Limitations of a Greenhouse Assay for Determining Potential of Aphanomyces Root Rot in Sugarbeet Fields[†]

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ABSTRACT

Soil was collected in October and November of 1985 through 1989 from 100 fields (scheduled for planting to sugarbeet the following season) in west central Minnesota (WC MN) and in the southern Red River Valley (RRV) and evaluated for presence of *Aphanomyces cochlioides* by a sugarbeet seedling assay in the greenhouse. All dying seedlings were examined microscopically to confirm infection by the fungus. At 4 to 5 wk after planting, surviving seedlings were rated for root rot; these values were used to calculate a root rot index (1 to 100 scale, 0 = all plants healthy, 100 = all plants dead). Root-rot-index values ranged from 6 to 100 in the 100 fields, and *A. cochlioides* was detected in 64. Of 100 fields sampled, 57 were planted to sugarbeet the following season (*A. cochlioides* had been detected in 42 of these fields). In the 2 yr where conditions were favorable for *Aphanomyces*, root- rot-index values correlated with sucrose yields (kg/ha) in WC MN ($P = 0.02$) and the RRV ($P = 0.02$). Weather conditions were dry in 3 of 5 yr, so root rot rarely developed in fields. Reliability of the greenhouse index also was confounded by production practices, pests, and other diseases that affect sugarbeet yield and/or *A. cochlioides*. Thus, the index has potential to predict Aphanomyces root rot in the field, but application of the index is affected by numerous factors in sugarbeet production.

Additional Key Words: *Beta vulgaris, Aphanomyces cochlioides.*

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Soilborne fungal pathogens of sugarbeet *(Beta vulgaris* L.) in Minnesota and North Dakota include *Aphanomyces cochlioides* Drechs., *Pythium* species, and *Rhizoctonia solani* Kühn. Of these pathogens, *A. cochlioides* is the most destructive. The fungus infects roots in warm (20 to 30 C) wet soil and causes damping-off of seedlings and root rot of older plants. Under conditions favorable for disease, entire fields of 2- to 5-wk-old plants can be destroyed (McKeen, 1949). Surviving seedlings resume growth when fields become drier, but roots may be scarred, distorted, and produce numerous lateral roots. Later in the season, root rot can develop in plants that were infected as seedlings or from new infections on sound, older roots. Root rot of older beets results in low yields, reduced sucrose content, and high levels of impurities compared with healthy beets (Papavizas and Ayers, 1974).

Fields infested with *A. cochlioides* pose serious, long-term problems for sugarbeet producers. The fungus produces thick- walled oospores, which persist in soil for years, even in the absence of a sugarbeet crop. Control strategies include cultural practices, varieties with partial resistance to *Aphanomyces,* and the seed treatment fungicide hymexazol (Duffus and Ruppel, 1993; Whitney and Duffus, 1986). However, when disease pressure is severe, these control measures can be insufficient to result in economic yields.

Fink and Buchholtz (1954) developed a greenhouse assay of field soil where the number of dying sugarbeet seedlings infected by *A. cochlioides* was related to yield loss in fields in northern Iowa. Use of root rot indices for soilborne pathogens also has been reported for *Verticillium* on strawberry (Wilhelm, 1957) and potato (Hoyos et al., 1991), *Sclerotium rolfsii* on sugarbeet (Leach and Davey, 1938; Backman et al., 1981), and root rot of pea (Reiling et al., 1960; Sherwood and Hagedorn, 1958) and snap bean (Kobriger et al., 1983).

In the four decades since Fink and Buchholtz described their soil assay procedure, production practices and varieties have been improved to maximize yield and quality of sugarbeet. To aid in crop management decisions, sugarbeet producers in Minnesota and North Dakota need to identify fields with potential for Aphanomyces root rot. Our objectives were to index field soils in the greenhouse to determine potential for Aphanomyces root rot and then to assess these fields during the growing season for Aphanomyces root rot and sucrose yields. A brief report has been published (Windels and Nabben-Schindler, 1991).

MATERIALS AND METHODS

Soil sample collection. Soil was collected from 100 fields in October and November of 1985 through 1989 in west central Minnesota (WC MN) and the Red River Valley (RRV) (scheduled for planting to sugarbeet the following season) (Table 1). Some fields had a documented or uncertain history of root rot and others had no history of root rot. Soil samples were collected within 1- to 3-ha areas of fields with a hand trowel to depths of 15 to 20 cm for each of 30 to 40 stops, spaced at equal intervals in a triangular- or diamond- shaped pattern. At least 4 L of soil were collected per field. Soil was air-dried if wet and stored at 18 C for no longer than 2 wk before processing.

Greenhouse assay. Soil samples were screened (0.6-cm mesh) and thoroughly mixed. Screens and containers were washed in soapy water and surface-treated with 70% ethanol between soil samples to avoid cross-contamination. Soil was dispensed into plastic pots (10.5 x 10.5 x 9.2 cm) to within 3.5 cm of the rim, gently packed, planted with 25 seeds of sugarbeet 'Maribo Ultramono' (treated with 0.3 g a.i. metalaxyl + 2.1 g a.i. thiram/kg seed to protect against *Pythium* and *R. solani),* and covered with 200 cc of soil. For each sample, four to six pots containing raw field soil were prepared; additional soil from the same sample was autoclaved for 1 hr on each of 2 consecutive days, and one pot was planted for comparison with the raw field soil. Two additional controls included soil with a known high potential for Aphanomyces root rot and soil with no history of root rot. Soil was watered at least daily to keep moist, and radiation averaged 400 μmole m^{-2} s^{-1} for 14 hr per day. Greenhouse temperatures were set at 18 $\pm$ 2 C until emergence and then increased to 27 $\pm$ 3 C to favor damping-off. Soil samples collected each fall were assayed concurrently.

Stand counts were made at emergence and then every 3 to 4 days until 4 to 5 wk after planting. Dying seedlings were removed with tweezers, washed, and severed below the cotyledons. Roots were surface--treated in 0.5% NaOCl for 15 to 20 sec and rinsed twice in sterile distilled water. Each root was placed in a section of a quad petri dish containing 5 ml of sterile distilled water, incubated at 22 C, and microscopically examined for characteristic structures of *A. cochlioides, Pythium* spp., and *R. solani* after at least 24 to 48 hr. If these fungi were not observed, the root was blotted dry on a paper towel and placed on potato-dextrose agar (PDA; Difco Laboratories, Detroit, MI) or metalaxyl-benomyl-vancomycin sulfate agar (MBVA), which is semi-selective for *A. cochlioides* from plants (Pfender et al., 1984).

A modification (Sherwood and Hagedorn, 1958) of the Fink and Buchholtz (1954) assay that included an assessment of root rot was done at 4 to 5 wk after planting. Seedlings were removed from soil,

washed in tap water, and rated for disease with a 0 to 3 category system: 0 = hypocotyl and root healthy (white and firm); 1 = light brown discoloration of hypocotyl; 2 = moderate discoloration of hypocotyl; and 3 = hypocotyl severely rotted or plant dead. Seedlings that died before plants were assessed for root rot were assigned a rating of 3. A root rot index (0 to 100 range) was calculated for each pot of soil:

$$\frac{\Sigma(\text{Category value x number of plants in category}) \times 100}{\text{Total number of plants emerged x 3}}$$

Then, a mean root rot index was calculated for each field (based on all pots assayed per soil sample).

Field evaluation. Sugarbeet plants within the area of a field where the soil sample had been collected the previous fall were assessed for symptoms of Aphanomyces root rot in June and August. If foliar symptoms (undersized plants with yellowing of lower leaves, plants sometimes wilted) were observed, at least 10 of these plants were selected (not adjacent or in the same row) and examined for root rot. If no foliar symptoms were observed, 10 plants were selected at random and roots were examined.

Yields were determined in late September by randomly selecting six rows in the 1- to 3-ha areas where soil samples had been collected. Plants were removed until 10 marketable-size roots (>5 cm diameter) were collected per row; row length was measured; and roots were rated for Aphanomyces root rot, which often rots the lower portion of roots. Roots were rated on a scale of 0 to 7, where 0 = clean roots; 1 = root large, crown slightly scurfy; 2 = root large, tip or root infections (brown, scarred surface) superficial and affect <5% root surface; 3 = <25% root constricted and rotted, <25% rotted root remains in soil, or brown scars cover 6 to 25% root surface; 4 = 26 to 50% root constricted and rotted, 26 to 50% rotted root remains in soil, or brown scars cover 26 to 50% root surface; 5 = 51 to 75% root constricted and rotted, 51 to 75% rotted root remains in soil, or brown scars cover 51 to 75% root surface; 6 = >75% root constricted and rotted, >75% rotted root remains in soil, or brown scars cover >75% root surface; and 7 = root completely rotted. American Crystal Sugar Quality Laboratory, East Grand Forks, MN, determined yield of sucrose (Carruthers and Oldfield, 1961).

RESULTS

Greenhouse assay: seedling stands and isolations. Of 100 fields sampled over 5 yr, *A. cochlioides* was detected in 64 by the greenhouse assay (Table 1). Emergence averaged 94% in soil samples from 64 fields where *A. cochlioides* was detected and 95% in soil samples from 36 fields where the fungus was absent (data not shown).

Variation in stands occurred among the 64 soil samples where *A. cochlioides* was detected by 4 to 5 wk after planting (Figure 1). For example, Aphanomyces damping-off was severe in 12 soil samples and stands averaged 1 to 10% (percent seedlings surviving based on number of seeds planted), whereas Aphanomyces damping- off was negligible in three soil samples and stands averaged >90%. Of 3,000 dying seedlings, *A. cochlioides* was isolated from 82% (data not shown). Sometimes seedlings with typical symptoms of Aphanomyces damping-off yielded no fungi, or *R. solani* (present in 40 fields) and species of *Pythium, Alternaria, Fusarium,* or *Penicillium* were isolated.

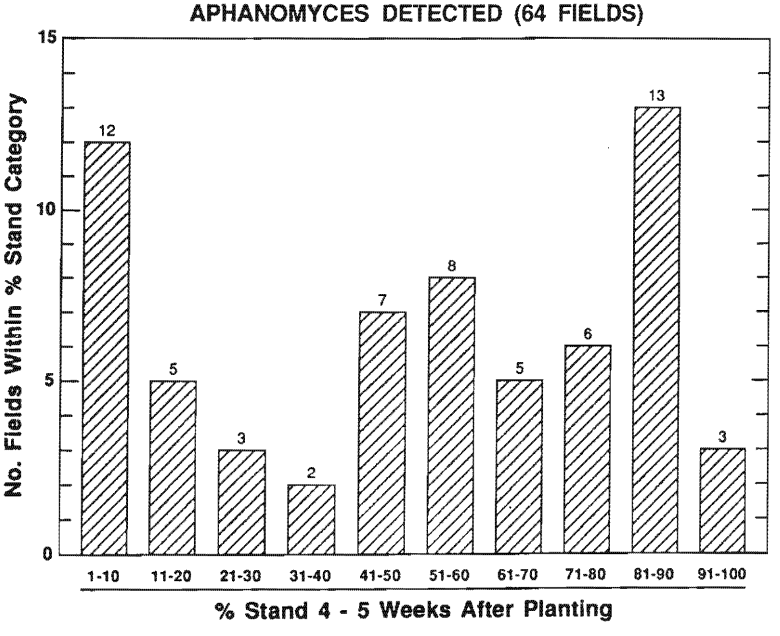

Figure 1. Number of fields where *Aphanomyces cochlioides* was detected in the greenhouse assay and categorized into 10 stand ranges; stand is based on percent sugarbeet seedlings alive at 4 to 5 wk after planting compared to number of seeds planted.

In the 36 soil samples where *A. cochlioides* was not detected, stand loss averaged 4% at 4 to 5 wk after planting (data not shown). *Pythium* was occasionally isolated. *R. solani* was isolated from one or more dying seedlings in 11 soil samples and, in one of these samples, 30% of the seedlings died and were infected by *R. solani.*

Greenhouse assay: root rot index. Root-rot-index values in the 64 soil samples where *A. cochlioides* was detected varied from less than 10 (low disease level) to 100 (maximum disease) (Figure 2). Data are not shown for individual fields, but in four fields, *A. cochlioides* was identified in only one dying plant per soil sample; in five fields, all seedlings died before the assay was completed; and one soil sample was heavily infested by *R. solani (A. cochlioides* was not detected) and had a root rot index of 45.

Field evaluation: wet seasons. Only 57 of the 100 fields evaluated in the greenhouse were planted to sugarbeet the following season (Table 1), because producers changed their planting plans. In the greenhouse assay, *A. cochlioides* had been detected in 42 of the 57 fields.

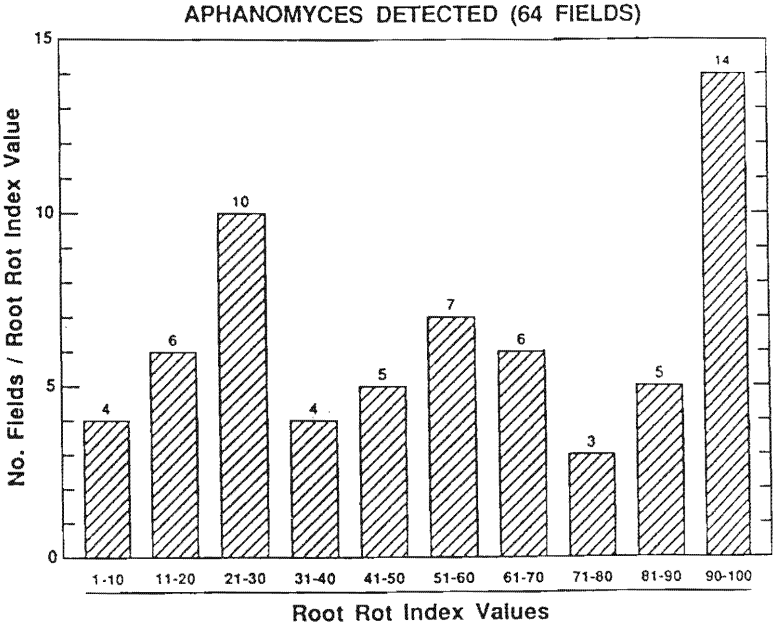

Figure 2. Number of fields where *Aphanomyces cochlioides* was detected in the greenhouse assay and categorized into 10 root-rot-index ranges based on a 0-100 index.

Table 1. Occurrence of *Aphanomyces cochlioides* in fields sampled in October-November, 1985 through 1989 in west central Minnesota (WC MN) and the southern Red River Valley (RRV) based on the greenhouse assay and the number of these fields planted to sugarbeet the following season.

	No. Fields Tested in Greenhouse Assay				No. Fields Planted to Sugarbeet			
	WC MN		RRV		WC MN		RRV	
Year[a]	Sampled	Aph[b]	Sampled	Aph	Sampled	Aph	Sampled	Aph
1985-1986	11	8	1	0	8	6	1	1
1986-1987	13	8	1	0	9	6	0	0
1987-1988	9	7	13	6	8	6	0	0
1988-1989	11	9	9	8	8	8	6	5
1989-1990	11	9	21	9	6	4	11	6
Total	55	41	45	23	39	30	18	12

[a] Soil samples were collected and assayed in the greenhouse in November-December. Fields planted to sugarbeet the following season were sampled for disease, yield, and quality.

[b] Number of fields where *A. cochlioides* (Aph) was detected in the greenhouse assay.

In 1986 and in select regions in 1990, rainfall was normal to above normal. In May-September, 1986, 57.7 cm of rain fell in Olivia, MN (WC MN), compared with the 30-yr average of 44.4 cm for this period. This precipitation favored Aphanomyces root rot, which was observed in six fields where the fungus had been detected in the greenhouse assay but was not observed in two fields where *A. cochlioides* was not detected in the greenhouse assay. There was a significant negative correlation between root- rot-index values in the greenhouse and kg sugarbeet/ha ($r = -0.75$, $P = 0.02$, $n = 9$), root rot index and percent sucrose ($r = 0.68$, $P = 0.04$), and root rot index and kg recoverable sucrose/ha ($r = -0.76$, $P = 0.02$) (data not shown).

Precipitation at Wahpeton, ND (RRV), in May-September 1990 was 29.9 cm, which is below normal for this period (38.9 cm), but 10.6 cm of rain fell in June, which favored Aphanomyces root rot early in the season. Disease occurred in four of six fields where the fungus had been detected in the greenhouse; symptoms were not observed in five fields where *A. cochlioides* was not detected in the greenhouse assay. There were significant negative correlations between root-rot-index values in the greenhouse and kg sugarbeet/ha ($r = -0.56$, $P = 0.07$, $n = 11$) and between root-rot-index values and kg recoverable sucrose/ha ($r = -0.67$, $P = 0.02$) (data not shown).

The regression line and individual observations (fields) for root-rot-index values in the greenhouse assay and recoverable sucrose in the field for WC MN in 1986 and the RRV in 1990 are shown in Figure 3. Two sites in WC MN with high root-rot-index values (54 and 62, Figure 3) in the greenhouse assay had high recoverable sucrose; these fields had been tiled and were adequately drained so Aphanomyces root rot was not a problem.

Field evaluation: dry seasons. Dry weather severely limited Aphanomyces root rot and also confounded sugarbeet development, yield and quality, regardless of the presence or absence of *A. cochlioides* for fields evaluated in WC MN in 1987 through 1990 and in the RRV in 1989. In WC MN, precipitation was below average (43 cm in 1987, 26.4 cm in 1988, and 31.9 cm in 1989) in May-September at Olivia, MN. During 1987 through 1989, a few plants with symptoms of *Aphanomyces* were noted in nine of 20 fields where the fungus had been detected in the greenhouse; symptoms were not observed in the five fields where *A. cochlioides* had not been detected in the greenhouse assay. Rainfall was favorable for root rot in 1990 in the southern portion of WC MN (50 cm from May through September at Olivia, MN), but the fields sampled in

the fall of 1989 were located at the northern end of the cooperative where rainfall was low. Of these six fields, a few infected seedlings were observed in three of four fields where *A. cochlioides* had been detected in the greenhouse assay, and disease was not observed in the other two fields. Correlation coefficients calculated for the greenhouse root rot index versus yield, percent sucrose, or recoverable sucrose/ha were not significant ($P < 0.05$) for WC MN fields sampled in 1987-1990 (data not shown).

In the southern RRV, precipitation at Wahpeton, ND, during May-September 1989 was 34.8 cm. Only 3.3 cm of precipitation fell in June, and symptoms of *Aphanomyces* were not observed. Rainfall (16.6 cm) in July and August 1989 favored some development of Aphanomyces root rot, which was observed on a few plants in five of six fields where the fungus had been detected in the greenhouse. A low level of Aphanomyces root rot was observed in one field where the fungus had not been detected in the greenhouse assay. There were no significant

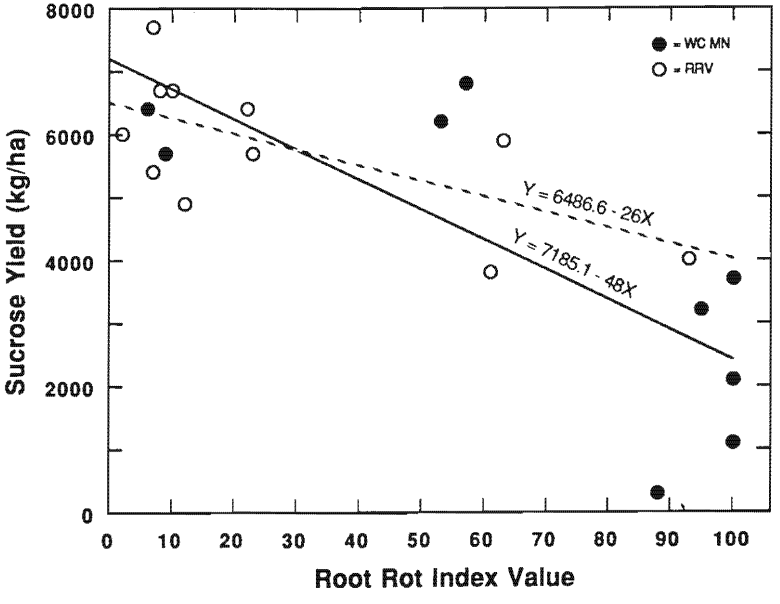

Figure 3. Regression of root-rot-index values (0 to 100) in the greenhouse assay and sucrose yields (kg/ha) in the field: data presented for west central Minnesota (WC MN), 1986 (---, $r^2 = 0.57$) and for the southern Red River Valley (RRV), 1990 (---, $r^2 = 0.45$).

correlations ($P < 0.05$) between root rot index versus yield, percent sucrose, or recoverable sucrose (data not shown).

In fields where *A. cochlioides* was observed during 1986 through 1990, damping-off was more common than root rot. Of the 48 fields harvested in 1987 through 1990, root rot indices (0 to 7 scale) were low and averaged ≤ 1 for 38 fields, > 1 to 2 for seven fields, and > 2 to 3 for three fields. These values may be slightly inflated because a root occasionally was infected by A. cochlioides and *R. solani* and it was impossible to rate for damage caused by *A. cochlioides* alone.

In addition to dry weather, reliability of the greenhouse assay was compromised by a number of factors that affected sugarbeet yield and/or Aphanomyces root rot in the field. These observed factors included: Cercospora leaf spot, root maggot, root aphids, grasshoppers, herbicide damage, weeds, planting practices (e.g., planting date, plant populations), hail damage, soil drainage, and variety selection.

DISCUSSION

In wet seasons (WC MN in 1986 and RRV in 1990), the greenhouse root rot index was a reasonable indicator of Aphanomyces root rot and correlated with yields of recoverable sucrose. However, application of the greenhouse index in the field often was limited by other confounding factors, e.g., dry weather and cultural practices that affected yield and/or Aphanomyces root rot. Fink and Buchholtz (1954) reported a correlation between number of sugarbeet seedlings infected by *A. cochlioides* in the greenhouse and yield loss in the field, but their studies occurred in two seasons when Aphanomyces root rot commonly occurred.

Reliability of the greenhouse assay is dependent on assaying a soil sample that represents the field. In this study, fields were sampled after fall tillage (plowing and disking) and were as close to spring planting conditions as possible. McKeen (1949) reported that populations of *A. cochlioides* decrease in the soil profile to depths of 15- to 20-cm. We avoided sampling fields that were plowed but not disked because plowing moves soil from the bottom of the furrow to the surface, and soil on the surface is placed deeper in the soil profile. Thus, the fungus would not be distributed uniformly or represent placement of inoculum at planting.

When sampling fields with low levels of inoculum, or nonrandomly distributed (aggregated) inoculum, it is possible to miss localized concentrations of *A. cochlioides*. One field in this study

developed Aphanomyces root rot although the fungus had not been detected in the greenhouse assay. Fink and Buchholtz (1954) also reported that Aphanomyces root rot occasionally developed in fields when it had not been detected in the greenhouse assay. Either the fungus was not present in the soil sample that was assayed or the assay was not sensitive enough to detect the fungus.

Methods for quantifying populations of *A. cochlioides* are inadequate and are limited to baiting the fungus from soil with sugarbeet seedlings. This approach is a relative "fungus activity index" and a number of problems affect its sensitivity and reliability. Boosalis and Scharen (1959) found that viable oospores of *Aphanomyces* have low levels of germination, so the fungus may be present but not active or detectable. Also, *A. cochlioides* is not consistently isolated from seedlings, even when symptoms of disease are typical. Thus, the assay developed by Fink and Buchholtz (1954) (based on percentage of seedlings from which *A. cochlioides* was isolated) was supplemented with a root rot index based on relative stand loss and severity of infection (Sherwood and Hagedorn, 1958; Kobriger, et al., 1983). Also, we were concerned about plant-to-plant spread (Pfender and Hagedorn, 1983) in the greenhouse assay, which would inflate values of the index. However, several instances were observed where a single seedling was infected, whereas adjacent plants remained healthy. Apparently, regular collection of dying seedlings minimized spread of *A. cochlioides* from diseased to healthy plants, although this possibility cannot be ignored.

The implications of detecting *A. cochlioides* in a field depends on occurrence of warm wet weather and on when and how long those conditions persist. Oospores and zoospores of *Aphanomyces* infect roots within 2 hr under ideal conditions (Cunningham and Hagedorn, 1962; Papavizas and Ayers, 1974). Root rot can develop after a single rainstorm if soil remains wet long enough for *Aphanomyces* to infect roots. Under prolonged wet periods, this primary inoculum results in production of more inoculum and of multiple secondary infections. The polycyclic nature of the disease accounts for root rot in a wet season when populations of the fungus are low or when the fungus is not detected by the greenhouse assay. Pfender and Hagedorn (1983) found that low levels of inoculum of *Aphanomyces euteiches* required more time to reach a given level of root rot on peas compared with high levels of inoculum. Thus, during prolonged wet weather, fields initially identified with low levels of inoculum could sustain a buildup of disease over the season, whereas fields initially identified with high levels of *A. cochlioides* would suffer severe disease early in the season. Also, Aphanomyces damping-off can have a greater impact on crop yield than late-season root rot.

The greenhouse root rot index has limitations, but it still has a place in sugarbeet production. It can be used to identify fields with high levels of infestation that should not be planted to sugarbeet. It can be used to discern whether fields with a history of stand establishment problems are infested by *A. cochlioides* or if another factor is responsible. When producers know that *A. cochlioides* is present in certain fields, they can make more prudent decisions in selection of cultivars, seed treatment with hymexazol, duration and crops in rotations, planting dates, sanitation measures, and regulation of soil moisture. There is a continuing need for a sensitive, reliable, and inexpensive method to quantify propagules of *A. cochlioides* in soil to assist producers in making these management decisions.

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