Soilborne Pathogens of *Euphorbia lathyris: Macrophomina phaseolina, Pythium aphanidermatum,* and *Rhizoctonia solani*

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ABSTRACT

Young, D. J., and Alcorn, S. M. 1982. Soilborne pathogens of *Euphorbia lathyris: Macrophomina phaseolina, Pythium aphanidermatum,* and *Rhizoctonia solani.* Plant Disease 66:236-238.

Macrophomina phaseolina, Rhizoctonia solani, and *Pythium aphanidermatum* were isolated from *Euphorbia lathyris* grown in fields near Tucson, AZ. *R. solani* occurred as a damping-off organism in the fall. During the summer, *M. phaseolina* was a major pathogen, particularly infecting plants at least several months of age; sclerotia frequently formed on roots and in pith tissues. Some plants infected with *M. phaseolina* were growing in an area newly cleared of native desert vegetation. It was also isolated from *E. lathyris* field plants dying during the summer in western Arizona and Utah. *Pythium aphanidermatum* infected seeds, seedlings, and mature plants in laboratory and greenhouse tests. Although *P. aphanidermatum* was infrequently isolated from field plants in Arizona, it was a major pathogen of greenhouse plants growing at high temperatures in nonsterile soil. *Fusarium* spp. were frequently isolated from root lesions, but their role as pathogens is not yet known.

Additional key words: gopher plants, hydrocarbon source

Concern regarding the future availability of petroleum has stimulated investigations of possible alternative or supplementary sources of hydrocarbons. One suggested source is the gopher plant or caper spurge, *Euphorbia lathyris* L. (6). Reportedly originating in the Orient, this plant has become established as a weed in sections of western Europe and the United States (10). It has also been cultivated for seed in Japan and China (10).

Under the auspices of the Office of Arid Lands Studies, University of Arizona, and the Diamond Shamrock Corporation, experiments in the field and in the greenhouse were initiated in southern Arizona in 1979 to determine the feasibility of raising this species for its hydrocarbons. We report here proof of the pathogenicity of *Rhizoctonia solani* Kuehn, *Pythium aphanidermatum* (Edson) Fitz., and *Macrophomina phaseolina* (Tassi) Goid.

Paper 3390 of the University of Arizona Agricultural Experiment Station.

Accepted for publication 4 June 1981.

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0191-2917/82/03023603/$03.00/0
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MATERIALS AND METHODS

Isolation procedures. Diseased *E. lathyris* plants of varying ages were collected from March 1979 through August 1980 from plantings in greenhouses in Arizona and from several fields in Arizona, New Mexico, Nevada, and Utah.

Sections of roots or lower stems were washed in running tap water for 3 min, 0.5% sodium hypochlorite for 30–60 sec, and distilled water for 1 min, then placed on 2% water agar + streptomycin sulfate at 100 ppm. The tissues were incubated in the dark at 20, 30, and 34 C for 14 days. Pure cultures, obtained from hyphal tips, were subsequently maintained on V-8 agar (10% V-8 juice + 2% Bacto agar), Difco potato-dextrose agar (PDA), or Difco Czapek's agar.

Growing test plants. *E. lathyris* seeds (from plants grown in Chico, CA) were planted in metal trays (48 × 35 × 8 cm) or 10- or 15-cm-diameter pots, respectively containing 3,800, 260, and 300 g of a 1:1:2 mixture of perlite, peat, and Baccto potting medium (Michigan Peat Co., P. O. Box 66388, Houston, TX 77006). As appropriate, the planting medium was steam-sterilized for 12 hr, then allowed to cool for 24 hr before use. Seeded containers were placed on greenhouse benches or at 21 C in growth chambers with a 12-hr photoperiod until plant emergence.

Additionally, forty 4-mo-old *E. lathyris* plants, planted in nonsterile sand, were observed for disease. These plants were grown in a greenhouse with diurnal temperatures of 38 C (high) and 21 C (low) with high humidity.

Inoculation procedures. The following procedures were followed for all three fungi. Plants 1 and 2 mo old were grown in 10-cm-diameter pots; 100 ml of inoculum was added to each pot; 10 test and 10 control plants were used per experiment. Six-month-old plants were grown in 15-cm-diameter pots, to each of which was added 375 ml of inoculum; six test and six control plants were used per experiment. Isolations were made from plants showing symptoms. Experiments were conducted twice; percentages were taken from the pooled data. Experiments with *R. solani* and *P. aphanidermatum* were terminated after 30 days; tests with *M. phaseolina* were run for 60 days.

Tests with *R. solani*. *R. solani* was grown on PDA plates for 5 days at 30 C in the dark, then comminuted for 30 sec in distilled water. The resulting suspension of hyphal fragments and agar was used as a soil drench. In preemergence tests, 100 *E. lathyris* seeds were planted in a metal tray, after which 2,000 ml of inoculum, containing 10^3, 10^4, or 10^5 hyphal fragments per milliliter, was poured on the soil surface. Trays were incubated at 21 C. In postemergence tests, 1-mo-old plants were inoculated by adding suspensions of 10^4 hyphal fragments per milliliter to the soil surface. Plants were then incubated at 21, 30, and 36 C. Two-month-old *E. lathyris* were inoculated with suspensions containing 10^5 hyphal fragments per milliliter and incubated at 30 and 36 C. Six-month-old plants were inoculated with 10^5 hyphal fragments per milliliter and incubated at 21 and 30 C.

Tests with *M. phaseolina*. Pathogenicity of *M. phaseolina* was determined by two methods. Wooden toothpicks were autoclaved for 1 hr in distilled water, then placed in a broth containing 0.5% tryptone, 0.25% yeast extract, 0.1% dextrose, and 2 parts soil extract

(prepared by autoclaving 1,000 g of field soil with 1,500 ml of distilled water for 1 hr and then filtering the suspension into sterile flasks through Whatman No. 1 filter paper). The broth with toothpicks was autoclave-sterilized for 20 min, after which it was inoculated with *M. phaseolina* and incubated at 34 C, in the dark, for 21 days. By this time, sclerotia had formed on the toothpicks. Stems (10–15 mm diam) of plants to be inoculated were wiped with 2% amphyl (Sterling Drug Inc., Lehn and Fink Div., Montvale, NJ 07645) and pierced with a sterile needle 3 cm above the soil line. Toothpicks with sclerotia were inserted into the holes. Sterile toothpicks were similarly inserted into control plants. Plants were placed in growth chambers with 14 hr of light at 34 C and 10 hr of dark at 26 C (11) and watered only when the top 8 cm of soil was dry.

For the second procedure, *M. phaseolina* was grown for 7 days on PDA at 34 C in the dark. A suspension of hyphal fragments and sclerotia (10^5/ml) was prepared as described for *R. solani.* Two to 5 cm of the tap and lateral roots were pruned from plants to be inoculated, after which the remaining roots were soaked for 1–2 min in 100 ml of inoculum per plant. Inoculated plants were then placed singly in sterile soil in 10-cm-diameter pots; the remaining inoculum was poured on the soil. Tap water was substituted for the inoculum in treating control plants.

Tests with *P. aphanidermatum.* *P. aphanidermatum* was grown for 3 days at 34 C in the dark on V-8 agar plates, then comminuted for 30 sec in distilled water. This suspension contained hyphal fragments, oospores, and sporangia, each of which was counted as one propagule. Seeds in metal trays were exposed to the same inoculum concentrations and procedures as for *R. solani.* Trays were incubated at 21 C or in the greenhouse. Plants 1 and 2 mo old were inoculated by drenching the soil surface with suspensions ranging from 10 to 1×10^5 propagules per milliliter. Plants were then placed in growth chambers at 30–33 or at 35–36 C. Six-month-old plants were similarly inoculated with suspensions containing 10^4 propagules per milliliter and were incubated at 37 C.

RESULTS

Naturally infected greenhouse plants. Four-month-old plants (180–240 cm in height, some in flower) growing in the greenhouse with diurnal temperatures of 38 and 21 C (day-night) were found to be infected with *P. aphanidermatum.* A blue purple color developed in stems near the soil line; lower leaves became chlorotic. Chlorosis and bluing of the stem progressed upward. Within 4 wk of the initial symptoms, most plants were dead or nearly so. *P. aphanidermatum* was consistently isolated from roots and from all portions of affected stems.

Naturally infected field plants. Stems of *E. lathyris* up to 2 mo old became discolored at the soil line and collapsed; black lesions occurred on taproots. *R. solani* was the primary organism associated with these infections; damping-off was most common in November and December 1979 in field plots (Table 1). *R. solani* also caused discrete brown lesions, discoloration, and dieback of roots to approximately 30-cm depths on older, field-grown *E. lathyris.* This occurred particularly in June 1979 and March, June, and July 1980 (Table 1). Affected plants had necrotic lower leaves, chlorotic upper leaves, and were stunted.

During 1979 and 1980, most established plants that died were infected with *M. phaseolina.* Some of these were from plots previously fumigated with methyl bromide (0.97 kg/10 m^2). Infection occurred largely during the warm months (Table 1). Lower leaves became chlorotic and wilted, then necrotic; the necrosis proceeded acropetally. Brown lesions occurred on larger roots and at the soil line on hypocotyls. The cortex of the tap and lateral roots often sloughed off. Sclerotia formed under the root cortex and in the stem pith. In one sampling of 30 plants in June 1980, 60% were infected at the soil line but 13.3% had infections on roots at 15–20 cm depths.

P. aphanidermatum was isolated during summer months from field-grown plants that received frequent irrigations or rains (Table 1). Such plants were flaccid and had foliar symptoms similar to those caused by *R. solani* and *M. phaseolina.* However, none had the blue color exhibited by the infected greenhouse-grown plants.

Fusarium spp. and *Cephalosporium* spp. were also isolated from *E. lathyris* roots (Table 1). Tests are in progress to determine the pathogenicity of these isolates.

Inoculations with *R. solani.* When separate pots of 100 seeds were planted in metal trays and inoculated with 0, 10^3, 10^4, or 10^5 hyphal fragments per milliliter, an average of 89, 84, 60, and 43 seedlings emerged, respectively, after 19 days. *R. solani* was consistently recovered from inoculated seeds and seedlings.

One-month-old *E. lathyris* plants in infested soil were wilted after 3 days at all temperatures tested. After 7 days, 100, 90, and 60% of the plants at 21, 30, and 36 C, respectively, were dead. *R. solani* was recovered only from affected plants. Additional symptoms included black lesions on the taproots and lower stems; hypocotyls frequently collapsed. Similar results were obtained using 2-mo-old plants incubated at 30 and 36 C.

No symptoms occurred on aerial parts of 6-mo-old plants, although scattered, small, brown lesions were present on lateral roots of inoculated plants. *R. solani* was recovered from these lesions on 100 and 83% of the inoculated plants at 21 and 30 C, respectively.

Inoculation with *M. phaseolina.* One-month-old *E. lathyris* plants, inoculated by the drench procedure and incubated either in the greenhouse or at 34 and 26 C (day-night) in a growth chamber, exhibited no stem or foliar symptoms by 2 mo. However, brown lesions were scattered on the taproots of 50 and 60% of the inoculated plants, respectively, from which *M. phaseolina* was consistently isolated. Some darkening of vascular bundles at the soil line of inoculated stems also occurred. Six-month-old plants similarly inoculated and incubated at 34 and 26 C for 2 mo also did not show foliar symptoms. However, 50 and 40%

Table 1. Recovery of *Rhizoctonia solani* (Rs), *Macrophomina phaseolina* (Mp), *Pythium aphanidermatum* (Pa), and *Fusarium* and *Cephalosporium* spp. (F) from representative *Euphorbia lathyris* exhibiting symptoms in the field

Isolation dates	Plants 1-2 mo old[a]					Plants 3-4 mo old					Plants 5-9 mo old				
	Total no.[b]	Rs	Mp	Pa	F	Total no.	Rs	Mp	Pa	F	Total no.	Rs	Mp	Pa	F
June 1979	10[c]	0[c]	0	0	1	14	7	5	0	2	0	0	0	0	0
July–Sept. 1979	19	2	2	0	1	37	1	19	0	10	0	0	0	0	0
Oct.–Dec. 1979	37	8	2	0	19	8	1	0	0	3	2	0	0	0	0
Jan.–Mar. 1980	0	0	0	0	0	0	0	0	0	0	25	12	1	0	9
Apr.–June 1980	14	0	2	0	6	8	0	4	0	3	107	15	57	1[d]	24
July–Aug. 1980	18	0	2	2[d]	11	16	1	6	4[d]	3	27	9	7	3[d]	4
Totals	98	10	8	2	38	83	10	34	4	21	161	36	65	4	37

[a] Plant age determined from time of seeding; emergence generally 2 wk later.
[b] Total number of plants with symptoms from which isolations were attempted.
[c] Number of plants with symptoms from which designated pathogen was isolated. Differences between total plants and numbers of plants with the designated fungi represent those from which *Aspergillus* sp., *Dactylaria* sp., *Penicillium* sp., *Rhizopus* sp., or *Trichoderma* sp. was isolated.
[d] Collected from areas with excess water.

of these plants had root lesions, as described, from which *M. phaseolina* was isolated. Control plants remained symptomless.

Separate lots of 4-mo-old *E. lathyris* plants were inoculated by the toothpick and soil-drench methods and then incubated at 34 and 26 C. Symptoms developed within 7 days on all toothpick-inoculated plants, but only after 17 days on drench-inoculated plants. Early symptoms included wilting and chlorosis of leaves, beginning with the tips of the lower leaves. Stems turned dark at the soil line. In the affected stem region, vascular bundles were dark, and sclerotia formed in the hollow center. In advanced stages of the disease, cortical tissues were sloughed from roots; sclerotia had formed on the inside of these tissues.

Inoculations with *P. aphanidermatum*. In tray tests, seedling emergence at 21 C after 19 days was 96, 56, 23, and 7% following inoculations with 0, 10^3, 10^4, and 10^5 propagules per milliliter. Seedling emergence from similarly inoculated trays at greenhouse temperatures after 19 days was 85, 44, 16, and 0%, respectively.

Plants 1, 2, and 6 mo old became infected under all experimental conditions. Symptoms occurred on younger plants as early as 3 days following inoculation. Tap and lateral roots darkened; black lesions occurred on the stems at the soil line; and yellowing, then dying of leaves proceeded acropetally. The apical portions of stems became flaccid. Unlike plants inoculated with *R. solani* or *M. phaseolina,* however, stems of greenhouse plants inoculated with *P. aphanidermatum* developed a blue color at the soil line, which in some instances progressed to the shoot tip. At 30 C, 6-mo-old plants died within 14 days.

DISCUSSION

R. solani, M. phaseolina, and *P. aphanidermatum* were pathogenic on *E. lathyris*. In the greenhouse, infections by *R. solani* were favored by air temperatures of 21 C; in the field, this fungus was associated particularly with losses of young plants (under 2 mo) following October plantings. *R. solani* generally did not kill older *E. lathyris* plants; however, it caused root lesions to soil depths of at least 30 cm.

M. phaseolina is a serious pathogen of established *E. lathyris* plants in the vicinity of Tucson during the hot season. The recovery of *M. phaseolina* from representative, naturally infected *E. lathyris* grown in the summer in western Arizona and Utah indicates that this fungus is also a pathogen in these areas. *M. phaseolina* was rarely isolated from plants during the cool months of the year. Infection by *M. phaseolina* is favored by high temperature and stress (4).

Sclerotia are the primary inocula of *M. phaseolina* (8) and are formed only on colonized organic matter (7). Although the majority of infection sites on *E. lathyris* occurred at the soil line, infections did occur at depths of 15–20 cm. Survival of sclerotia at these depths should be recognized in making field surveys for the occurrence of *M. phaseolina* (3) and in placing chemicals for control.

Of considerable interest and practical importance is the fact that a number of *E. lathyris* plants growing in newly cleared desert were infected with this fungus in the first season. Although *P. aphanidermatum* has been recovered from nonagricultural soils in Arizona (12) and *R. solani* is thought to occur in arable land worldwide (1), we are not aware of similar reports for *M. phaseolina,* nor are we aware of reports of this fungus causing infections of native flora of Arizona. That these fungi are indigenous in uncultivated lands is important because of the interest in using such lands for diverse "new" crops (eg, jojoba [5], guayule [9], and buffalo gourd [2]), in addition to *E. lathyris.*

P. aphanidermatum was isolated from occasional field plants exposed to excess water during the hot summer. However, based on observations and isolations from naturally infected and inoculated *E. lathyris* growing in the greenhouse, *P. aphanidermatum* decayed seeds and killed plants at least through the flowering stage.

The most favorable time for planting *E. lathyris* in the field in the vicinity of Tucson is either fall or spring (T. R. Peoples, Department of Plant Sciences, University of Arizona, *personal communication*). Because chemicals may provide protection against damping-off, fall plantings of *E. lathyris* seem most appropriate to achieve the most biomass by the following season. To avoid infection by *M. phaseolina* and to conserve the amount of water needed to grow the crop, harvest in early summer should be contemplated.

ACKNOWLEDGMENTS

The assistance of Deborah Bird and Katherine Clay is gratefully acknowledged.

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