Effects of Soil Temperature and Moisture on Activity of *Phytophthora megasperma* f. sp. *medicaginis* and Alfalfa Root Rot in the Field

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ABSTRACT

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Activity of *Phytophthora megasperma* f. sp. *medicaginis* was determined for four soil irrigation treatments applied to first-year and to 1- and 2-yr-old stands of Iroquois alfalfa. PA was measured with an alfalfa seedling baiting assay every 10 days from May through October. Incidence and severity of Phytophthora root rot, based on root destruction and plant survival, were assessed every 10 days from the second trifoliolate leaf stage through the first-year growing season. PA was not detected below ~12–15 C, but at higher temperatures it was substantial, even in soil not continually irrigated to a moisture level of ≤0.1 bars. PA activity was lower in soil maintained at ≤0.1 bars than in drier soil. Root rot was directly proportional and plant survival was inversely proportional to soil moisture. Root lesions appeared ~8 wk after seeding in soil continually maintained at ≤0.1 bars. Extensive root rot developed in soil irrigated to ≤0.1 bars either continually or for 10 days on a 20-day schedule from the first trifoliolate leaf stage. Severe root destruction also occurred in soils continually wetted to that moisture level starting 14 wk after the first trifoliolate leaf stage.

In the northeastern USA, ~500,000 hectares (ha) are planted to alfalfa. Individual plantings remain commercially productive for ~3–4 yr, when the number of productive plants in a stand often decreases below an economic threshold. This stand decline has been attributed to many biotic and abiotic factors; eg, pest damage, mechanical injury, and winter injury (7).

Phytophthora root rot (PRR), which is caused by *Phytophthora megasperma* f. sp. *medicaginis* (10), is a major factor in alfalfa stand decline during extended periods of high soil moisture (1–6, 8,13). *Phytophthora megasperma* f. sp. *medicaginis* appears to be widespread in soils cropped to alfalfa.

Soils vary in activity of *P. megasperma* f. sp. *medicaginis* (PA) (13). Pratt and Mitchell (13) observed that planting alfalfa in a soil would increase the PA. There is little information on the effects of soil moisture and temperature on PA in the field. Pratt and Mitchell (13) reported that seedling infection was greater at 15 or 20 C than at 25 C while no infection occurred at 30 C. They, however, were examining the effects of temperature on infection of seedlings in a baiting technique, not on inoculum in the field. Similarly, no investigations on the relationship between PA and PRR development in the field have been reported. Gray and Hine (6) examined various aspects of PRR development in the first-year stands of alfalfa and, in particular, described the infection process and root rot development under field conditions. In their study, they did not attempt to control soil moisture in the field to determine its effect on PA and PRR.

The objectives of this work were to quantify the effects of soil moisture and temperature on PA and PRR development in the field.

MATERIALS AND METHODS

Site characteristics. A field on the research farm, Department of Agronomy, Cornell University, Aurora, NY, that had been planted to corn from 1963 through 1975, was seeded with Iroquois alfalfa in 1976. Severe PRR was observed in late summer of that year.

Soils typical of four classes occurred in the field: Honeoye loam, Honeoye loam (eroded), Kendaia silt loam, and Lima loam. Drainage ranged from poor to fair and slope ranged from 0–6%. The soil had a pH of ~7.4 and by analysis contained 37 kg/ha P, 146 kg/ha K, 1,482 kg/ha Mg, 4,818 kg/ha Ca, 44 kg/ha Mn, 2 kg/ha Zn, 1 kg/ha B.

The stand was cut and the hay removed three times each year. In 1977, 16 plots (13.5 m^2) spaced 10 m apart were located as a strip running lengthwise in the field; this strip was designated 2-yr-old alfalfa. In 1978, an adjacent comparable strip provided plots of 3-yr-old plants. In addition, alfalfa established in 1979 as a strip immediately adjacent to the 3-yr-old stand provided plots of first-year alfalfa.

Soil moisture control. An overhead irrigation system was constructed to permit supplemental irrigation of the plots as needed. Tensiometer measurements taken every second day at two locations 10 cm deep were used to estimate the matric potentials in each plot. In unirrigated plots, soil moisture at 10 cm was monitored every second day by measuring the electrical resistance of buried gypsum blocks (14). Soil moisture was measured from 30 April through 28 October each year. Natural precipitation was sufficient during May, September, and October to maintain the soil water suction at ≤0.5 bars each year. Irrigation was applied, therefore, only during the months of June, July, and August. Four irrigation treatments were used: NW, no irrigation; C2, soil irrigated ≤0.1 bars after 7 July; C, soil irrigated to maintain ≤0.1 bars for the entire sampling period; C10, soil irrigated to maintain ≤0.1 bars for 10 days at 20-day intervals.

Soil temperature. Soil temperature was monitored in each plot with two thermisters placed 10 cm deep. Measurements were recorded at 1000–1200 hours on alternate days. Soil temperature at 10 cm was also continuously monitored in one plot. During June–September, it varied ~7 C between daily high and low readings.

Soil and plant sampling. Soil and plants were collected every 10 days starting 21 April each year. Ten soil cores (2.5 cm in diameter) from the top 15–20 cm of the soil profile were taken randomly and bulked for each plot. Soil samples were assayed for PA on the day

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that they were collected. Sampling of the first-year plants was started at the first trifoliolate leaf stage. Fifteen to 20 plants randomly selected were removed with the root system intact from each plot.

***Phytophthora megasperma* activity assay.** PA describes the infection of alfalfa seedling baits by propagules of *P. megasperma* f. sp. *medicaginis* in soil. Differences in infection of baits are presumed to represent different numbers of propagules in soil that are directly or indirectly capable of rapid infection. PA was assayed by the procedure of Marks and Mitchell (11) except that incubation was for ~72 hr and uninjured alfalfa seedlings were used. Soil samples from a plot were sieved through a 0.45-cm mesh screen and mixed well. Dilutions (1:0, 1:2, 1:8, 1:26, 1:49 v/v) of the soil were made with sterile masonry sand. Thirty milliliters of each soil dilution was placed in a 7.5-cm glass petri dish. Forty milliliters of distilled water was added to cover the soil to a depth of ~2.0 mm. Six Iroquois alfalfa seedlings (72-hr-old) were placed in the water on the soil. The soil, water, and seedlings were covered and incubated at 21–23 C. All soil dilutions were assayed in triplicate. Each seedling was examined for the presence of *P. megasperma* sporangia. A seedling with one or more sporangia was scored as infected. PA was calculated as the sum of the product of the dilution factor × average number of infected seedlings per plate; ie,

$$PA = 1A + 3B + 9C + 27D + 50E$$

in which A, B, C, D, and E equal the average number of seedlings infected per plate at the 1:0, 1:2, 1:8, 1:26, and 1:49 dilutions, respectively.

Disease estimation. Phytophthora root rot was assessed only for plants in the first-year stand. Plants were free of symptoms at the time water treatments were started. The following observations were made for all plants: general root rot rating (12); taproot length; number of taproot lesions; number of lateral root lesions; lesion length; distance between lesion and crown; and number of adventitous roots immediately above the taproot lesion.

Plant survival. Plant densities were estimated five times during the year in each plot. On each sampling date a wire frame (929 cm^2) was tossed into each plot and the number of plants were counted; the process was repeated four times for each plot.

Determination of PA in soil profiles. Soil cores from several plots with high PA were taken on three occasions. The cores measured 20 cm^2 × 40 cm long; the depth of the hole from which the core was taken was 50 cm. The core was sectioned into 10 4.0-cm lengths. Each section was screened through a 2.0-mm screen and assayed by using 10 alfalfa seedlings per dish in the bathing assay. To relate the presence of *P. megasperma* in each soil section to their location in the soil profile, each section length was adjusted by 1.2. This extrapolation was based on the premise that the top and bottom of the core represented the actual soil surface and soil at a depth of 50 cm. Due to compaction of 20% (40 cm core/50 cm hole × 100). The depth each section represented in the soil profile could be approximated by multiplying each section length by 1.2.

RESULTS AND DISCUSSION

PA in general. Although the alfalfa seedling assay measures the relative activity of *P. megasperma* in soil, neither the types of propagules responsible for infection of the seedlings nor their source were identified by the assay. Zoospores usually are considered the principal propagule responsible for seedling infection by *Phytophthora* spp. However, our understanding of whether zoospores are the only infectious propagules and from which structure the zoospores are ultimately produced, is incomplete. Therefore, although factors affecting PA were identified, the basis of these effects remains unknown.

Soil temperature and PA. From late October through early May, soil temperatures ranged 0–15 C, and soil moisture was high (0.0–0.5 bars). In the spring, PA was detected in soil only after the field soil temperature reached 15 C. By mid-June, soil temperature had increased to ~18–20 C, soil moisture decreased (≥ 1.0 bars), and PA increased. In the fall, PA was no longer detectable in soil after the temperature fell below 12 C. These results, which were obtained regardless of the age of the stand, indicate that soil temperature significantly affects PA in early spring and late autumn.

From 16 June through 3 September, soil temperature remained above 18 C and did not significantly affect PA. In this same period, the irrigation treatments were applied and significant differences between soil moisture and PA were observed (Table 1). Therefore, apparently after soil temperature increases to ≥18 C, PA is affected more by changes in soil moisture than by temperature.

Soil moisture and PA. During the irrigation treatments, plots continually maintained at ≤0.1 bars had lower PA than plots not irrigated and were even lower in PA than the level recorded when the irrigation treatments were begun (Table 1).

The adverse effects on PA of irrigating the plots during the summer was clearly evident in the fall in samples taken after irrigation was terminated. Highest PA was recorded for plots not irrigated; treatments that involved irrigation of the plots regardless

TABLE 1. Activity of *Phytophthora megasperma* f. sp. *medicaginis* (PA) in soil from first-, second-, and third-year stands of alfalfa under four irrigation treatments

Sampling dates	Irrigation treatments[a]	Relative PA after: 1 yr	2 yr	3 yr
21 April–16 June	C	7.88 A	3.48 A	4.07 A
	C10	4.15 B	2.95 A	5.11 A
	C2	3.61 B	···	3.63 A
	NW	2.55 B	2.45 A	3.67 A
17 June–3 September	C	−2.85 B	−2.10 C	0.05 B
	C10	21.60 A	8.34 B	1.38 A
	C2	11.15 A	···	3.56 A
	NW	10.20 A	14.58 A	1.68 A
4 September–24 October	C	−8.23 C	−0.92 C	−1.53 B
	C10	9.28 B	11.14 B	2.30 A
	C2	8.66 B	···	−1.92 B
	NW	18.94 A	17.83 A	2.40 A

[a] Irrigation treatments: C = soil moisture suction maintained at ≤0.1 bars; C10 = soil moisture suction maintained at ≤0.1 bars for 10 days beginning every 20 days; C2 = soil moisture suction maintained at ≤0.1 bars starting on 27 July; NW = no supplemental water. Irrigation treatments were only applied from 17 June–3 August of each year.

[b] Numbers are mean values of relative PA in soil samples as determined according to Wilkinson (15). Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

TABLE 2. Percentage of diseased alfalfa plants as determined in a first-year stand of alfalfa under four irrigation treatments

Sampling period[a]	Irrigation treatment[b]	Percentage of diseased plants[b]
21 April–16 June	C	0.0 A
	C10	0.0 A
	C2	0.0 A
	NW	0.0 A
17 June–3 September	C	19.95 A
	C10	18.71 A
	C2	13.81 A
	NW	4.33 B
4 September–10 October	C	24.27 A
	C10	21.20 A
	C2	16.20 A
	NW	4.27 B

[a] Irrigation treatments: C = soil moisture suction maintained at ≤0.1 bars; C10 = soil moisture suction maintained at ≤0.1 bars for 10 days beginning every 20 days; C2 = soil moisture suction maintained at ≤0.1 bars starting 27 July; and NW = no supplemental water. Irrigation treatments were only applied from 17 June–3 August of each year.

[b] Means followed by the same letter did not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

TABLE 3. Severity of Phytophthora root rot in a first-year stand of alfalfa under four irrigation treatments

Irrigation treatments[a]	Sampling date	Mean disease scores[b]	Sampling date	Mean disease scores[b]
C	27 July	2.00 A	14 September	3.50 A
C10		1.67 A		2.83 B
C2		1.00 B		2.17 C
NW		1.00 B		1.00 B
C	6 August	2.50 A	24 September	3.50 A
C10		2.00 B		2.83 B
C2		1.00 C		2.17 C
NW		1.00 C		1.00 D
C	16 August	3.34 A	5 October	4.00 A
C10		2.50 B		2.83 B
C2		1.00 C		2.50 B
NW		1.00 C		1.33 C
C	26 August	3.50 A	15 October	4.00 A
C10		2.67 B		3.00 B
C2		1.17 C		2.67 B
NW		1.00 C		1.33 C
C	4 September	3.34 A	24 October	3.67 A
C10		2.83 A		3.00 AB
C2		1.50 B		2.67 B
NW		1.00 B		1.50 C

[a] Irrigation treatments: C = soil moisture suction maintained at ≤0.1 bars; C10 = soil moisture suction maintained at ≤0.1 bars for 10 days beginning every 20 days; C2 = soil moisture suction maintained at ≤0.1 bars starting 27 July; NW = no supplemental water. Irrigation treatments were only applied from 17 June–3 August of each year.

[b] Disease severity ratings were basedon a scale by Pratt and Mitchell (12). Means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

TABLE 4. Survival of alfalfa plants in a first-year stand under four irrigation treatments

Sampling dates	Irrigation treatments[a]	Mean number of surviving plants[b]
5 June	C	24.1 A
	C10	24.27 A
	C2	24.67 A
	NW	25.03 A
25 June	C	21.3 A
	C10	23.17 A
	C2	23.3 A
	NW	24.5 A
15 August	C	11.0 A
	C10	14.5 A
	C2	20.5 B
	NW	21.1 B
11 October	C	5.8 A
	C10	5.0 AB
	C2	11.5 B
	NW	12.3 B
24 October	C	3.7 A
	C10	4.8 A
	C2	5.0 A
	NW	10.0 B

[a] Irrigation treatments: C = soil moisture suction maintained at ≤0.1 bars; C10 = soil moisture suction maintained at ≤0.1 bars for 10 days beginning every 20 days; C2 = soil moisture suction maintained at ≤0.1 bars starting on 27 July; NW = no supplemental water. Irrigation treatments were only applied from 17 June–3 August of each year.

[b] Numbers are means for plants counted within five random areas (each 929 cm^2). Numbers followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

of the regime used had lower PA, and the lowest was recorded for plots maintained with high soil moisture for the longest period.

Disease development. PRR was initially assessed at the first trifoliolate leaf stage in first-year stands. Second- and third-year stands were affected by PRR in previous years; therefore, the incidence of infection, lesion development, and root decay could not be readily assessed. Others also have recognized this difficulty and assessed PRR only in first-year stands of alfalfa (6,9).

No lesions were observed on roots of plants examined before irrigation. When irrigation treatments were started, soil matric potential was 0.8 bar. This matric potential was probably too dry for PRR development. However, on the secondary and taproots of the first plants (8-wk-old) sampled after irrigation treatments were applied, faint yellowish-brown lesions (2.0 mm) were observed. That the lesions were due to PRR was verified by observing sporangia produced from them. The number of diseased alfalfa plants increased significantly ($P = 0.05$) as a result of irrigation (Table 2). Plots in which high soil moisture was maintained continually (C) or intermittently (C10) had significantly more plants with PRR than did plots that received no water. Delaying the onset of irrigation by 6 wk (C2) slowed, but did not preclude, significant PRR development.

Taproot length of healthy plants was not significantly affected by water treatments. Taproots of diseased plants from the irrigated plots were significantly shorter than healthy roots. However, the lengths of diseased roots did not differ significantly for the irrigation schedules.

Root destruction was assessed according to the scale described by Pratt and Mitchell (12). Analysis of these data for sampling dates after 17 July indicated that there was a significant difference ($P = 0.05$) among the water treatments (Table 3). Root rot was greater in the irrigated plots and was related to the length of the period in which high soil moisture was maintained (ie, C treatment >C10 >C2). Pratt and Mitchell (12) reported that greater root destruction occurred with intermittent watering than when soil was continually saturated. That their results differed slightly from ours probably is due to different conditions associated with the two investigations including moisture levels, direct seeding vs transplanting, and soil type. An important observation from our study is that even if soil moisture is inadequate for PRR during the first several months following seeding, severe PRR may still develop if soil moisture increases to ≤0.1 bars.

Lesions induced by *Phytophthora* occurred principally on roots other than the primary taproot. These lesions, measured longitudinally, ranged 0.2–0.4 cm, but neither lesion location nor length of lesions was affected by the irrigation treatments.

Lesions on the taproot (roots ~5–30 cm long) occurred at depths of 5–15 cm. Similar data were reported by Gray and Hine (7) and by Elgin et al (2). Frosheiser (4) observed PRR on roots as deep as 80 cm. In our studies, PA was detected in soil cores down to a depth of 50 cm. At depth increments 0–5, 6–10, 11–15, 16–20, 21–25, 26–30, 31–35, 36–40, 41–45, and 46–50 cm PAs were 10.0, 10.0, 9.1, 8.8, 7.9, 6.5, 5.0, 2.1, 1.1, and 0.6, respectively. It is likely that, in the zone 5–30 cm from the surface, favorable edaphic conditions occur for periods long enough to permit root infection and development of lesions. At depths less than 5 cm or greater than 30 cm, soil moisture, temperature, or aeration favorable for infection may have been too transient or occurred too infrequently to allow infection and lesion development.

Survival of plants. The number of alfalfa plants per unit area of plot decreased over the growing season in all irrigation treatments (Table 4). Before irrigation treatments were applied, plant densities decreased only slightly and did not differ significantly among treatments. By 15 August, plant densities differed significantly and were related to soil moisture content (ie, C irrigation treatment or C10 >C2, or NW). By 24 October, the mortality of plants in plots under the C2 water irrigation matched those for the C and C10 treatments. These data underscore the importance of high soil moisture for PRR and indicate that so long as soil moisture and temperature are favorable, plants apparently remain fully susceptible and are subject to severe PRR throughout the first year of the stand.

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