
Characterization and Spatial Distribution of *Aphanomyces* in Sugarbeet Fields

A.T. Dyer^{1*}, L.J. Szabo², and C.E. Windels³

¹Former Research Assistant, University of Minnesota, Department of Plant Pathology, St. Paul 55108; ²USDA-ARS Cereal Disease Laboratory, St Paul 55108; and ³University of Minnesota, Northwest Research and Outreach Center, Crookston 56716

ABSTRACT

To study phenotypes and spatial distribution of *Aphanomyces* in sugarbeet fields with histories of root rot, 80 soil cores were collected 1-m apart along a transect in one field each in Minnesota (MN), North Dakota (ND), and Texas (TX). *Aphanomyces* was isolated from 52, 84, and 49% of the soil cores collected in MN, ND, and TX, respectively. Randomly amplified polymorphic DNA (RAPD) analysis was completed on 41, 41, and 39 isolates from MN, ND, and TX, respectively, and *A. euteiches* as an outgroup. A single polymorphic (1400 bp) RAPD product was specific to 56% of the Texas isolates. Oogonia of isolates from sugarbeet had a mean diameter of 23.2 μm compared to two isolates of *A. euteiches* which had a mean diameter of 26.1 μm . Isolates of *Aphanomyces* from sugarbeet were highly pathogenic on sugarbeet and root rot indices (0 to 100 scale) ranged from 67 to 100 ($n=12$); the same isolates were not pathogenic on pea, oat, or tomato. Isolates of *A. euteiches* were pathogenic only on pea. RAPD analyses, morphology, and pathogenicity of our isolates from sugarbeet were consistent with the description of *A. cochlioides*. Ordinal runs analysis detected aggregation of *A. cochlioides* in MN and TX fields ($P = 0.029$, $P = 0.002$, respectively) while isolates at the ND site were uniform ($P = 0.89$).

Additional key words: Oomycete, root rot, *Beta vulgaris* L.

* Corresponding author: adyer@montana.edu

A*phanomyces cochlioides* Drechs. causes significant perennial losses in sugarbeet (*Beta vulgaris* L.) yield in the United States and

throughout the world (Didelot et al., 1995; Drechsler, 1929; Papavizas and Ayers, 1974; Payne et al., 1994; Smith, 1997; Szymczak and Banaszak, 1994; Windels and Lamey, 1998). When soil is warm (15 - 35°C) and wet, *A. cochlioides* infects seedlings as well as older roots and results in root rot and stand losses. The Red River Valley (RRV) of Minnesota and North Dakota plus west central Minnesota is the leading region in sugarbeet production in the United States with about 300,000 ha planted annually (Bangsund and Leistritz, 1999). Unusually wet weather in this region for the last 10 years has resulted in increased prevalence and severity of *Aphanomyces* root rot. A recent survey estimates that *A. cochlioides* infests about 50% of the land planted to sugarbeet in the RRV and west central Minnesota (B. J. Jacobsen, Montana State University, Bozeman, *personal communication*). In 2000, the economic impact of *Aphanomyces* root rot on about 200,000 ha of sugarbeet in the RRV was conservatively estimated at \$18 million in direct losses to producers from abandonment of 8,000 ha and yield losses in other infested fields (A. Cattanaach, American Crystal Sugar Co., Moorhead, MN, *personal communication*).

Oospores of *A. cochlioides* form in diseased roots and persist in soil for many years (Papavizas and Ayers, 1974) so disease management is essential for continued production of sugarbeet. Controls consist of partially resistant cultivars, seed treatment (hymexazol), early planting, control of weed hosts, long rotations between sugarbeet crops, and avoidance of heavily infested fields (Windels and Lamey, 1998). In wet seasons when *Aphanomyces* root rot is severe, these control measures have not resulted in economic returns to producers. There also are concerns that possible variability in populations of *A. cochlioides* affect efficacy of hymexazol or stability of cultivar resistance to root rot. If such variability occurs, then spatial distribution of the pathogen also needs to be understood as a factor in disease management.

Isolates of *Aphanomyces* cultured from diseased sugarbeet roots often are assumed to be *A. cochlioides* but other species also are reported as pathogenic to sugarbeet (Larrison, 1994; Scott, 1961). Three species have nearly identical morphological traits but *A. cochlioides* is highly pathogenic to sugarbeet while *A. camptostylus* Dresch. (primary host is oat, *Avena sativa* L.) and *A. cladogamus* Dresch. (primary host is tomato, *Lycopersicon esculentum* L.) are marginally pathogenic to sugarbeet (Scott, 1961). A fourth potential species isolated from spinach (*Spinacia oleracea* L.) and barley (*Hordeum vulgare* L.) in Europe, reported as "A3", has larger oospores than the other three species and is moderately pathogenic on sugarbeet (Larrison, 1994). It is unknown if species other than *A. cochlioides* attack sugarbeet in the RRV and west

central Minnesota. Cereals are grown in the region, but spinach and tomato are not grown commercially.

Molecular markers provide an important tool for understanding variability between and within species of *Aphanomyces*. Cultures isolated from spinach, sugarbeet, and several weed and crop species were assigned to four species by isozyme analyses (Larrison, 1994). More recently, randomly amplified polymorphic DNAs (RAPDs) distinguished variability within *A. euteiches*, which correlated with host preference (Malvick et al., 1998).

In the research reported here, three fields with a history of *Aphanomyces* root rot were sampled to determine: 1) if cultures of *Aphanomyces* isolated from sugarbeet have characteristics consistent with *A. cochlioides* based on RAPDs, morphology, and pathogenicity and 2) the spatial distribution of *Aphanomyces* inoculum within these fields. A preliminary report has been published (Dyer et al., 1998).

MATERIALS AND METHODS

Aphanomyces isolations

Soil samples were collected in June or July, 1997 from one field each near Buffalo Lake, Minnesota; Cavalier, North Dakota; and Bushland, Texas. The Minnesota and the Texas sites had histories of over 30 years of sugarbeet production and *Aphanomyces* root rot. The North Dakota site had a history of small grain and dry bean production but when planted to sugarbeet for the first time in 1996, the crop was severely affected by *Aphanomyces* root rot. At each site, starting approximately 30 m from the edge of the field, a soil core was collected every meter along a transect for a total of 80 samples. For Minnesota and North Dakota samples, soil cores were approximately 7.5 cm in diameter and 15 cm in depth. For Texas samples, cores were 2.5 cm in diameter and 15 cm in depth. Individual cores were placed in paper bags and stored at 23°C.

Within 1 week of collection, each soil core was passed through a 6-mm galvanized wire mesh screen and 20 cm³ of soil was dispensed into an individual cell (2.5 x 2.5 x 8 cm) of a plastic cutting tray. Soil was added to alternating cells in a row, with every other row skipped to avoid cross-contamination. Sugarbeet seeds of American Crystal 'Maribo Ultramono' were treated with metalaxyl and pentachloronitrobenzene (0.3 and 1 g a.i. kg⁻¹, respectively) to avoid infection by *Pythium* spp. and *Rhizoctonia solani* Kühn. Two seeds were planted per cell and trays were placed in a growth chamber at 24 ± 1°C with a 16 h photoperiod. Soil was watered as needed to keep moist. Seedlings

were monitored daily. Dead and dying seedlings were removed, washed, and surface-treated in 0.5% NaOCl for 15 sec, rinsed twice with sterile distilled water (SDW), and placed in Petri dishes containing SDW. Seedlings were examined after 24, 48, and 72 h and those with microscopic structures typical of *Aphanomyces* were placed on a semi-selective medium (Pfender et al., 1984). At 30 days after planting, all surviving seedlings in the cutting trays were removed and assayed for *Aphanomyces* following the same procedures.

Isolates collected from the seedling assay were transferred to corn meal agar (CMA, Difco, Detroit, MI) supplemented with rifampicin (38 mg/L). For each isolate, a single hyphal tip was transferred to a new CMA plate to insure only one genotype per isolate. Cultures were stored in SDW and were replaced in storage every 6 to 9 months by culturing on CMA with rifampicin to repeat the process for storage (Windels, 2000).

RAPD analyses

Mycelium for DNA extractions was grown from mycelial plugs in stationary culture for 3 days on 10% V8 broth amended with rifampicin (50 mg/L), penicillin G (50 mg/L) and CaCO₃ (1 g/L). These culture conditions adequately controlled bacterial contaminants (Dyer and Leonard, 2000). Mycelia were separated from broth with Whatman #1 filter paper using a vacuum pump. Mycelial mats were rinsed once with SDW to remove residual broth. Two mats were harvested per isolate; one mat was processed immediately for DNA extractions and the other was stored at -20°C as a backup.

One hundred and forty-seven decamer primers with G+C contents ranging from 60 to 80% were screened for use in RAPD analyses. Primers were obtained from three commercial primer sets (Genosys Biotechnologies Inc., The Woodlands, TX; Operon Technologies Inc. Alameda, CA; University of British Columbia, Canada) and one previously reported set (Kubelik and Szabo, 1995). Primers were screened against DNA collected from one isolate of *A. cochlioides* each from Texas and Colorado (provided by Dr. C.M. Rush, Texas A & M, Bushland) and three isolates from Minnesota. Based on results of the initial screening, six primers were selected (Table 1) to analyze the populations of *Aphanomyces* isolated from sugarbeet.

RAPD analyses (Welsh and McClland, 1990; Williams et al., 1990) were conducted on 41, 41, and 39 isolates of *Aphanomyces* collected in Minnesota, North Dakota, and Texas, respectively. Two isolates of *A. euteiches* (supplied by Dr. D.K. Malvick, University of Illinois, Urbana) were included as an outgroup in all experiments.

Attempts to acquire isolates of *A. camptostylus*, *A. cladogamus*, and "A3" for this study were unsuccessful.

Preparation and quantification of DNA for RAPD analyses followed the protocol of Malvick et al. (1998) except NaCl was not added to the extraction buffer. The RAPD reactions were performed in a total volume of 25 μ l containing 4 mM MgCl₂; 50 mM KCl; 10 mM Tris -HCl (pH = 8.3); 0.001% gelatin; 50 mM dATP, dTTP, dCTP and dGTP; 10 pmoles of primer; 5 ng of genomic DNA; and 1 unit Amplitaq polymerase (Perkin Elmer, Foster City, CA). Amplifications were performed in either a PTC 100 or a PTC 200 thermocycler (MJ Research Inc. Watertown, MA) programmed as follows: 1) 2 min at 94°C for initial melting, 2) 1 min at 94°C, 3) 1 min at 36°C, and 4) 2 min at 72°C. Steps 2 through 4 were repeated 39 times followed by a final 10 min at 72°C.

Morphology

Twenty isolates of *Aphanomyces* from sugarbeet and two isolates of *A. euteiches* were measured with a micrometer at 400X for diameter of hyphae, oogonia, and zoospore cysts and for number of antheridia surrounding the oogonia. To measure diameters of hyphae, isolates were grown on CMA amended with rifampicin (25 μ g ml⁻¹) for 3 to 5 days in the dark at 23 \pm 1°C. A 3-mm diameter piece of agar was removed at the margin of each actively growing culture, compressed under a cover slip, and the diameter of 10 hyphae per isolate were measured. To measure the diameter of oogonia, cultures were grown in 30 ml of oat broth (Parke and Grau, 1992) in 25 x 100 mm test tubes and incubated in the dark from 33 to 37 days at 23 \pm 1°C. Ten randomly selected oogonia were measured per isolate and numbers of antheridia surrounding each oogonium were counted. Zoospores were produced following the protocols of Mitchell and Yang (1966), as modified by Windels (2000). A 2-ml aliquot of the resulting zoospore suspension was added to a test tube and placed on a vortex mixer (Labline Instruments, Inc., Melrose Park, IL) for 30 sec to initiate encystment of zoospores. Diameters of ten zoospores were measured in a Spiers-Levy Esinophil counting chamber.

Pathogenicity tests

Twelve isolates of *Aphanomyces* from sugarbeet and two of *A. euteiches* (same as evaluated for morphology) were evaluated for pathogenicity on sugarbeet, oat, tomato, and pea. Isolates were grown on 20 ml water agar (WA) amended with 100 mg/L CMA (to encourage growth of *A. euteiches*, which grows poorly on unamended WA) in a 10-cm diameter petri dish. Uninoculated plates of WA were included as controls.

Table 1. Number and size of scorable RAPD products obtained from *Aphanomyces cochlioides* and *A. euteiches*.

Primer [†]	Sequence 5'-3'	Number products A. <i>cochlioides</i>	Number products specific to <i>A. cochlioides</i>	Number products specific to <i>A. euteiches</i>	Number products specific to <i>A. euteiches</i>	Total number products	Size range (base pairs)
Gen-80-7	GCACGCCGGA	8	5	4	1	9	500-2550
Gen-80-11	GCAGCAGCCG	5	2	5	2	7	500-1750
Gen-80-29	ACGGCGGCTC	5	1	6	2	7	650-1400
Gen-80-34	CGCCACGAGC	6	1	6	1	7	600-1800
OP-D-8	GTGTGCCCCA	4	1	4	1	5	600-2350
CRL-33	CTCGCGCCCC	9	6	6	3	12	500-2500
Total		37	16	31	10	47	500-2550

[†] Designated by primer source: Gen 80 = Genosys 80% GC content primers, OP = Operon primers and CRL = a set previously reported by Kubelik and Szabo (1995).

A soil mixture (Waukegan silt loam field soil: sand: peat: composted manure, 6:6:5:2, v:v:v:v, pH = 7.2) was pasteurized for 2 h at 75°C. Soil was added to 10-cm diameter plastic pots (350 cm³/pot), watered, and then a 7-day-old culture of *Aphanomyces* on WA amended with CMA was placed on the surface. For oat ('Rodney') and pea ('Little Marvel'), five seeds were placed equidistantly on the agar surface and covered with 100 cm³ of soil. For sugarbeet, 50 cm³ of soil was placed over the agar, sown with 20 seeds of 'American Crystal 9363' (treated with metalaxyl and thiram at 0.625 g and 2.5 g a.i. kg⁻¹ of seed, respectively), and then were covered with 50 cm³ of soil. Tomato seed ('Rutgers') was placed on moistened paper towels in the dark for 48 h to prime germination and then planted (10 seeds/pot) following the procedure described for sugarbeet. Pots were placed in a randomized design in a greenhouse set at 18°C (night) and 26°C (day) with a 12-h photoperiod. Soil was watered to keep moist. There were three replicates per isolate and crop. When the pathogenicity experiment was repeated, several modifications were made: *A. euteiches* isolate 467 was substituted for Ae174 (which had died in storage); the soil mixture was a field soil and sand mixture (3:1, v:v) that was autoclaved (1 h on two consecutive days); there were four replicates per isolate and crop; and the experiment was performed in a growth chamber with a 16-h photoperiod at 20°C for the first week, followed by 25°C.

Stand counts were made at emergence and every 2 or 3 days thereafter until 3 weeks after planting. Dying seedlings were removed and assayed for *Aphanomyces* as previously described. Three weeks after planting, roots of remaining seedlings of all crops were rated for root rot on a 0 to 3 scale where 0 = completely healthy and 3 = dead or dying. A root rot index (RRI) then was calculated (Windels and Nabben-Schindler, 1996). Roots were collected from one pot per isolate per crop and assayed for *Aphanomyces*, as previously described.

Statistical methods

The two-sample t-test was used to compare morphology and pathogenicity among isolates sorted by location and RAPD phenotypic group. Variability among individual isolates for morphology and pathogenicity was performed with a single-factor analysis of variance. When data were significant, a Tukey-Kramer multiple comparison test was performed ($P = 0.05$). Root rot indices were subjected to square root, arc sin transformations and analyzed by a single-factor analysis of variance. If the analysis showed significant variation among isolates, a Tukey-Kramer multiple comparison test was performed to identify differences ($P = 0.05$). Runs analyses (Madden et al., 1982) were performed to

determine spatial distribution of *Aphanomyces* along transects at the three sites. To test for genotypic independence among closest neighbors, a χ^2 test of independence was conducted.

RESULTS

Cultures

The seedling assay of soil samples collected from sugarbeet fields in Minnesota, North Dakota, and Texas resulted in isolation of 42, 67, and 39 cultures of *Aphanomyces*, respectively, and represented isolations from 52, 84, and 49% of the soil cores, respectively.

RAPD analysis

In initial screening of 147 primers against five isolates of *Aphanomyces* from sugarbeet (three, one, and one isolates from Minnesota, Texas, and Colorado, respectively) only one polymorphic product was detected by the primer CRL-33. Five other primers produced between five and 12 clearly amplified DNA products and sizes ranged from 500 to 2550 base pairs (bp) (Table 1). These six primers were selected for analysis of 121 isolates of *Aphanomyces* from sugarbeet and two isolates of *A. euteiches*. A total of 47 RAPD products were scored. Sixteen were specific to *Aphanomyces* isolates from sugarbeet seedlings and 10 were specific to *A. euteiches*. To assure reproducibility, RAPD analysis of the North Dakota population was repeated and no change in amplified products



Fig. 1. Primer CRL-33 amplified products from left to right; six isolates of *Aphanomyces* from sugarbeet collected in Minnesota (B), six from North Dakota (C), and six from Texas (T). Lanes at the left and right margins are a 500 base pair ladder (LAD); (←) identifies the 1400 bp RAPD product for isolates T1, T51, and T71.

was detected. A single polymorphic 1400 bp product amplified by primer CRL-33 was detected among the sugarbeet isolates (Fig. 1). It was not detected within the Minnesota and North Dakota populations but was a unique subset of the Texas population. Twenty-two of the 39 Texas isolates (56%) displayed the 1400bp product.

Morphology

Irrespective of geographic source or RAPD phenotype, isolates did not significantly differ for number of antheridia per oogonia (mean 3.0, s.d. = 0.30, $P = 0.78$), encysted zoospores (mean = 10.2 μm , s.d. = 0.55, $P = 0.92$), or for diameter of hyphae (mean = 7.0 μm , s.d. = 0.45, $P = 0.63$) (Table 2). No significant differences were identified for oogonial diameter of isolates from sugarbeet (averaged 23.2 μm , s.d. = 0.86) among locations or among RAPD phenotypes ($P > 0.05$). Comparison analysis among isolates, however, showed that oogonia of isolate B1 from Buffalo Lake, MN, were significantly larger than oogonia of isolate T71 from Bushland, TX (Tukey-Kramer multiple comparison test, $P = 0.05$).

Oogonia diameters for *A. euteiches* isolates averaged 26.1 μm (s.d. = 0.98) and were significantly larger than oogonia of *Aphanomyces* isolates from sugarbeet ($P < 0.001$). All *Aphanomyces* isolates from sugarbeet produced smaller oogonia than those of *A. euteiches* and most (65%) were significantly smaller (Tukey-Kramer multiple comparison test, $P = 0.05$).

Pathogenicity

In the first experiment, sugarbeet sown in soil inoculated with *Aphanomyces* isolates obtained from sugarbeet had an average RRI of 94.4 (range: 64 - 100) and most isolates were equally virulent (Table 3). Isolate B22 and T71 (a 1400 bp RAPD phenotype), however, had indices of 92 and 86 respectively, which were significantly lower than most isolates but were significantly greater than isolate T72 (RRI = 64, $P = 0.05$). At time of inoculation, mycelium had reached margins of the plates for all isolates except isolate T71. In the second experiment, sugarbeet sown into soil inoculated with *Aphanomyces* had a mean RRI of 97.7 (range: 87 to 100) and only isolate B14 had a significantly lower RRI compared to other isolates from sugarbeet (Table 3).

In both experiments, all isolates of *Aphanomyces* from sugarbeet were not pathogenic on pea (Table 3) but isolate B13 was re-isolated from one pea seedling (data not shown).

Cultures of *A. euteiches* were pathogenic on peas but not on sugarbeet (Table 3). None of the isolates were pathogenic on oat or

Table 2. Characteristics of selected *Aphanomyces* isolates collected from one sugarbeet field each in Minnesota (MN), North Dakota (ND), and Texas (TX) and two *A. euteiches* (Ae) isolates.

Isolate designation	Source	RAPD 1400 bp product [†]	Number antheridia	Size (μm)		
				Oogonia [‡]	Zoospore	Hyphae
B1	Buffalo Lake, MN	-	3.5	25.1	10.8	8.1
B13	Buffalo Lake, MN	-	2.8	23.1	9.9	7.0
B14	Buffalo Lake, MN	-	3.0	23.6	9.7	6.3
B19	Buffalo Lake, MN	-	3.1	22.6	9.9	8.1
B22	Buffalo Lake, MN	-	2.9	22.7	10.8	7.6
B24	Buffalo Lake, MN	-	3.9	24.3	10.4	6.8
C2	Cavalier, ND	-	3.1	22.5	10.0	7.0
C6	Cavalier, ND	-	3.2	22.8	10.1	6.8
C10	Cavalier, ND	-	2.8	24.8	10.1	7.4
C13	Cavalier, ND	-	2.5	22.4	9.5	6.7
C22	Cavalier, ND	-	3.3	24.3	9.4	7.0
C23	Cavalier, ND	-	2.7	22.6	10.5	7.0
T1	Bushland, TX	+	3.0	23.1	10.0	6.9
T8	Bushland, TX	-	2.3	22.3	11.1	6.9
T24	Bushland, TX	-	3.0	23.1	11.0	6.5
T30	Bushland, TX	-	2.8	23.8	9.9	6.9
T47	Bushland, TX	-	3.4	23.6	10.0	6.6
T51	Bushland, TX	+	3.7	22.7	9.7	7.3
T71	Bushland, TX	+	2.6	22.0	10.5	7.1
T72	Bushland, TX	-	<u>3.0</u>	<u>22.8</u>	<u>9.9</u>	<u>7.1</u>
			$\bar{\chi} = 3.0$	23.2	10.2	7.0
Ae35	Malvick, Univ. Ill.	-	2.5	26.8	9.2	7.0
Ae174	Malvick, Univ. Ill.	-	<u>2.2</u>	<u>25.4</u>	<u>11.2</u>	<u>6.5</u>
			$\bar{\chi} = 2.3$	26.1	10.2	6.7

[†] Indicates presence (+) or absence (-) of the 1400 bp RAPD product from primer CRL33.

[‡] Tukey-Kramer Multiple Comparison Test ($T_{0.05} = 2.92$).

Table 3. Root rot indices of sugarbeet and pea seedlings grown in pasteurized soil inoculated with cultures of *Aphanomyces* isolated from sugarbeet soils from Minnesota and Texas or *A. euteiches* (Ae) compared to an uninoculated control.

Isolate	Source [†]	RAPD Phenotype [‡]	Root Rot Index [§]			
			Experiment 1		Experiment 2	
			Beet [#]	Pea	Beet [#]	Pea
B13	Minn.	-	100a	2	100a	4
B14	Minn.	-	100a	0	87b	3
B24	Minn.	-	100a	0	96a	7
T24	Texas	-	100a	2	100a	12
B1	Minn.	-	99a	0	99a	12
T1	Texas	+	99a	2	100a	10
T51	Texas	+	98a	0	99a	3
B19	Minn.	-	98a	0	99a	5
T8	Texas	-	97a	5	98a	3
B22	Minn.	-	92b	0	99a	2
T71	Texas	+	86b	0	98a	3
T72	Texas	-	64c	0	98a	7
Ae 35			2	62	3	98
Ae 174			0	71	--	--
Ae 467			--	--	0	85
Control			1	0	0	10

[†] Cultures of *Aphanomyces* were isolated from sugarbeet seedlings in soil collected in Minnesota (Minn.) and Texas; cultures of *A. euteiches* (Ae) were from pea.

[‡]Indicates presence (+) or absence (-) of the 1400 bp RAPD product for CRL33.

[§]Root Rot Index (RRI)= $[\sum(\text{disease class} \times \text{no. of seedlings per class}) / 3(\text{total emergence})] \times 100$; disease class = seedlings rated on a 0-3 scale, 0 = completely healthy and 3 = dead or dying.

[#]Values in columns followed by the same letter are not statistically significant, Tukey-Kramer Multiple Comparison Test ($P = 0.05$); root rot indices for the control and isolates of *A. euteiches* were excluded from analysis because of lack of pathogenicity on sugarbeet.

tomato seedlings in both experiments (data not shown).

Spatial distribution

Spatial patterns of *A. cochlioides* along the sampling transects were aggregated at the Minnesota and Texas sites ($P = 0.029$ and $P = 0.0023$, respectively, Fig 2); soil cores yielded 52 and 49% isolates, respectively. Spatial distribution at the North Dakota site, where most soil cores (84%) yielded isolates, approached uniformity ($P = 0.89$, Fig 2). For the Texas site, where two RAPD phenotypes occurred, a χ^2 test of independence indicated that the phenotype of an isolate had no effect on the phenotype of an adjacent isolate ($P = 0.12$).

DISCUSSION

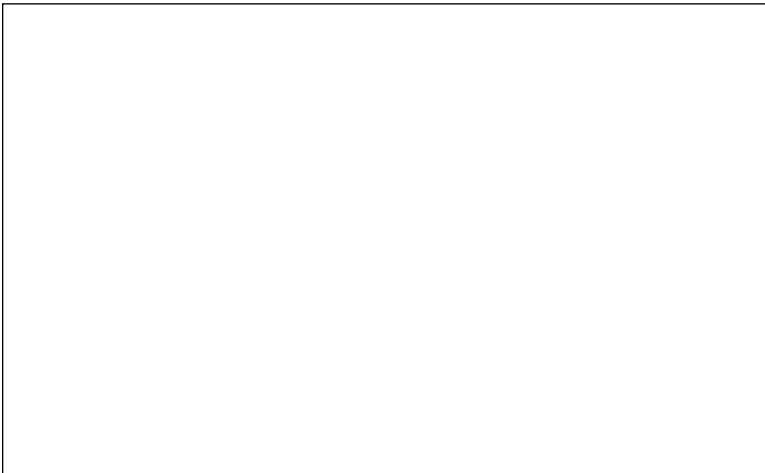


Fig. 2. Spatial positions of *Aphanomyces* recovered from soil collected in sugarbeet fields along 79-m transects. Isolates are aggregated for Buffalo Lake, Minnesota (runs analysis of spatial distribution: $P = 0.029$) and Bushland, Texas ($P = 0.0023$) but are uniformly distributed at Cavalier, North Dakota ($P = 0.89$); ○ = isolates that produce the 1400 bp product with primer CRL33 and ● = isolates that do not produce this product.

Assessment of RAPD analyses, morphologic characters, and pathogenicity trials consistently revealed that *Aphanomyces* cultures isolated from soil collected in Minnesota, North Dakota, and Texas sugarbeet

fields were a single species of limited variation, *A. cochlioides*. Isolates in this study had considerably smaller oogonial diameters (mean 23.2 μm) compared to the "A3" population (mean 34 μm) reported in Europe (Larrson, 1994). With the morphology of *A. cochlioides*, *A. camptostylus*, and *A. cladogamus* being nearly identical (Scott, 1961), pathogenicity tests were relied on to distinguish the isolates. Isolates from sugarbeet were not pathogenic on tomato and oat, the preferred hosts of *A. cladogamus* and *A. camptostylus*, respectively (both marginal pathogens of sugarbeet) and were highly pathogenic on sugarbeet. From these results we concluded that the isolates examined represented one species, *A. cochlioides*. It would have been interesting to explore the genetic relatedness of *A. cochlioides*, *A. cladogamus*, and *A. camptostylus*.

The only significant genetic variation found among and within the populations was the 1400 bp product specific to some isolates in Texas. Our study, however, included isolates from only three fields, which may not represent all the RAPD phenotypes present in *A. cochlioides*-infested fields in Minnesota, North Dakota, and Texas. Preliminary analyses of 16 isolates from the three populations employing amplified fragment length polymorphisms (AFLPs), produced 159 scorable products and distinguished nine molecular phenotypes (*unpublished*). These results indicate that RAPDs are limited in detecting genetic phenotypes.

Although all three fields sampled had histories of severe *Aphanomyces* root rot, inoculum was aggregated in the Minnesota and Texas sites and uniform in the North Dakota site. These patterns may be explained, in part, by cropping history. Sugarbeet had not been grown in the Texas (C. Rush, Texas A & M, *personal communication*) or Minnesota sites the previous two years but had been grown at the North Dakota site the previous year. Inoculum densities of *A. cochlioides* may be reduced over time when a field is rotated to other crops and thereby, result in patchy or aggregated distribution patterns. Beale et al. (2002) found that inoculum of *A. cochlioides* was aggregated early in the season in sugarbeet fields on a 3-year rotation but as the season progressed, incidence of *Aphanomyces* root rot was uniformly distributed. Changes in distribution of *A. cochlioides* are supported by direct observation of oospore decomposition in soil (Brantner et al., 2002). Redroot pigweed (*Amaranthus retroflexus* L.), a known host of *A. cochlioides* (Schneider 1965), was frequently observed when sampling the North Dakota site and also may have contributed to a uniform and high population of the pathogen.

In summary, the isolates of *Aphanomyces* collected from soil in three sugarbeet fields with a history of root rot represent a single spe-

cies, *A. cochlioides*. Based on morphology, pathogenicity, and RAPD data, genetic diversity within this species is limited. Distribution of inoculum of *A. cochlioides* was highly aggregated (Minnesota and Texas sites) but when inoculum levels were high, distribution was uniform (North Dakota site).

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