls by zoospores of an isolate of *Phytophthora megasperma* f. sp. *medicaginis* (UQ125). **1,** Zoospore cysts accumulating in the root-hair zone of cultivar ICC6334. **2,** Zoospore cyst germ tube penetrating the root epidermis, showing conical structure (arrow) associated with production of the germ tube. **3 and 4,** Serial sections showing intracellular penetration of root epidermal cells of cultivar ICC6334 via an infection peg emerging from a swollen germ-tube tip produced by an encysted zoospore. **5,** Swollen germ-tube tips produced by encysted zoospores on the root-hair zone of cultivar CPI56564. **6,** Germ tubes from vacuolate zoospore cysts entering a stomatal pore in the hypocotyl region of cultivar CPI56564. Bars = 10 μm.

surface water, especially if the propagules were poured on top of the soil surface. Thus, cultivars that express resistance in the root tissue would appear susceptible if the resistance also were not expressed in the hypocotyl and the lower epicotyl. This may explain the unsuccessful attempt to develop a greenhouse resistance assay for chickpea seedlings with zoospores poured around the base of the plants under continually saturated soil conditions, which produced highly variable results (6). An assay developed with oospores incorporated into the soil and flooded periodically, however, was successful (6). Stossel et al (27) found that resistance of soybean hypocotyls to inoculation with zoospores of *P. m. glycinea* increased from the top (youngest) to the bottom (oldest) but related this expression of resistance to maturation of the tissue and possibly an increase in the production of glyceollin.

Zoospores move limited distances through soil, largely carried by moving water (15). Pfender et al (20) reported that zoospores of *P. megasperma* were able to migrate upward through soil but the distance moved depended on soil type. They concluded that only lesions near the soil surface would be a source of secondary inoculum, which could contaminate surface water. Zoospores of *P. megasperma* and other species of *Phytophthora* have been detected in irrigation water (14,28). Because *P. m. medicaginis* frequently produces lesions at the base of the chickpea stem, it is possible that the disease could spread via zoospores liberated from these lesions into surface water flowing over flooded fields and that these zoospores could reinfect neighboring chickpeas through stomata at or below the soil line, producing new lesions. This would account for the rapid spread of root rot, which often follows a watercourse through chickpea fields. In addition, organic matter colonized by the fungus could be brought to the surface by tilling, thereby enabling the liberation of zoospores into surface water during flooded conditions. There is growing epidemiological and biological evidence that some soilborne diseases are polycyclic (3,4,19,26). Direct observation of the infection process of plants in the field will be required to investigate these hypotheses.

The infection process involved the accumulation, encystment, and germination of zoospores on the root surface, followed by penetration after growth of the germ tube. Zoospores were attracted mainly to the root-hair zone, in contrast to reports of *P. m. medicaginis* on alfalfa and for other *Phytophthora* species, where zoospores generally were attracted to the region of elongation (8,13,15,16). Goode (8) reported that, although zoospores of *P. fragariae* accumulated in great numbers on the root-hair zone of strawberries, none were seen to penetrate. In the present study, more than 80% of cysts in the root-hair zone germinated and penetrated the host. In the majority of cases, the germ tube penetrated intercellularly, but intracellular penetration also occurred via an infection peg produced from a swollen germ-tube tip. Photomicrographs revealed that the germ-tube swellings described here appear identical to structures produced by other *Phytophthora* spp., which have been called appressoria by some previous authors (12,16–18). Beagle-Ristaino and Rissler (1) considered that these structures were best termed swollen germ-tube tips rather than appressoria because no investigation of their adherence was conducted. We also adopt this view. The presence of swollen germ-tube tips during the infection process has not been reported previously by authors studying the infection of alfalfa by *P. m. medicaginis*.

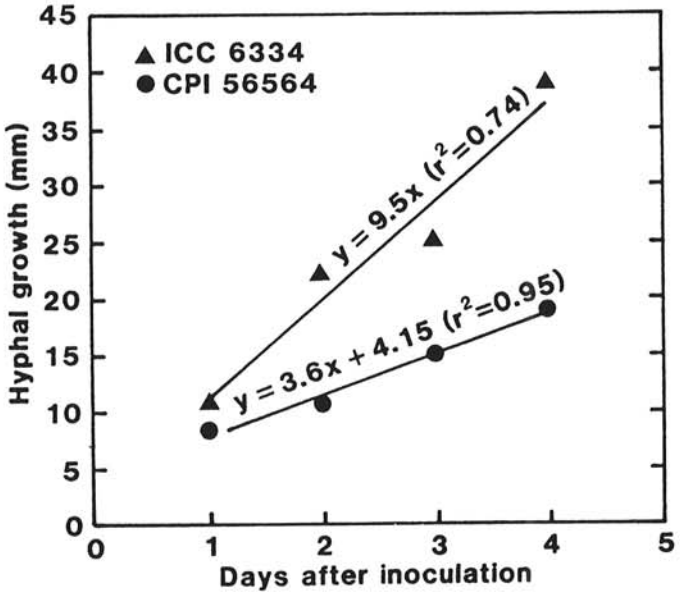


Fig. 7. Growth of hyphae of *Phytophthora megasperma* f. sp. *medicaginis* through roots of chickpea cultivars ICC6334 (susceptible) and CPI56564 (field resistant). The simple linear regression equations and coefficients of determination (r^2) were calculated with data from all replicates, but each point on the graph represents the mean of three replicates. The r^2 values were significant at $P < 0.05$.

Germ-tube swellings usually occurred when penetration was through rather than between cell walls, similar to the swelling of ramification hyphae before wall penetration. This phenomenon occurs with other species of *Phytophthora* (11,16,23).

Prepenetration and penetration events appeared similar for the field resistant and susceptible cultivars. The accumulation of zoospores was similar for both cultivars, as reported by Irwin (9) for *P. m. medicaginis* on alfalfa. Comparable results were obtained for the interaction of *P. m. glycinea* and soybean (1). The growth rate of hyphae of *P. m. medicaginis* from a block of agar through wounded roots was greater for the susceptible cultivar when compared with that in the field resistant cultivar, although there was no significant difference in the growth rate of hyphae through similarly wounded epicotyl tissue of the two cultivars. Resistance in this case apparently is activated during colonization and is expressed only in the root tissue. Work with *P. m. glycinea* in soybean roots also indicated that resistance was effective during colonization (1). Our study provides the basis for future biochemical and physiological investigations into the mechanisms of resistance to *P. m. medicaginis* in chickpea.

LITERATURE CITED

1. Beagle-Ristaino, J. E., and Rissler, J. F. 1983. Histopathology of susceptible and resistant soybean roots inoculated with zoospores of *Phytophthora megasperma* f. sp. *glycinea*. Phytopathology 73:590-595.
2. Brinsmead, R. B., Rettke, M. L., Irwin, J. A. G., Ryley, M. J., and Langdon, P. W. 1985. Resistance in chickpea to *Phytophthora megasperma* f. sp. *medicaginis*. Plant Dis. 69:504-506.
3. Campbell, C. L., Pennypacker, S. P., and Madden, L. V. 1980. Progression dynamics of hypocotyl rot of snapbean. Phytopathology 70:487-494.
4. Campbell, C. L., and Powell, N. T. 1980. Progression of diseases induced by soilborne pathogens: Tobacco black shank. Prot. Ecol. 2:177-182.
5. Chi, C. C., and Sabo, F. E. 1978. Chemotaxis of zoospores of *Phytophthora megasperma* to primary roots of alfalfa seedlings. Can. J. Bot. 56:795-800.
6. Dale, M. L., and Irwin, J. A. G. 1990. Estimation of inoculum potential of *Phytophthora megasperma* f. sp. *medicaginis* in chickpea fields and the development of a glasshouse resistance assay. Aust. J. Exp. Agric. 30:109-114.
7. Feder, N., and O'Brien, T. P. 1968. Plant microtechnique: Some principles and new methods. Am. J. Bot. 55:123-142.
8. Goode, P. M. 1956. Infection of strawberry roots by zoospores of *Phytophthora fragariae*. Trans. Br. Mycol. Soc. 39:367-377.
9. Irwin, J. A. G. 1976. Observations on the mode of infection of lucerne roots by *Phytophthora megasperma*. Aust. J. Bot. 24:447-451.
10. Irwin, J. A. G., and Dale, J. L. 1982. Relationships between *Phytophthora megasperma* isolates from chickpea, lucerne, and soybean. Aust. J. Bot. 30:199-201.
11. Klarman, W. L., and Corbett, M. K. 1974. Histopathology of resistant and susceptible soybean hypocotyls inoculated with *Phytophthora megasperma* var. *sojae*. Phytopathology 64:971-975.
12. Lazarovits, G., Stossel, R., and Ward, E. W. B. 1981. Age-related changes in specificity and glyceollin production in the hypocotyl reaction of soybeans to *Phytophthora megasperma* var. *sojae*. Phytopathology 71:94-97.

13. Marks, G. C., and Mitchell, J. E. 1971. Penetration and infection of alfalfa roots by *Phytophthora megasperma* and the pathological anatomy of infected roots. Can. J. Bot. 49:63-67.
14. McIntosh, D. L. 1966. The occurrence of *Phytophthora* spp. in irrigated systems in British Columbia. Can. J. Bot. 44:1591-1596.
15. Mehrotra, R. S. 1970. Techniques for demonstrating accumulation of zoospores of *Phytophthora* species on roots in soil. Can. J. Bot. 48:879-882.
16. Milholland, R. D. 1975. Pathogenicity and histopathology of *Phytophthora cinnamomi* on highbush and rabbiteye blueberry. Phytopathology 65:789-793.
17. Miller, C. R., Dowler, W. M., Petersen, D. H., and Ashworth, R. P. 1966. Observations on the mode of infection of *Pythium ultimum* and *Phytophthora cactorum* on young roots of peach. Phytopathology 56:46-49.
18. Miller, S. A., and Maxwell, D. P. 1984. Light microscope observations of susceptible, host resistant and nonhost resistant interactions of alfalfa with *Phytophthora megasperma*. Can. J. Bot. 62:109-116.
19. Pfender, W. F., and Hagedorn, D. J. 1983. Disease progress and yield loss in Aphanomyces root rot of peas. Phytopathology 73:1109-1113.
20. Pfender, W. F., Hine, R. B., and Stanghellini, M. E. 1977. Production of sporangia and release of zoospores by *Phytophthora megasperma* in soil. Phytopathology 67:657-663.
21. Pochard, E., Clerjeau, M., and Pitrat, M. 1976. La résistance du piment, *Capsicum annum* L., a *Phytophthora capsici* Léon. I. Mise en évidence d'une induction progressive de la resistance. Ann. Amelior. Plant. 26:35-50.
22. Robinson, R. A. 1969. Disease resistance terminology. Rev. Appl. Mycol. 48:593-603.
23. Ruppel, E. G. 1966. Histology of resistant and susceptible seedlings of *Tephrosia vogelii* inoculated with *Phytophthora parasitica* var. *nicotianae*. Phytopathology 56:489-492.
24. Shipton, W. A., and Brown, J. F. 1962. A whole-leaf clearing and staining technique to demonstrate host-pathogen relationships of wheat stem rust. Phytopathology 52:1313.
25. Sidman, R. L., Mottla, P. A., and Feder, N. 1961. Improved polyester wax embedding for histology. Stain Technol. 36:279-284.
26. Stack, R. W. 1980. Disease progression in common root rot of spring wheat and barley. Can. J. Plant Pathol. 2:187-193.
27. Stossel, P., Lazarovits, G., and Ward, E. W. B. 1980. Penetration and growth of compatible and incompatible races of *Phytophthora megasperma* var. *sojae* in soybean hypocotyl tissues differing in age. Can. J. Bot. 58:2594-2601.
28. Thomson, S. V., and Allen, R. M. 1974. Occurrence of *Phytophthora* species and other potential plant pathogens in recycled irrigation water. Plant Dis. Rep. 58:945-949.
29. Ward, H. M. 1887. Illustrations of the structures and life history of *Phytophthora infestans*, the fungus causing the potato disease. Q. J. Micros. Sci. 27:413-425.
30. Zar, J. H. 1984. Biostatistical Analysis. 2nd ed. Prentice-Hall, Englewood Cliffs, NJ. 718 pp.