Pathogenicity of *Phoma medicaginis* var. *medicaginis* to Roots of Alfalfa

R. RODRIGUEZ, Department of Plant Pathology, The Pennsylvania State University, and K. T. LEATH and R. R. HILL, JR., USDA Regional Pasture Research Laboratory, University Park, PA 16802

ABSTRACT

Rodriguez, R., Leath, K. T., and Hill, R. R., Jr. 1990. Pathogenicity of *Phoma medicaginis* var. *medicaginis* to roots of alfalfa. Plant Dis. 74:680-683.

The pathogenicity of *Phoma medicaginis* var. *medicaginis* on roots of alfalfa (*Medicago sativa*) was investigated in greenhouse and growth chamber experiments. Conidia were applied to roots of 4- to 6-mo-old plants grown in agar, nutrient solution, or greenhouse potting mix. All inoculations caused necrosis of main and lateral roots. *P. m.* var. *medicaginis*, in the absence of other organisms, penetrated intact roots and caused a black, dry necrosis. Wounding increased the extent and frequency of necrosis, and disease from root inoculations reduced stem number, crown diameter, and dry weight of roots and foliage of greenhouse-grown plants. *P. m.* var. *medicaginis* should be considered a primary pathogen of alfalfa roots.

Additional keywords: root rot complex

Root diseases are a major cause of yield loss and stand decline of alfalfa (*Medicago sativa* L.) worldwide (12). Some root diseases are caused by specific pathogens and have characteristic symptoms that are easily recognized. Diseases caused by *Phytophthora megasperma* (Drechs.) f. sp. *medicaginis* Kuan & Erwin, *Stagonospora meliloti* (Lasch.) Petr., and *Rhizoctonia crocorum* (Pers.:Fr.) DC. are examples of such diseases. Alfalfa is also afflicted with a less distinct root disease that is often referred to as the crown and root rot complex. Numerous fungi (3,4,15,21,24) and two bacteria (16–18) have been implicated as causal agents. This generalized type of root rot occurs under varied climatic and edaphic conditions, and the organisms causing the disease vary with time of year and geographic location. Usually, the severity of root rot increases with the age of the stand through several growing seasons until the stand is thinned and no longer profitable (11,12).

Phoma medicaginis Malbr. & Roum. var. *medicaginis* Boerema, a recognized pathogen of alfalfa leaves and stems (12), has been implicated in crown and root rots of alfalfa (2,5,6,10,19,20), and *Phoma* spp. are also considered to be primary root pathogens of other legumes (1,2). However, the reports on the pathogenicity of *P. m.* var. *medicaginis* to alfalfa roots are inconsistent. Stivers et al (27) failed to induce rot symptoms by inoculating alfalfa roots with *P. m.* var. *medicaginis*, whereas Cormack (5) reported slight to moderate rot symptoms in field-inoculated alfalfa roots and consistently recovered *P. m.* var. *medicaginis*. Cormack believed *P. m.* var. *medicaginis* to be a secondary pathogen of roots, because it was always associated with other fungi. *P. m.* var. *medicaginis* has been recognized as an important pathogen of crown buds (26) but was shown to cause root rots (2) only when freshly isolated from leaf lesions. Although *P. m.* var. *medicaginis* has not been considered an important crown and root pathogen of alfalfa, its potential in this regard has been recognized (23), and it was rated by one researcher in Germany (22) as second only to *Verticillium albo-atrum* Reinke & Berthier as a root pathogen of alfalfa.

A definitive study of *P. m.* var. *medicaginis* as a root pathogen of alfalfa has not been reported. The objective of this research was to determine the pathogenicity of this fungus to roots of alfalfa.

MATERIALS AND METHODS

Plant production. The alfalfa cultivar Iroquois was used in all experiments. Plants were grown from seed previously cleaned by washing in flowing tap water for 1 hr, soaking in 70% ethanol for 5 min and mercury bichloride (1 g/L of water) for 20 min, and rinsing three times (20 min each) in autoclaved, distilled water. Seed was checked for microbial growth after 48 hr on water agar. Only seeds free of microorganisms were selected for experiments. Clonal plants were produced by rooting stem cuttings in 1% of agar containing half-strength Hoagland's solution (HSA) (7).

The slant-board system (9) of growing plants was used to facilitate access to plant roots. Gnotobiotic plant growth was achieved in glass culture tubes 2.8 cm in diameter and 19.8 cm in height. The tubes were capped with filter paper. Plant growth media consisted of HSA or a mixture of dried, ground alfalfa tops (1% by weight in sand and water, 20% wt/vol) (AS). Each tube contained 30 ml of medium. Tubes and media were autoclaved before aseptic placement of the seeds. Slant boards and growth tubes were maintained in a controlled environment chamber that provided a photoperiod of 15 hr at 21 C and 9 hr of darkness at 15 C. Light intensity was 125 $\mu E \cdot s^{-1} \cdot m^{-2}$ for the gnotobiotic and 200 $\mu E \cdot s^{-1} \cdot m^{-2}$ for the slant-board experiments.

Inoculum production. *P. m.* var. *medicaginis* was cultured on oatmeal agar (OMA) or on 1-cm-long pieces of alfalfa stem that had been sterilized by soaking in 70% ethanol for 5 min and mercury bichloride (1 g/L) for 20 min, rinsing three times (10 min each) in autoclaved, distilled water, and microwaving for 4 min. Sterility of stem pieces was verified in nutrient and potato-dextrose broths. Agar discs with mycelium were used to transfer the fungus to the OMA plates and the stem pieces, which were laid on the surface of 1% water agar. Conidia were collected from OMA cultures by rinsing with autoclaved, distilled water after cultures had grown for 1 mo at 21 C in darkness. Desired concentrations of spore suspensions were achieved by hemacytometer counts and dilution.

Gnotobiotic culture pathogenicity tests. One-month-old plants were inoculated with 5 ml of an aqueous suspension containing 2.5×10^6 conidia per milliliter, pipetted into a 5-mm-diameter vertical hole in the agar or on the surface of the AS medium. An isolate of *P. m.* var. *medicaginis* from alfalfa roots from field-grown plants in Lycoming County, Pennsylvania, was used. Control plants received autoclaved, distilled water. Duration of each experiment was 30 days, and each treatment had 24 single-plant replications.

Present address of first author: Department of Crop Protection, College of Agricultural Sciences, University of Puerto Rico, Mayaguez P. R. 00708.

Contribution 8934 of the U.S. Regional Pasture Research Laboratory, and Journal Series 8297 of the Pennsylvania Agricultural Experiment Station.

Accepted for publication 21 February 1990 (submitted for electronic processing).

Isolations were made from each symptomatic root system and randomly from asymptomatic control roots on potato-dextrose agar incubated at 21 C.

Slant-board culture pathogenicity tests. Cuttings from clonal plants grown in the growth chamber were immersed in 70% of ethanol for 1 min and in undiluted hydrogen peroxide for 20 min, given three 10-min rinses in autoclaved, distilled water, and then planted into autoclaved HSA in culture tubes as described in the methods for the gnotobiotic culture. Plants were removed from the culture tubes after 1 mo and placed in slant boards. After 2 wk, roots were spread fanlike to facilitate inoculation of individual roots (14). Pieces of alfalfa stem, colonized with the *P. m.* var. *medicaginis* isolate used in the gnotobiotic experiments, were used as inoculum. Stem pieces were placed in contact with roots at the tip or 2 or 10 cm above the tip. Control roots were placed in contact with noncolonized stem pieces. In a separate experiment, disease development was compared in wounded and intact roots. Wounding was done either by pressing roots with a file card (a stiff wire brush used for cleaning files) or by scraping the root surface with a sterile dissecting needle. Symptom development in nonwounded lateral and main roots was evaluated in a third experiment. The length of necrotic lesions was measured with a ruler. A completely randomized design, with four roots on each of four plants per treatment, was used in each experiment. Data were subjected to an analysis of variance with means compared by the Tukey studentized range test (25) and with orthogonal contrasts.

Greenhouse culture pathogenicity tests. One-month-old seedling plants were carefully removed from vermiculite-peat growing medium and their roots washed in flowing tap water. Treatments included nonwounded control, wounded control, nonwounded inoculated, and wounded inoculated. Wounding was done by pressing a file card against the roots of each plant, which made multiple small wounds. Inoculation consisted of immersing roots for 1 hr in a conidial suspension (2×10^6 spores per ml of 50 ppm Tween 20 in water). An isolate of *P. m.* var. *medicaginis* from alfalfa roots from Bradford County, Pennsylvania, was used. Control roots were immersed in the Tween 20 solution, and treated plants were repotted in a vermiculite-peat mixture in plastic containers (4-cm diam. $\times$ 21-cm tall). Tops were cut back to 4 cm and *Rhizobium meliloti* Dangeard was applied to the growing medium immediately after treatment. Plants were kept in saturated humidity at 21 $\pm$ 1 C in darkness for 48 hr after treatment and then returned to the greenhouse. Fifty single-plant replications were used per treatment in a completely randomized design. Dry weight of roots and foliage, number of stems, and diameter of crowns were recorded 30 days after inoculation. Data were subjected to analysis of variance, and means were compared with the Bonferroni multiple comparison technique (8).

RESULTS

Gnotobiotic culture pathogenicity tests. The rot symptom caused by *P. m.* var. *medicaginis* was a blackish, dry necrosis often accompanied by collapsed root tissue at or around the infection site. *P. m.* var. *medicaginis* caused necrosis of alfalfa roots growing in HSA and AS growth media in the absence of other organisms. Incidence of plants with root necrosis was 85% for those growing in HSA and 90% for those growing in the AS medium. Infections occurred at root tips and at loci where lateral roots initiated, and entire roots of some plants were stunted and discolored (Fig. 1). Withering, wilting, chlorosis, and reddening of foliage occurred on plants with severely diseased roots. *P. m.* var. *medicaginis* was consistently recovered from inoculated plants in all trials. No microorganisms were isolated from tissue of control plants.

Slant-board culture pathogenicity tests. Roots severely infected by *P. m.* var. *medicaginis* were often collapsed, with necrosis extending above and below the inoculation site (Fig. 2). *P. m.* var.

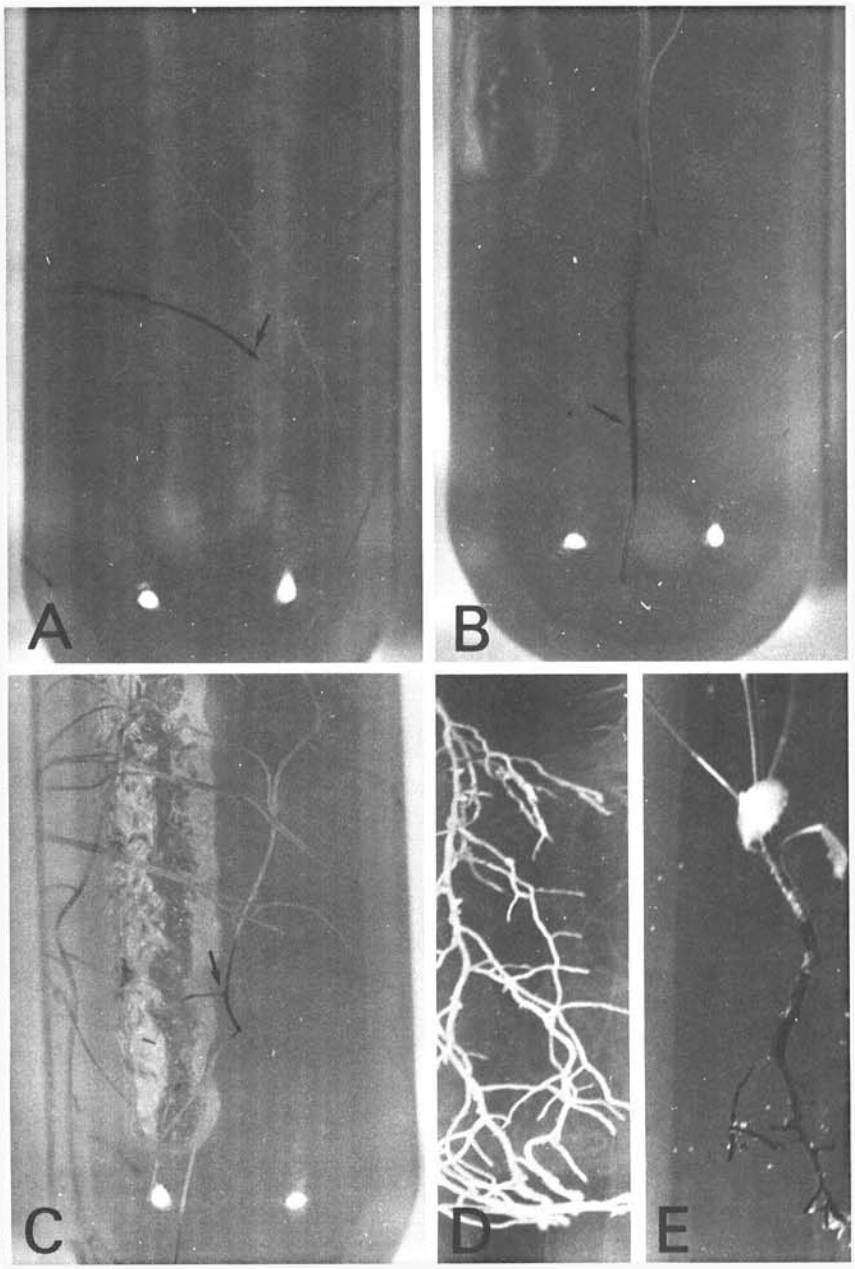

Fig. 1. Root rot symptoms induced by *Phoma medicaginis* var. *medicaginis* in alfalfa grown under gnotobiotic conditions. **(A–C)** Different sites of infection on roots inoculated with conidia in the Hoagland's solution agar medium (arrows) (×2), **(D)** noninoculated control from the alfalfa-sand (AS) mixture showing density of root system, and **(E)** symptoms induced by the fungus in roots of alfalfa grown in the AS mixture.

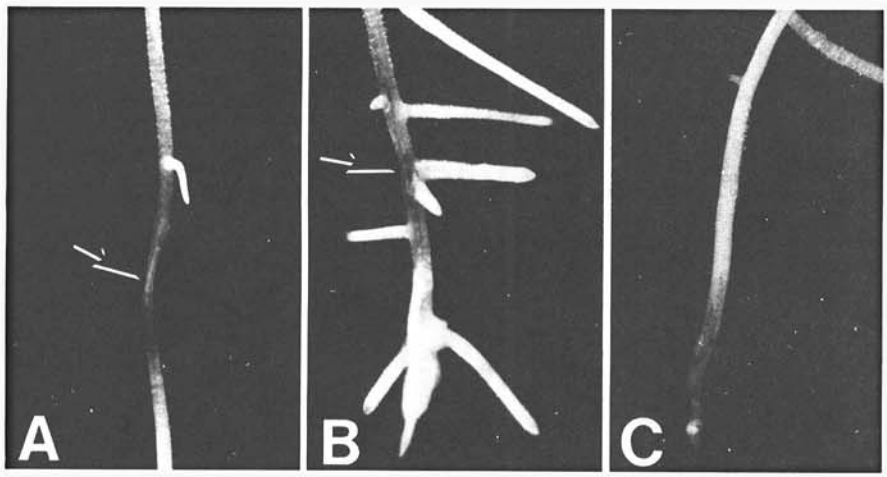

Fig. 2. Root rot symptoms induced by *Phoma medicaginis* var. *medicaginis* in alfalfa grown in the slant-board system. **(A)** Response of main root to inoculation showing bidirectional spread of necrosis from the inoculation site (arrow), **(B)** proliferation of lateral roots below the inoculation site (arrow), and **(C)** necrosis of root tip caused by inoculation with the fungus.

Table 1. Comparison of necrosis in wounded and nonwounded roots of 6-wk-old alfalfa plants inoculated with *Phoma medicaginis* var. *medicaginis* in slant boards

Treatment		Length of necrosis (mm)[x]	Sympto-matic roots (%)
Wounding method	Inoculum		
File card[y]	−	0.0 a[z]	0
File card	+	4.9 c	94
Needle	−	0.0 a	0
Needle	+	4.3 c	94
None	+	2.4 b	69

[x] Mean of 16 measurements (four roots on each of four plants).
[y] A stiff wire brush was used to clean files.
[z] Means followed by the same letter are not different at $P < 0.05$, using orthogonal analysis.

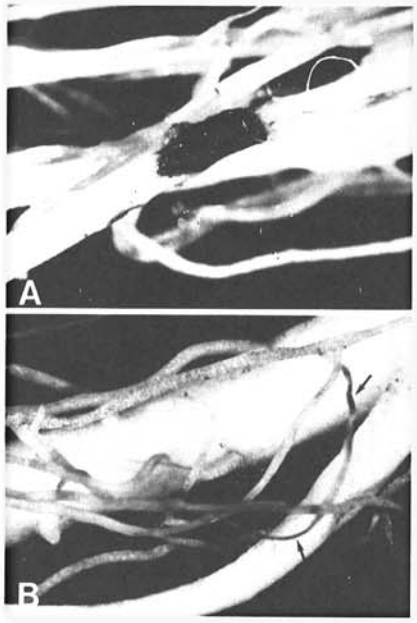

Fig. 3. Symptoms caused by inoculation of alfalfa roots with *Phoma medicaginis* var. *medicaginis*. **(A)** Rot lesion on wounded main root (×5), and **(B)** rotted, nonwounded lateral roots (arrows).

medicaginis readily infected root tips and caused necrosis (Fig. 2). Inoculations at 2 cm above the root tip often resulted in the initiation of lateral roots distal to the necrotic area. Wounding significantly increased the length of necrotic lesions (Table 1), although *P. m.* var. *medicaginis* caused necrosis in the absence of wounding. Control roots appeared healthy. Lesions had a mean length of 14.5 mm on lateral roots and 9.5 mm on main roots, a difference significant at $P < 0.05$. Isolations from necrotic areas consistently yielded *P. m.* var. *medicaginis*. Bacteria were isolated from diseased and control roots; most were *Bacillus*-like. Frequency and type of bacteria appeared constant across treatments, and none were able to rot injured potato tuber tissue, which suggested a lack of pathogenicity.

Greenhouse culture pathogenicity tests. Necrosis was most severe around wound sites but also occurred in nonwounded tissues, especially on lateral roots (Fig. 3). Inoculation with *P. m.* var. *medicaginis* reduced stem number, crown diameter, and stem and root dry weights (Table 2). As in the slant-board inoculations, wounding increased disease severity. *P. m.* var. *medicaginis* was consistently isolated from disease sites. *Trichoderma* sp. was also recovered along with bacteria. Both were common to all treatments, and bacteria tested negatively in potato soft rot assays.

DISCUSSION

It is quite difficult to diagnose by visual appearance the causal organisms involved in root rot of alfalfa, however, rot symptoms caused by *P. m.* var. *medicaginis* are somewhat distinctive. *P. m.* var. *medicaginis* causes a black necrosis that should be discernible from the brown to reddish brown appearance of rots caused by *Fusarium* spp., although it would be more difficult to differentiate the necrosis caused by *P. m.* var. *medicaginis* from the more similar blue-black necrosis caused in crowns and upper taproots by *Colletotrichum trifolii* Bain & Essary.

Symptoms of root rot caused by *P. m.* var. *medicaginis* in the absence of other organisms testify to the ability of this fungus to act as a primary root pathogen of alfalfa. In roots of older plants grown on slant boards or in the greenhouse, *P. m.* var. *medicaginis* caused disease and demonstrated a significant adverse impact on plant performance. Although *P. m.* var. *medicaginis* infected intact roots, disease severity was increased by wounding.

Under field conditions, *P. m.* var. *medicaginis* is commonly associated with the soil, most frequently as a colonizer of plant debris. It seems quite likely that root-feeding insects such as the clover root curculio, *Sitona hispidulus* F., could serve as a vector for *P. m.* var. *medicaginis* or predispose roots to infection through feeding wounds. *P. m.* var. *medicaginis* has been isolated from such feeding sites (13). Roots are subjected to abiotic injuries under field conditions, and wound-enhanced disease caused by *P. m.* var. *medicaginis* is probably a common occurrence in roots of field-grown alfalfa.

We believe that these experiments demonstrate that *P. m.* var. *medicaginis* can be a primary root pathogen of alfalfa and can severely impair plant performance. The root-rot potential of *P. m.* var. *medicaginis* should be considered when evaluating the etiology of the root-rot complex of alfalfa and in setting priorities for breeding programs.

LITERATURE CITED

1. Barbetti, M. J. 1984. Subterranean clover foliage fungi as root pathogens. Australas. Plant Pathol. 13:38-40.
2. Boerema, G. H., Dorenbosch, M. M. J., and Leffring, L. 1965. A comparative study of the black stem fungi on lucerne and red clover and

Table 2. Effect of inoculation with *Phoma medicaginis* var. *medicaginis* and wounding on growth parameters of 1-mo-old, greenhouse-grown alfalfa plants

	Wounded		Nonwounded		
Parameter	Control	Inoculated	Control	Inoculated	SE[a]
Stem no.	5[b]	4	5	4	I
Crown diam. (mm)	3	2	3	2	I
Root dry wt. (g)	141	70	145	100	I,W
Foliage dry wt. (g)	239	125	251	157	I,W

[a] Significant effect at $P < 0.05$; I = inoculation, W = wounding.
[b] Data are means of 48 plants, with each plant a replicate.

the footrot fungus on pea. Neth. J. Plant Pathol. 71:79-89.
3. Broadfoot, W. C., and Cormack, M. W. 1941. A low temperature basidiomycete causing early spring killing of grasses and legumes in Alberta. Phytopathology 31:1058-1059.
4. Cherewick, W. J. 1948. Two important diseases of alfalfa in Manitoba. (Abstr.) Phytopathology 38:5.
5. Cormack, M. W. 1945. Studies on *Ascochyta imperfecta*, a seed- and soil-borne parasite of alfalfa. Phytopathology 35:838-854.
6. Czaplinska, S. 1963. Investigations on the biology of fungi causing diseases of Lucerne, in particular wilt in Lower Silesia. Acta Agro. Bot. 14:101-130.
7. Hoagland, D. R., and Arnon, D. E. 1938. The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347. 39 pp.
8. Johnson, R. A., and Wichern, D. W. 1982. Applied Multivariate Statistical Analysis. Prentice-Hall, Englewood Cliffs, NJ. 607 pp.
9. Kendall, W. A., and Leath, K. T. 1974. Slant-board culture methods for root observations of red clover. Agron. J. 14:317-320.
10. Leach, G. M. 1959. A survey of root deterioration of *Medicago sativa* in Oregon. Plant Dis. Rep. 43:622-625.
11. Leath, K. T. 1989. Diseases and forage stand persistence in the United States. Pages 465-479 in: Persistence of Forage Legumes. G. C. Marten, ed. Am. Soc. Agron., Madison, WI.
12. Leath, K. T., Erwin, D. C., and Griffin, G. D. 1988. Diseases and Nematodes. Pages 621-670 in: Alfalfa and Alfalfa Improvement. A. A. Hanson et al, eds. Agron. Monogr. 29 Am. Soc. Agron., Madison, WI. 1084 pp.
13. Leath, K. T., and Hower, A. A. 1988. Role of *Sitona hispidulus* on the development of Fusarium wilt. No. Am. Alfalfa Improv. Conf. 31:24.
14. Leath, K. T., and Kendall, W. A. 1978. Fusarium root rot of forage species: Pathogenicity and host range. Phytopathology 68:826-831.
15. Leath, K. T., Lukezic, F. L., Crittenden, H. W., Elliot, E. S., Halisky, P. M., Howard, F. L., and Ostazeski, S. A. 1971. The Fusarium root rot complex of selected forage legumes in the Northeast. Pa. Agric. Exp. Stn. Res. Bull. 777. 64 pp.
16. Leath, K. T., Lukezic, F. L., Pennypacker, B. W., Kendall, W. A., Levine, R. G., and Hill, R. R., Jr. 1989. Interaction of *Fusarium avenaceum* and *Pseudomonas viridiflava* in root rot of red clover. Phytopathology 79:436-440.
17. Lukezic, F. L., Hildebrand, D. C., Schroth, M. N., and Shinde, P. A. 1982. Association of *Serratia marcescens* with crown rot of alfalfa in Pennsylvania. Phytopathology 72:714-718.
18. Lukezic, F. L., Leath, K. T., and Levine, R. G. 1983. *Pseudomonas viridiflava* associated with root and crown rot of alfalfa and wilt of birdsfoot trefoil. Plant Dis. 67:808-811.
19. McDonald, W. C. 1955. The distribution and pathogenicity of the fungi associated with crown and root rotting of alfalfa in Manitoba. Can. J. Agric. Sci. 35:309-321.
20. Pegg, G. F., and Parry, D. W. 1983. Infection of lucerne (*Medicago sativa*) by *Fusarium* species. Ann. Appl. Biol. 103:45-55.
21. Roberts, D. L. 1986. Destructive disease of alfalfa in north Florida. Soil Crop Sci. Soc. Fla. Proc. 45:107-109.
22. Rossner, H. 1968. Studies on the causal agent of the Phoma disease of Lucerne (*P. medicaginis*). Phytopathol. Z. 63:101-123.
23. Schenck, N. C., and Gerdemann, J. W. 1956. Taxonomy, pathogenicity and host-parasite relations of *Phoma trifolii* and *Phoma herbarum* var. *medicaginis*. Phytopathology. 46:194-200.
24. Sleeth, B. 1951. Diplodia crown rot of alfalfa. Plant Dis. Rep. 35:50-51.
25. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. McGraw-Hill Inc, New York. 633 pp.
26. Stephen, R. C., Saville, D. J., Harvey, I. C., and Hedley, J. 1982. Herbage yields and persistence of lucerne (*Medicago sativa* L.) cultivars and the incidence of crown and root diseases. N. Z. J. Exp. Agric. 10:323-332.
27. Stivers, R. K., Jackson, W. A., Ohlrogge, A. J., and Davis, R. L. 1947. The relationship of varieties and fertilization to observed symptoms of root rots and wilt of alfalfa. Agron. J. 48:71-73.