Variability in Sensitivity to Metalaxyl in vitro, Pathogenicity, and Control of *Pythium* spp. on Sugar Beet

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ABSTRACT

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Pythium ultimum var. *sporangiferum* (76 isolates) and *P. aphanidermatum* (21 isolates) cultured from diseased sugar beet seedlings in Minnesota and North Dakota were tested for sensitivity to metalaxyl, pathogenicity on sugar beet, and disease control by metalaxyl seed treatment. Sensitivity to metalaxyl (effective concentration causing 50% growth inhibition [EC₅₀]) was determined by linear growth on corn meal agar amended with 0, 0.01, 0.1, 1, 10, and 100 µg a.i. metalaxyl ml⁻¹ after 48 h in the dark at 21 ± 1°C. Variation among isolates was significant ($P = 0.05$) within and between species, and EC₅₀ values averaged 0.16 (range: 0.05 to 1.30 µg ml⁻¹) for *P. ultimum* var. *sporangiferum* and 2.06 (range: 1.19 to 3.12 µg ml⁻¹) for *P. aphanidermatum*. In pathogenicity tests on sugar beet, most isolates of *P. ultimum* var. *sporangiferum* (72 of 76) and all of *P. aphanidermatum* significantly ($P = 0.05$) decreased final stands compared to the noninoculated control. There was no correlation between aggressiveness in the absence of metalaxyl and in vitro sensitivity to metalaxyl. When *Pythium*-infested soil was planted with seed treated with metalaxyl at the standard (0.625 g a.i. kg⁻¹) or half rate, some isolates that were least sensitive to metalaxyl in vitro resulted in a significant ($P = 0.05$) reduction in disease control. These results may explain, at least in part, why producers do not attain expected stands when they plant metalaxyl-treated sugar beet seed.

Additional keywords: *Beta vulgaris*

Sugar beet (*Beta vulgaris* L.) is susceptible to numerous diseases, including seed rot, preemergence and postemergence damping-off, and root rot (18). In the Red River Valley (RRV) of Minnesota and North Dakota and in west-central Minnesota, *Pythium* spp. are isolated from dying seedlings, particularly in wet seasons or when fields are poorly drained, soil is compacted, or emergence is delayed. Identification of 37 *Pythium* isolates from dying seedlings (randomly selected from a collection of over 100) included 28 isolates of *P. ultimum* Trow. var. *sporangiferum* Drechs., 7 of *P. aphanidermatum* (Edson) Fitzp., and 1 each of *P. irregulare* Buisman and *P. acanthicum* Drechs. (15). These species (excluding *P. acanthicum*) have been reported on sugar beet (27), but *P. ultimum* and *P. aphanidermatum* are the most common pathogens (18).

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specific field is impossible to diagnose because the damage occurs before the problem is noticed by the producer.

Metalaxyl is a phenylamide fungicide which inhibits rRNA synthesis of species in the Peronosporales (9). Variation in sensitivity to metalaxyl has been reported among and within species of *Pythium* (7,28), *Phytophthora* (4,5,8,10,13,25), and other related genera (6) and has been associated with reduced disease control (6,7,19,20). Resistance to metalaxyl has been induced in the laboratory with species of *Pythium*, *Phytophthora*, and *Peronospora* (2,3). These reports have raised the question of whether poor sugar beet emergence may be attributed, at least in part, to insensitivity of *Pythium* spp. to metalaxyl.

This study was conducted to evaluate cultures of *P. ultimum* var. *sporangiferum* and *P. aphanidermatum* isolated from diseased sugar beet seedlings for sensitivity to metalaxyl in vitro; to determine if aggressiveness and metalaxyl sensitivity of isolates was related by correlating pathogenicity of these isolates on sugar beet with in vitro metalaxyl sensitivity; and to evaluate the control by metalaxyl seed treatment of isolates with different sensitivities to metalaxyl in vitro. A brief report has been published (1).

MATERIALS AND METHODS

***Pythium* isolates.** Cultures were isolated from dying sugar beet seedlings collected in 1991 to 1993 from 47 fields in Minnesota and North Dakota (usually within 3 weeks after emergence) or from seedlings grown in the greenhouse in field-collected soil (within 2 weeks after emergence). All isolates were from fields where metalaxyl had never been used or where metalaxyl had been used as a seed treatment for the first time. Seedlings were surface-treated with 0.5% NaOCl for 15 s, rinsed twice with sterile distilled water (SDW), and then placed in SDW or on potato dextrose agar (PDA). Tips of hyphae were transferred to PDA; 97 isolates were identified (27) and stored at -80°C (16).

In vitro tests for sensitivity to metalaxyl. Corn meal agar (CMA) at 56°C was amended with 0, 0.01, 0.1, 1, 10, and 100 µg a.i. metalaxyl ml⁻¹ and dispensed (20 ml/1.5- by 9-cm diameter plastic petri plate). A 6-mm-diameter disk was transferred from the margin of a 3-day-old *Pythium* culture on CMA to the inside edge of a petri dish containing the medium (4 rep-

Sugar beet is very susceptible to *Pythium* seed rot and damping-off (17). All seed sold to producers in the RRV and west-central Minnesota are pretreated with fungicide and, since 1992, have been treated with metalaxyl (Apron; Novartis, Greensboro, NC) to control *Pythium* spp. Other seed treatment fungicides are added to control *Aphanomyces cochlioides* Drechs., *Phoma betae* (A. B. Frank), and *Rhizoctonia solani* (Kühn). Metalaxyl (Ridomil; Novartis) also is available as a supplemental in-furrow treatment at planting, but generally is unnecessary and not commonly applied. Viability of commercial seed is between 90 and 95%, yet emergence typically is considerably lower. Emergence of 42 commercial cultivars treated with Apron + Thiram (0.625 + 2.5 g a.i. kg⁻¹ seed, respectively) evaluated at five locations in 1996 averaged 61%, and for individual cultivars ranged from 49 to 71% (Dave Hilde, American Crystal Sugar Co., personal communication). This rate of emergence is not perceived as a problem when producers plant seed to attain high plant populations and then thin to desired stands. When producers plant directly to desired stand, an increasingly popular practice, reduced emergence results in sparse and uneven stands. Factors contributing to reduced emergence include agronomic practices, environmental conditions, pesticide damage, insects, and diseases. Frequently, the cause of reduced stand in a

licate plates per medium for each of 97 isolates). Dishes were incubated in the dark at $21 \pm 1^\circ\text{C}$ using a randomized block design with time as the blocking factor. After 48 h, linear growth of fungal colonies was measured. The concentration of metalaxyl causing 50% growth inhibition compared to growth on nonamended CMA (EC_{50}) was estimated for each isolate from the fitted regression line of the logit-transformed percent inhibition plotted against log-transformed fungicide concentration (14). The experiment was conducted twice.

Pathogenicity tests. The 97 isolates of *Pythium* were evaluated for pathogenicity on sugar beet seedlings by an inoculum-layer technique (12,21). Soil mix consisted of field soil, sand, and peat moss (3:1:1 by volume) that was moistened and autoclaved for 1 h on each of two consecutive days. Plastic pots (10-cm-diameter) were lined with paper toweling, and 250 cm^3 soil was added to each pot. A 3-day-old culture of a *Pythium* sp. grown on 2% water agar (WA, 9-cm-diameter) was placed on the soil surface, cut into quarter sections to allow passage of water, and then covered with 50 cm^3 soil. Twenty-five Maribo Ultramono sugar beet seeds were equidistantly placed on the soil surface and covered with 50 cm^3 soil. Controls included

WA added to soil and soil without WA. Pots were placed in incubators ($25 \pm 1^\circ\text{C}$, 12 h photoperiod) in a randomized block design with four replicates (one replicate per incubator). Soil was watered to keep it moist. Stand counts were taken at emergence and then every 3 to 4 days until 3 weeks after planting. The experiment was conducted twice.

To fulfill Koch's postulates, dying seedlings were removed at each stand count, and at least one plant from each pot was assayed to verify presence of the appropriate *Pythium* sp. Cotyledons were removed and the remainder of the seedling was washed in distilled water, surface-treated in 0.5% NaOCl for 15 s, rinsed twice in SDW, and placed in a section of a quad plate containing 5 ml SDW. After 24 to 48 h, seedlings were microscopically examined for structures characteristic of the *Pythium* species.

***Pythium* control with metalaxyl seed treatment.** Based on rankings from the in vitro screening of *Pythium* isolates for sensitivity to metalaxyl, 20 isolates were selected to evaluate disease control by seed treatment with metalaxyl. These isolates included the 5 least-sensitive isolates of *P. aphanidermatum* (EC_{50} values from 2.70 to 3.12 $\mu\text{g ml}^{-1}$), the 5 least-sensitive isolates

of *P. ultimum* var. *sporangiferum* (EC_{50} values from 0.60 to 1.30 $\mu\text{g ml}^{-1}$), and 10 isolates of *P. ultimum* var. *sporangiferum* that were sensitive to metalaxyl (EC_{50} values from 0.05 to 0.10 $\mu\text{g ml}^{-1}$). Sugar beet seed of Maribo Ultramono was treated with metalaxyl (Apron 50WP) at 0.625 (standard rate), 0.312 (half the standard rate, used to emphasize differences), and 0 (control) g a.i. kg^{-1} seed (11). Soil was prepared and individually infested with each of the 20 isolates as previously described. Pots were arranged in a randomized block design (six replicates per treatment) in a greenhouse at $25 \pm 3^\circ\text{C}$ and a 12-h photoperiod for 3 weeks. Stand counts and seedling assays for pathogen verification were done as previously described. The experiment was conducted twice.

Statistical analyses. Data from repeated experiments were combined for analysis. To stabilize variances, EC_{50} values were adjusted by log transformation and percent final stand values from pathogenicity tests and efficacy tests (with half-standard rate and no metalaxyl seed treatment) were subjected to inverse sine transformations (24). Experiments were analyzed by analysis of variance (SAS Institute, Cary, NC), and mean separations were according to Fisher's least significant difference test, $P = 0.05$.

RESULTS

In vitro tests for sensitivity to metalaxyl.

EC_{50} values for *P. ultimum* var. *sporangiferum* averaged 0.16 (range: 0.05 to 1.30 $\mu\text{g ml}^{-1}$). Most isolates (71 of 76) had EC_{50} values $\leq 0.2 \mu\text{g ml}^{-1}$; 2 isolates were within ≥ 0.5 to 1.0 $\mu\text{g ml}^{-1}$; and 3 isolates were within >1.0 to 1.4 $\mu\text{g ml}^{-1}$ (Fig. 1A).

EC_{50} values for *P. aphanidermatum* averaged 2.06 (range: 1.19 to 3.12 $\mu\text{g ml}^{-1}$). The distribution of EC_{50} values for isolates of *P. aphanidermatum* (Fig. 1B) was spread more evenly throughout a higher range than for isolates of *P. ultimum* var. *sporangiferum*.

Pathogenicity tests. At 3 weeks after planting, 73 of 76 isolates of *P. ultimum* var. *sporangiferum* resulted in stands that were significantly ($P = 0.05$) lower ($\bar{x} = 7\%$; range 0 to 82%) than the noninoculated control ($\bar{x} = 96\%$; data not shown). Sixty-two isolates resulted in stands of $<10\%$.

When *P. aphanidermatum* was evaluated for pathogenicity on sugar beet, the 21 isolates resulted in stands that were significantly ($P = 0.05$) lower ($\bar{x} = 9\%$; range 0 to 60%) than the noninoculated control ($\bar{x} = 96\%$) 3 weeks after planting (data not shown). Stands were $<10\%$ for 18 of the isolates.

Correlation coefficients for aggressiveness on sugar beet in the absence of metalaxyl (percent final stand values from pathogenicity tests) and in vitro sensitivity to metalaxyl (EC_{50} values) were not significant ($P = 0.05$) for isolates of *P. ultimum* var. *sporangiferum* ($r = 0.184$) or *P. aphanidermatum* ($r = -0.029$; data not shown).

Fig. 1. Distribution of EC_{50} values (concentration of metalaxyl causing 50% growth inhibition 48 h after inoculation of corn meal agar with the fungicide) for (A) 76 isolates of *Pythium ultimum* var. *sporangiferum* and (B) 21 isolates of *P. aphanidermatum*.

Pythium control with metalaxyl seed treatment. At the standard rate of metalaxyl seed treatment, average final stands were 91% for isolates of *P. aphanidermatum* and 88 and 94% for the least- and most-sensitive isolates of *P. ultimum* var. *sporangiiiferum*, respectively (Table 1). Sugar beet stands were significantly ($P = 0.05$) lower for isolates 55, 21, and 10 of *P. ultimum* var. *sporangiiiferum* and isolate 70 of *P. aphanidermatum* compared to the noninoculated control (Table 1); these isolates had EC_{50} values among the least sensitive to metalaxyl. There were no significant differences ($P = 0.05$) in stand for the other isolates of *P. ultimum* var. *sporangiiiferum* and *P. aphanidermatum* when metalaxyl was applied at the standard rate compared to the noninoculated control. The correlation coefficient between fungicide sensitivity (EC_{50} values) and final stand with the standard metalaxyl seed treatment was significant only for isolates of *P. ultimum* var. *sporangiiiferum* ($r = -0.549$, 13 df). When seed was not treated with metalaxyl, the 20 isolates resulted in significantly ($P = 0.05$) lower final stands (range 0 to 58%) compared to the noninoculated control (91%).

At half the standard rate of metalaxyl seed treatment, the average final stand was 83% for isolates of *P. aphanidermatum* and 77 and 90% for the least- and most-sensitive isolates of *P. ultimum* var. *sporangiiiferum*, respectively (Table 1). Isolates 70, 72, 78, and 79 of *P. aphanidermatum* and isolates 55, 35, 21, 10, and 95 of *P. ultimum* var. *sporangiiiferum* resulted in significantly ($P = 0.05$) lower stands of sugar beet compared to the noninoculated control (Table 1), and all but isolate 95 had EC_{50} values among the least sensitive to metalaxyl. These isolates were pathogenic to sugar beet ($P = 0.05$) when seed was not treated with metalaxyl, although isolate 35 of *P. ultimum* var. *sporangiiiferum* was less aggressive, resulting in a final stand of 58%. Isolate 51 of *P. ultimum* var. *sporangiiiferum* was the only culture from the 5 least-sensitive isolates of this species that resulted in stands not significantly different from the noninoculated control at the half-rate of metalaxyl seed treatment. This isolate was less aggressive, however, on untreated seed. The correlation coefficient between fungicide sensitivity (EC_{50} values) and final stand with half the standard rate of metalaxyl seed treatment was significant

only for isolates of *P. ultimum* var. *sporangiiiferum* ($r = -0.549$, 13 df).

DISCUSSION

The range of sensitivity to metalaxyl that occurred among isolates of *P. ultimum* var. *sporangiiiferum* (EC_{50} values ranged from 0.05 to 1.30 $\mu\text{g ml}^{-1}$) and *P. aphanidermatum* (EC_{50} values ranged from 1.19 to 3.12 $\mu\text{g ml}^{-1}$) from sugar beet seedlings is consistent with those reported for *Pythium* species (7,28) and for species and varieties of *Phytophthora* (4,5,8,13,22). Cook and Zhang (7) reported a narrow range of sensitivity to metalaxyl of *P. ultimum* var. *sporangiiiferum* pathogenic to wheat (EC_{50} values were estimated as ≤ 0.3 ppm), but tested only a few isolates. White et al. (28) found an isolate of *P. aphanidermatum* from carrot that had a low EC_{50} value (0.39 $\mu\text{g ml}^{-1}$) compared to our isolates of this species. Sanders (20), on the other hand, reported that 60 to 75% of *P. aphanidermatum* isolates from turf grass grew without inhibition on a medium containing 50 and 100 ppm metalaxyl, but these isolates were from a site where metalaxyl had been applied for 3 years.

Table 1. Percent final stand 3 weeks after planting sugar beet seed treated with 0.625, 0.312, and (control) 0 g a.i. kg^{-1} seed metalaxyl (Apron 50WP) into soil infested with 1 of 5 isolates of *Pythium aphanidermatum* or 1 of 15 isolates of *P. ultimum* var. *sporangiiiferum* that represent different levels of sensitivity to metalaxyl (5 least-sensitive and 10 sensitive based on in vitro EC_{50} values)

Isolate	EC ₅₀ µg ml ⁻¹ (Log EC ₅₀) ^c	Final stand (%) ^b		
		0.625 g kg ^{-1d}	0.312 g kg ^{-1d} (sin ⁻¹) ^e	Control (sin ⁻¹) ^e
<i>P. aphanidermatum</i>				
71	3.12 (2.41)	91	90 (1.16)	13 (0.13)
70	2.85 (2.44)	89	78 (0.90)	5 (0.05)
78	2.76 (2.40)	91	79 (0.94)	11 (0.11)
72	2.73 (2.43)	94	88 (1.09)	3 (0.03)
79	2.70 (2.40)	90	82 (0.97)	5 (0.05)
$\bar{x}$	2.83	91	83	7
<i>P. ultimum</i> var. <i>sporangiiiferum</i>				
Least sensitive isolates				
51	1.30 (2.08)	95	95 (1.34)	42 (0.47)
55	1.21 (2.04)	80	65 (0.72)	2 (0.02)
35	1.03 (1.99)	92	81 (0.98)	58 (0.63)
21	0.86 (1.87)	85	72 (0.82)	3 (0.03)
10	0.60 (1.76)	88	74 (0.84)	4 (0.04)
$\bar{x}$	1.00	88	77	22
Sensitive isolates				
50	0.10 (0.97)	94	91 (1.22)	0 (0.00)
45	0.10 (0.94)	92	90 (1.16)	0 (0.00)
6	0.10 (0.96)	97	93 (1.24)	2 (0.02)
95	0.10 (0.94)	94	79 (0.94)	1 (0.01)
8	0.10 (0.96)	92	91 (1.24)	2 (0.02)
34	0.07 (0.75)	96	94 (1.31)	1 (0.01)
11	0.06 (0.70)	91	88 (1.16)	2 (0.02)
9	0.05 (0.65)	92	89 (1.17)	3 (0.03)
27	0.05 (0.64)	94	96 (1.35)	1 (0.01)
38	0.05 (0.66)	94	90 (1.15)	9 (0.10)
$\bar{x}$	0.08	94	90	2
Noninoculated		94	95 (1.31)	91 (1.18)
Standard error	(0.22)	6	(0.21)	(0.13)
LSD, (<i>P</i> = 0.05) ^f	(0.21)	5	(0.17)	(0.10)

^a EC_{50} is the concentration of fungicide causing 50% growth inhibition 48 h after inoculation of corn meal agar amended with metalaxyl. Each value is an average of eight replicates combined from two experiments.

^b Each value is an average of 12 replicates combined from two experiments (25 seeds planted per replicate).

^c Log transformation value.

^d Rate of metalaxyl.

^e Inverse sine transformation value.

^f LSD = least significant difference.

Isolates of the two *Pythium* spp. collected from sugar beet seedlings varied significantly in sensitivity to metalaxyl within and between species. Yet, these isolates were from fields where metalaxyl had not been applied or where metalaxyl had been used as a seed treatment for the first time. A wide range of innate variation among isolates of *Pythium*, even in fields with no prior history of metalaxyl use, has been reported previously (7). White et al. (28) noted variability in sensitivity to metalaxyl among isolates of *Pythium* spp. from carrots, and this variability was not related to previous use of metalaxyl.

Based on the evidence in this study, the relationship is unclear between in vitro metalaxyl sensitivity and aggressiveness on sugar beet (an important component of pathogen fitness that influences the risk of developing fungicide resistance in a pathogen population, 23). There was no significant correlation between in vitro metalaxyl sensitivity of all isolates of *P. ultimum* var. *sporangiferum* or *P. aphanidermatum* and their aggressiveness on untreated sugar beet. In efficacy tests of select isolates, however, 2 of the 5 least-sensitive isolates of *P. ultimum* var. *sporangiferum* were less aggressive in the presence and absence of metalaxyl seed treatment compared to most isolates (Table 1). Also, for the subsample of isolates of *P. ultimum* var. *sporangiferum* that were the least and most sensitive to metalaxyl in vitro, the correlation between fungicide sensitivity and aggressiveness on sugar beet with no metalaxyl seed treatment was significant ($P = 0.05$).

The shape of the frequency distributions of EC_{50} values for *P. ultimum* var. *sporangiferum* and *P. aphanidermatum*, along with results from the tests of control with metalaxyl seed treatment, indicate that biologically meaningful levels of metalaxyl sensitivity are different for the two species. For isolates of *P. ultimum* var. *sporangiferum*, EC_{50} values were unevenly distributed, with most isolates being very sensitive and a few isolates being much less sensitive (Fig. 1A). Isolates of *P. aphanidermatum* had higher EC_{50} values but were more evenly distributed (Fig. 1B). The in vitro EC_{50} values of *P. ultimum* var. *sporangiferum* isolates were a better indicator of in vivo disease control with metalaxyl than those of *P. aphanidermatum*. When sugar beet seed treated with the standard rate of metalaxyl were sown into soil infested with *P. ultimum* var. *sporangiferum*, final stands were negatively correlated with EC_{50} values, and 3 isolates of *P. ultimum* var. *sporangiferum* that were least sensitive to metalaxyl resulted in significant stand reductions from the noninoculated control. Although the 5 isolates of *P. aphanidermatum* tested for control by metalaxyl seed treatment had the highest EC_{50} values, only 1 resulted in significant stand reductions from the noninoculated control at the standard rate. In

evaluations with half the standard rate of metalaxyl seed treatment, nearly all of the sensitive isolates of *P. ultimum* var. *sporangiferum* (based on EC_{50} values) were controlled, whereas many of the least-sensitive isolates of *P. ultimum* var. *sporangiferum* and *P. aphanidermatum* were not controlled at the same level. Similarly, Shew (22) found that in vitro responses corresponded to in vivo responses to metalaxyl in isolates of *Phytophthora parasitica* var. *nicotianae*. Variation in sensitivity to metalaxyl may explain, at least in part, why emergence of sugar beet seedlings often is lower than germination of seed. Other factors include variation in amount of fungicide on seed because of differences in seed size and application method (slurry, various coating materials), other pathogens, and soil temperature and moisture.

Although some isolates of *Pythium* are less sensitive to metalaxyl than others, and this factor may account for some reduction in seedling stands, the continued use of this fungicide as a seed treatment poses limited selection pressure for metalaxyl-resistant isolates (26). Only a small amount of fungicide is applied to seed; it persists in soil for a short time; and only a small proportion of the *Pythium* population in soil is exposed to metalaxyl. In addition, sugar beet crops are grown on a minimum of a 3-year rotation, frequently after crops where metalaxyl is not applied as a seed or soil treatment. If soil application of metalaxyl is used in addition to seed treatment to produce sugar beet and other rotational crops, however, the risk of selecting for naturally occurring strains with less sensitivity will increase, which may result in reduced disease control.

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LITERATURE CITED

- Brantner, J. R., and Windels, C. E. 1996. Variability in metalaxyl sensitivity of *Pythium* spp. pathogenic to sugar beet. (Abstr.) Phytopathology 86:520.
- Bruin, G. C. A., and Edgington, L. V. 1981. Adaptive resistance in Peronosporales to metalaxyl. Can. J. Plant Pathol. 3:201-206.
- Bruin, G. C. A., and Edgington, L. V. 1982. Induction of fungal resistance to metalaxyl by ultraviolet irradiation. Phytopathology 72:476-480.
- Coffey, M. D., and Bower, L. A. 1984. In vitro variability among isolates of six *Phytophthora* species in response to metalaxyl. Phytopathology 74:502-506.
- Coffey, M. D., Klure, L. J., and Bower, L. A. 1984. Variability in sensitivity to metalaxyl of isolates of *Phytophthora cinnamomi* and *Phytophthora citricola*. Phytopathology 74:417-422.
- Cohen, Y., and Coffey, M. D. 1986. Systemic fungicides and the control of Oomycetes. Annu. Rev. Phytopathol. 24:311-338.
- Cook, R. J., and Zhang, B.-X. 1985. Degrees of sensitivity to metalaxyl within the *Pythium*

- spp. pathogenic to wheat in the Pacific Northwest. Plant Dis. 69:686-688.
- Csinos, A. S., and Bertrand, P. F. 1994. Distribution of *Phytophthora parasitica* var. *nicotianae* races and their sensitivity to metalaxyl in Georgia. Plant Dis. 78:471-474.
- Davidse, L. C. 1988. Phenylamide fungicides: mechanism of action and resistance. Pages 63-65 in: Fungicide Resistance in North America. C. J. Delp, ed. APS Press, St. Paul, MN.
- Goodwin, S. B., and McGrath, M. T. 1995. Insensitivity to metalaxyl among isolates of *Phytophthora erythroseptica* causing pink rot of potato in New York. Plant Dis. 79:967.
- Hansing, E. D. 1978. Seed treatments. Pages 88-92 in: Methods for Evaluating Plant Fungicides, Nematicides, and Bactericides. E. I. Zehr et al., eds. APS Press, St. Paul, MN.
- Herr, L. J., and Roberts, D. L. 1980. Characterization of *Rhizoctonia* populations obtained from sugarbeet fields with differing soil textures. Phytopathology 70:476-480.
- Hunger, R. M., Hamm, P. B., Horner, C. E., and Hansen, E. M. 1982. Tolerance of *Phytophthora megasperma* isolates to metalaxyl. Plant Dis. 66:645-649.
- King-Watson, E. D. 1988. Sensitivity monitoring methods for phenylamide fungicides. Pages 61-62 in: Fungicide Resistance in North America. C. J. Delp, ed. APS Press, St. Paul, MN.
- Kuznia, R., and Windels, C. E. 1993. Identification and pathogenicity of *Pythium* species isolated from sugarbeet. 1992 Sugarbeet Res. Ext. Rep. 23:153-159.
- Kuznia, R., and Windels, C. E. 1994. Preservation of *Pythium* species and *Rhizoctonia solani* at -80 C. (Abstr.) Phytopathology 84:1149.
- Kuznia, R. A., Windels, C. E., and Stanghellini, M. E. 1992. Pathogenicity of *Pythium* species on sugar beet using a thermogradient plate. (Abstr.) Phytopathology 82:1088.
- Leach, L. D. 1986. Seedling diseases. Pages 4-8 in: Compendium of Beet Diseases and Insects. E. D. Whitney and J. E. Duffus, eds. APS Press, St. Paul, MN.
- Morton, H. V., and Urech, P. A. 1988. History of the development of resistance to phenylamide fungicides. Pages 59-60 in: Fungicide Resistance in North America. C. J. Delp, ed. APS Press, St. Paul, MN.
- Sanders, P. L. 1984. Failure of metalaxyl to control *Pythium* blight on turfgrass in Pennsylvania. Plant Dis. 68:776-777.
- Schmitthenner, A. F., and Hilty, J. W. 1962. A method for studying postemergence seedling root rot. Phytopathology 52:177-179.
- Shew, H. D. 1985. Response of *Phytophthora parasitica* var. *nicotianae* to metalaxyl exposure. Plant Dis. 69:559-562.
- Staub, T., and Sozzi, D. 1984. Fungicide resistance: a continuing challenge. Plant Dis. 68:1026-1031.
- Steele, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw-Hill Book Co., New York.
- Sujkowski, L. S., Fry, B. A., Power, R. J., Goodwin, S. B., Peever, T. L., Hamlen, R. A., and Fry, W. E. 1995. Sensitivities of Mexican isolates of *Phytophthora infestans* to chlorothalonil, cymoxanil, and metalaxyl. Plant Dis. 79:1117-1120.
- Urech, P. A. 1988. Phenylamide resistance management strategies. Pages 74-75 in: Fungicide Resistance in North America. C. J. Delp, ed. APS Press, St. Paul, MN.
- Van der Plaats-Niterink, A. J. 1981. Monograph of the Genus *Pythium*. Studies in Mycology 21. Centraalbureau voor Schimmelfcultures, Baarn, The Netherlands.
- White, J. G., Stanghellini, M. E., and Ayoubi, L. M. 1988. Variation in the sensitivity to metalaxyl of *Pythium* spp. isolated from carrot and other sources. Ann. Appl. Biol. 113:269-277.