Additional Hosts of the Beet Water Mold, Aphanomyces Cochlioides Drechs[1]

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Introduction

The beet water mold, *Aphanomyces cochlioides*, is one of the most important pathogens associated with black root disease of sugar beets in the Great Lakes region of the United States. Drechsler (5)[3] demonstrated its pathogenicity on sugar beets and Buchholtz (2) showed that *Chenopodium album* and *Amaranthus retroflexus* are hosts. McKeen (10) infected peppers (*Capsicum frutescens*) with *A. cochlioides* in the greenhouse. The following have been reported as nonsusceptible to *A. cochlioides*: barley, oats, corn, soybeans (2), alsike, alfalfa, white Dutch clover, yellow and white sweet clover, red clover, sweet peppers, garden pea, wild carrots, dandelion, wheat, yellow foxtail, and timothy (11).

The objectives of the study reported herein were: (1) to determine the reaction of plant species to *A. cochlioides* in addition to those previously reported, and (2) to compare the pathogenicity on sugar beets of isolates of the fungus from different host species.

Materials and Methods

Cultures of *A. cochlioides* used in inoculation tests to determine the host range were isolated from blighted sugar beet seedlings in accordance with a method outlined by Downie (4). Cultures fro msingle zoospores were obtained as follows: Droplets of an aqueous suspension of zoospores were sprayed with a micropipette onto a thin layer of water agar in a petri plate. Approximately 24 hours later blocks of agar, each containing one germinating zoospore, were cut out and removed from the petri plate with a micro-spatula and transferred to a nutrient medium. Cultures were maintained on slants of unstrained maize meal agar.

Zoospores for inoculation tests were obtained by growing the fungus in nutrient broth, then transferring the mycelial mats to flasks of sterile tap water in accordance with a previously

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[3] Numbers in parentheses refer to literature cited.

described method (13). Concentration of zoospores was determined with a bright-line counting chamber.

In order to reduce the likelihood of seedling infection by seed-borne fungi, seeds of most of the species included in inoculation tests were soaked in an aqueous suspension of ethyl mercury phosphate (1000 ppm) for 20 minutes, rinsed in running tap water for 30 minutes, then dried.

Bioassays to determine the occurrence of *A. cochlioides* in plants exposed to the fungus were conducted as follows: Small pieces of hypocotyls and roots were immersed in a shallow layer of sterile water in Syracuse dishes and incubated at 20°-25° C. The presence of *A. cochlioides* was indicated by the production of characteristic zoosporangia protruding from the tissues after approximately 16 hours, and by characteristic oospores or oogonia within the tissues. Identification was based on descriptions published by Drechsler (5) and Scott (4).

Host Range Survey

Seedlings of 98 species representing 40 plant families were exposed to zoospores of *A. cochlioides* in a series of tests. Included were many of the important crops and weeds of the Great Lakes region. In each test, seedlings of a highly susceptible sugar beet variety were included as a check on the infection potential of the zoospore inoculum.

Fifty-three species were first subjected to an extremely rigorous laboratory test. Seeds were germinated on moist blotting paper in petri dishes or glass jars. After germination, 20 ml water containing approximately 25,000 zoospores were poured into each dish or jar containing the seedlings and were decanted 2 days later. Within 3 days after exposure to zoospores, susceptible seedlings began to damp off. Although the extremely artificial environment doubtlessly predisposed plants of susceptible species to infection, the laboratory test was useful in that it provided a relatively quick method of isolating nonsusceptible species.

All species susceptible in the laboratory test were included in subsequent inoculation tests in the greenhouse. Also included were 48 species that were not tested previously in the laboratory. Plants were grown in 4-inch pots of steam-sterilized soil. When seedlings were emerging, 50 ml of water containing approximately 150,000 zoospores were poured into the soil of each pot. Total number of blighted seedlings was determined approximately 30 days after exposure to zoospores. These included plants with root rot and plants that had damped off.

Seedlings of 30 species in the following families became blighted when exposed to *A. cochlioides* zoospores in the greenhouse: Aizoaceae, Amaranthaceae, Caryophyllaceae, Hydrophyl-

laceae, Linaceae, Papaveraceae, Portulacaceae, and Solanaceae (Table 1). Species susceptible in the laboratory test were also susceptible in the greenhouse test.

Species susceptible to artificial exposures of the pathogen were subsequently grown in the greenhouse or field in soil naturally infested with *A. cochlioides*. Plants with symptoms of damping-off, wilting, root rot, and discoloration of the lower stem were bioassayed for the presence of *A. cochlioides*. *A. cochlioides* was identified in plants of 19 species, besides *Beta vulgaris*, that were grown in naturally infested soil (Table 1). Pure cultures of *A. cochlioides* were isolated from *Chenopodium album, Spinacia oleracea, Tetragonia expansa, Mollugo verticillata* and *Saponaria ocymoides* and were pathogenic on sugar beet seedlings in greenhouse inoculation tests.

A. cochlioides was nonpathogenic on the following species: Asclepiadaceae, *Asclepsis syriaca* L.; Balsaminaceae, *Impatiens balsamina* L.; Boraginaceae, *Anchusa azurea* Mill.; Compositae, *Ambrosia trifida* L., *Aster macrophyllus* L., *Calendula officinalis* L., *Carthamus tinctorius* L., *Solidago* sp., *Tagetes* sp., *Zinnia* sp.; *Convolvulaceae, Ipomoea purpurea* Lam.; Cruciferae, *Alyssum saxatile* L., *Brassica arvensis* (L.) Rabenh, *B. rapa* L., *Lepidium virginicum* L., *Raphanus sativus* L., *Thlaspi arvense* L.; Cucurbitaceae, *Cucumis melo* L., *C. sativus* L., Euphorbiaceae, *Euphorbia serpyllifolia* Pers., *Ricinus communis* L.; Geraniaceae, *Geranium* sp.; Gramineae, *Avena sativa* L., *Echinochloa crusgalli* (L.) Beauv., *Hordeum vulgare* L., *Secale cereale* L.; *Setaria glauca* L. (Beauv.), *Zea mays* L., Labiatae, *Monarda fistulosa* L.; Leguminoseae, *Astragulus canadensis* L., *Medicago sativa* L., *Melilotus alba* Desr., *Pisum sativum* L., *Glycine max* (L.) Merr., *Trifolium pratense* L.; Liliaceae, *Allium cepa* L.; Malvaceae, *Althaea rosea* Cav.; Nyctaginaceae, *Abronia umbellata* Lam., *Mirabilis jalapa* L.; Onagraceae, *Clarkia elegans* Dougl.; Pedaliaceae, *Sesamum indicum* L.; Pinaceae, *Pinus banksiana* Lamb.; Plantaginaceae, *Plantago major* L.; Plumbaginaceae, *Armeria pseud-armeria* Mansfeld; Polemoniaceae, *Phlox drummondii* Hook; Polygonaceae, *Fagopyrum esculentum* Moench, *Polygonum erectum* L., *P. pensylvanicum* L., *P. persicaria* L., *Rumex crispus* L., R. *mexicanus* Meisn.; *Primulaceae, Anagallis linifolia* L., Ranunculaceae, *Delphinium ajacis* L.; Rosaceae, *Geum chiloense* Balbis, *Potentilla arguta* Pursh; Scrophulariaceae, *Verbascum thapsus* L.; Solanaceae, *Lycopersicon esculentum* Mill., *Nicotiana rustica* L. *Petunia hybrida* Vilm., *Solanum melongena* var. *esculentum* Nees; *Tropealaceae, Tropeolum majus* L.; Umbelliferae, *Anethum graveolens* L.; Urticaceae, *Urtica gracilis* Ait.; Verbenaceae, *Verbena hybrida* Voss; Violaceae, *Viola tricolor* L.

Table 1. — Reaction of 30 plant species to artificial and natural exposures of *Aphanomyces cochlioides*.

Family, species, and common name[1]		Number of seedlings					
		In glass vessel		In artificially infested soil		In naturally infested soil	
		Damped off	Exposed to zoospores	Blighted	Exposed to zoospores	Infected with Aphanomyces	Bioassayed
Aizoaceae							
Mollugo verticillata L.	Carpet weed	----	----	9	50	4	61
Tetragonia expansa Murr.	New Zealand spinach	----	----	38	40	7	40
Amaranthaceae							
Amaranthus blitoides Wats.	Prostrate pigweed	45	45	49	61	2	59
A. retroflexus L.	Pigweed	74	80	34	55	10	310
Celosia argentea L.	Cockscomb	15	32	41	64	2	27
Gomphrena globosa L.	Globe amaranth	----	----	73	86	1	25
Caryophyllaceae							
Cerastium sp.	Mouse-ear chickweed	----	----	45	85	0	23
Dianthus chinensis L.	Rainbow pink	37	78	91	92	6	92
Lychnis alba Mill.	White cockle	37	41	71	104	5	101
L. chalcedonica L.	Maltese cross	----	----	3	12	0	7
Saponaria officinalis L.	Soapwort	4	7	101	151	0	34
S. ocymoides L.	Bouncing Bet.	----	----	18	18	39	61
Chenopodiaceae							
Beta lomatogona Fisch. and Mey.		----	----	6	7	----	----
B. macrocarpa Guss.		----	----	8	8	----	----
B. maritima L.		----	----	----	----	3	5
B. patellaris Moq.		20	20	29	41	2	7
B. patula Ait.		----	----	6	8	----	----
B. trigyna Wald and Kitt.		19	20	100	100	11	28
B. vulgaris L.	Sugar beet	36	36	132	155	389	795
B. vulgaris L.	Table beet	----	----	128	151	2	13
B. vulgaris L.	Mangel		----	150	163	3	5

Family, species, and common name[1]		Number of seedlings					
		In glass vessel		In artificially infested soil		In naturally infested soil	
		Damped off	Exposed to zoospores	Blighted	Exposed to zoospores	Infected with Aphanomyces	Bioassayed
B. vulgaris var *cicla* L.	Chard	----	----	128	177	11	20
Chenopodium album L.	Lamb's quarters	39	40	118	171	24	217
Kochia scoparia Schrad.	Fireweed	----	----	90	120	8	182
Kochia scoparia var. *culta* Farwell.	Mexican burning bush	28	28	7	8	1	20
Salsola kali L.	Russian thistle	8	8	30	46	9	121
Spinacea oleracea L.	Spinach, var. Bloomsdale Savoy	20	20	45	48	27	118
Hydrophyllaceae							
Phacelia campanularia Gray		17	21	35	74	0	11
Linaceae							
Linum usitatissimum L.	Flax, var. Bolley	----	----	9[2]	76	0	57
Papaveraceae							
Escholtzia californica Cham.	California poppy	----	----	3	51	----	----
Papaver rhoeas L.	Corn poppy	68	68	9	94	5	80
Portulacaceae							
Portulaca grandiflora Hook.	Moss rose	46	53	21	32	0	36
P. oleracea L.	Purslane	82	82	73	180	5	295
Solanaceae							
Capsicum frutescens L.	Peppers, var. California Wonder	70	70	59	109	0	135

[1] Authorities for scientific and common names are: Bailey (1), Engler and Prantl (7) and Fernald (8).
[2] Symptoms confined to a slight discoloration of the roots.

Differences in Susceptibility Between Some Host Species

Differences in degree of susceptibility to *A. cochlioides* between 8 species that had been infected in the host range survey were experimentally demonstrated in the greenhouse. Seedlings of the following species, representing 4 plant families, were grown in 4-inch pots of steam-sterilized soil and exposed to zoospores (approximately 150,000 per pot) about 3 weeks after planting, when all seedlings had emerged: *Amaranthus blitoides, Lychnis alba, Saponaria officinalis, Beta vulgaris, Chenopodium album, Salsola kali, Spinacia oleracea,* and *Portulacea oleracea.* Symptoms of disease included damping-off and root rot. Approximately 40 days after exposure to zoospores, significant differences between some species in susceptibility were apparent (Table 2) and ranged from 0% plants diseased, for *Lychnis alba* and *Portulacea oleracea,* to 100% for *Beta vulgaris.*

Table 2. — Relative susceptibility of 8 plant species exposed to *Aphanomyces cochlioides* zoospores in the greenhouse after emergence of seedlings.

Family and species	Plants exposed to zoospores	Plants diseased[1, 2]
	Number	Percent
Amaranthaceae		
Amaranthus blitoides prostrate pigweed	54	82.6
Caryophyllaceae		
Lychnis alba, white cockle	50	0
Saponaria officinatis, soapwort	52	50.3
Chenopodiaceae		
Beta vulgaris, sugar beet	35	90.0
Chenopodium album, lamb's quarters	59	84.9
Salsola kali, Russian thistle	40	27.3
Spinacia oleracea, spinach	31	78.9
Portulacaceae		
Portulacca oleracea	89	0
LSD (P = .05)		36.3

[1] Includes plants damped off and those with root rot.
[2] Results expressed as mean of two 4-inch pots. Percentage data were converted to degrees for statistical analysis.

Pathogenicity of Isolates from Different Hosts Compared

The pathogenicity of 10 cultures of *A. cochlioides,* isolated from *Beta vulgaris, Spinacia oleracea,* and *Chenopodium album* grown in soils from several sugar beet-growing areas of the United States[4] was compared in the greenhouse. Seedlings of moderately resistant sugar beet variety US 400 were exposed to zoospores of each culture (approximately 150,000 per pot) 14 days after

[4] Samples of Michigan and Montana soils were kindly furnished by H. W. Bockstahler and M. M. Afanasiev, respectively.

planting, when emergence was complete. Thirty days after exposure to zoospores, each plant was assigned one of the following numerical ratings according to severity of seedling blight: 0 (no symptoms; 1 (light symptoms); 2 (moderate symptoms); 3 (severe symptoms); 4 (dead). Incidence and severity of blight caused by each isolate were then converted to a single percentage value by a method similar to that outlined by LeClerg (9). The average infection type of the plants in each pot was obtained by adding the products of each infection type times the number of plants of that type and dividing this sum by the number of plants exposed to zoospores. This number was converted to a percentage value by dividing it by 4, the value of the highest infection type, and multiplying by 100.

Variety US 400 was susceptible to each culture (Table 3). No significant differences in virulence were noted between cultures from the 3 host species nor from the 5 locations.

Table 3. — Relative susceptibility of sugar beet variety US 400 to 10 cultures of *Aphanomyces cochlioides* isolated from sugar beet, spinach, and lamb's quarters *(Chenopodium album)* and from different areas.

Culture number and source[1]			Plants exposed to zoospores	Degree of susceptibility[2]
			Number	Percent
73-Q	Sugar beet	St. Paul, Minn.	101	78
75-A	do	Waseca, Minn.	113	79
75-D	do	do	107	78
91-E	do	East Lansing, Mich.	105	65
72-0	do	Bozeman, Mont.	105	72
98-E	do	do	106	76
97-N	do	Blooming Prairie, Minn.	105	71
40-Q	Spinach	Waseca, Minn.	103	78
111-A	Lamb's quarters	St. Paul, Minn.	111	72
111-Z	do	do	107	76
Control			117	0
LSD (P = .05)				N.S.

[1] Sub-cultures from single zoospores are designated by a capital letter following the culture number.

[2] Results expressed as mean of 4 pots. Incidence and severity of disease expressed as a single percentage value transformed to degrees for statistical analysis.

Discussion

A. cochlioides has been reported to occur in soils in which crops of sugar beets apparently have never been grown (4). Some of the common weeds shown to be hosts of *A. cochlioides* including *Chenopodium album, Amaranthus blitoides, A. retroflexus* and *Mollugo verticillata* may well aid in the survival of the fungus in agricultural soils. A reported increase in incidence

of black root disease of sugar beets on land previously occupied by a dense stand of Amaranthus (3) is cited as an example. Weed hosts that were shown to be highly resistant to infection by *A. cochlioides,* such as *Portulacea oleracea* and *Lychnis alba,* may play a less effective role in survival of the fungus than the more susceptible hosts. Spinach and ornamental plant hosts such as *Saponaria ocymoides,* although extremely susceptible, probably contribute little to the occurrence of the fungus in soils where the sugar beet crop is grown.

In this study and in studies of other investigators (2, 11), no major crop grown in rotation with sugar beets in the Great Lakes region was shown to be a natural host of *A. cochlioides.* Nevertheless, care must be exercised in the choice of crops to precede sugar beets, since it has been shown that black root disease is usually more severe when sugar beets follow late-plowed plantings of alfalfa or sweet clover than when sugar beets follow corn or rye (3). Inasmuch as there is no evidence that the legume crops are hosts of *A. cochlioides,* one explanation for the increase in black root disease when beets follow alfalfa or sweet clover may be that the residues of these crops and the accompanying microflora are more favorable for increase of the pathogen than those of corn or rye.

Summary

The reaction of 94 plant species to pure culture of *Aphanomyces cochlioides* was determined. Twenty-eight new experimental hosts from the following families are reported: Aizoaceae, Amaranthaceae, Caryophllaceae, Chenopodiaceae, Hydrophyllaceae, Linaceae Papaveraceae, Portulacaceae. Nineteen species in addition to *Beta vulgaris* were found to be natural hosts of the fungus. Differences in relative susceptibility to *A. cochlioides* between several host species were experimentally demonstrated. Cultures of *A. cochlioides* isolated from *Spinacia oleracea* and *Chenopodium album* were as pathogenic on *Beta vulgaris* as cultures isolated from *Beta vulgaris.*

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