Latent Infection of *Euphorbia lathyris* and Weeds by *Macrophomina phaseolina* and Propagule Populations in Arizona Field Soil

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ABSTRACT

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Infection of roots of *Euphorbia lathyris* by *Macrophomina phaseolina* occurred within 1 mo of an October 1980 planting in southern Arizona, but disease symptoms were not evident until May 1981. Propagule populations ranged from fewer than 1 to 246 sclerotia per gram of Gila silt loam, the lowest density sufficient to cause the death of more than 90% of field-grown plants in August 1981. The highest density of sclerotia occurred during a second consecutive cropping of *E. lathyris* in one field. Sclerotia of *M. phaseolina* survived in untilled soil for 1 yr. Six new weed hosts of *M. phaseolina* that were detected are *Amaranthus palmeri, E. hyssopifolia, E. prostrata, Ipomea coccinea, Sonchus oleraceus,* and *Tidestromia languinosa.*

Additional key words: charcoal rot

Euphorbia lathyris L., the gopher plant or caper spurge, produces large amounts of a hydrocarbonlike material that is a potential source of high-grade fuels and chemical feedstocks (10). Agronomic production of this plant was tested in the southwestern United States because 1) hydrocarbon production may be increased when the plant is exposed to high levels of solar radiation and 2) the plants can be grown with less water than many other agricultural crops (8).

Optimal seed germination of *E. lathyris* occurs when soil temperatures are between 16 and 26 C (10). Thus, in Arizona, plantings are best made in May or October, with harvests the following fall or summer, respectively.

Macrophomina phaseolina (Tassi) Goid. was the most important pathogen in field plantings of *E. lathyris* in Marana, AZ (16), causing extensive losses from March 1979 through August 1980. Early symptoms included chlorotic-necrotic leaf tips and wilt. As the disease progressed, there was extensive root rot and numerous black sclerotia formed in the stem pith. The extreme susceptibility of *E. lathyris* to *M. phaseolina* dictates that it be planted in the fall in Arizona as an annual, although it can be grown as a perennial.

After October plantings of *E. lathyris, M. phaseolina* was frequently recovered during the winter from the roots of apparently healthy plants, months before symptoms were expressed (May). This latent period is defined here as the time required for disease expression rather than Vanderplank's (14) definition: ". . . the time needed for a generation of the pathogen." Such latency may relate to host resistance or to some environmental factor that is unfavorable to the pathogen (15). Edmunds (5) also noted latent infection in charcoal rot of sorghum.

This paper reports results of studies of the latent period in disease development and of the relationships between disease incidence in *E. lathyris* and populations of sclerotia of *M. phaseolina* in soil.

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MATERIALS AND METHODS

Disease development. The occurrence of charcoal rot was followed in four noncontiguous field plots, each with a Gila silt loam soil type. The plot designations, dimensions, and the crop planted in the previous growing season were: plot A, 180 × 13 m, safflower (*Carthamus tinctorius* L.); plot B, 180 × 5 m, safflower; plot C, 180 × 5 m, cotton (*Gossypium hirsutum* L.); plot D, 180 × 5 m, soybean (*Glycine max* (L.) Merr.); and plot E, 180 × 25 m, *E. lathyris.*

Three thousand seeds of *E. lathyris* were planted 5 cm deep and 8 cm apart in plot A in October 1980. The plot was irrigated immediately after planting, received 11.05 cm of rain during the winter, and was furrow-irrigated every other week from May through July 1981.

Sixteen plants without symptoms on shoots or roots were randomly collected from plot A in April and again in June 1981 to determine whether taproots and lateral and/or feeder roots were infected from 0 to 28 cm below the soil surface. Roots from each plant were divided into four segments measuring 7–8 cm, washed in running tap water for 2 min, stirred in a solution of 0.5% sodium hypochlorite for 5 min, and rinsed in distilled water for 2 min. Isolations were made on modified Difco potato-dextrose agar (mPDA) (17). All samples were incubated for 1 wk in the dark at 34 C, then observed for the presence of *M. phaseolina* growing from the root segments.

By late August 1981, about 90% of the plants in plot A were dead. Subsequently, plants were mowed, then disked into the soil. At this time, roots were collected from weeds in the area (14 species, three plants per species) with no symptoms on aboveground or belowground parts. Root segments were plated on mPDA as before.

Seeds of *E. lathyris* were sown again into plot A on 20 October 1981. Irrigation procedures and schedules were the same as for the previous (fall) crop of *E. lathyris*; the plot received 9.44 cm of rain from the time of planting through February 1982. Twenty healthy-appearing plants (shoots and roots) were removed from the field at the time of emergence (3 November) and every other week until February 1982. Roots were cultured for *M. phaseolina* as described previously. The mean maximum and minimum temperatures (C) for this period were: November 1981, 20.6 and 4.3; December 1981, 19.5 and 1.0; January 1982, 12.9 and 0.3; and February 1982, 18.4 and 0.3.

One thousand seeds of *E. lathyris* were planted in plots B, C, and D, each consisting of two 180-m rows (1 m apart), in early May 1981. Plants were irrigated by furrows every 14 days. Healthy and diseased plants were counted about the first day of June, July, August, and September, with two additional readings in June. The occurrence of disease in field plots was determined as the percentage of plants with symptoms on aboveground plant parts. All diseased plants were removed from a plot when the data were recorded. Isolations from roots of representative diseased plants (about 10 plants per plot per sampling date) were made on 1.5% water agar + 100 ppm streptomycin sulfate.

Sclerotial populations of *M. phaseolina* in soil. The number of viable sclerotia per gram of air-dried soil was determined using a method modified from Mihail and Alcorn (9). A composite soil sample taken from each of the four field plots consisted of 10 cores, each 28 cm × 30 mm, taken at 1-m intervals in a diagonal path across each plot. The composites

were air-dried for 48–72 hr at 20 C, sifted through a 2-mm^2 screen sieve, and then mixed thoroughly by transferring the soil repeatedly between two beakers. Dried soils were stored at room temperature in lightly capped jars. Three 1-g replicates were assayed for *M. phaseolina* from each composite sample within 10 days of collection. First, each replicate was stirred for 10 min in 100 ml of 0.5% sodium hypochlorite. This treatment kills mycelia but allows sclerotia to survive (12). Next, each replicate was rinsed on a 45-μm^2 screen sieve with tap water for 2 min followed by distilled water for 1 min. The sclerotia and debris on the screen then were backwashed into 100 ml of molten mPDA that was poured into five petri dishes 100 × 15 mm. The number of colonies of *M. phaseolina* on each plate was determined after 5 days of incubation in the dark at 34 C. The means of these replicate counts and their standard deviations were determined.

Fluctuations in the sclerotial populations of *M. phaseolina* in plot A were determined by taking one composite sample each month from February 1981 through January 1982. The sclerotial populations in plots B, C, and D were similarly assayed each month from April (before spring planting of *E. lathyris*) through July 1981. Soils were alternately collected each month along the NE-SW and NW-SE diagonals of each plot. Additionally, in February 1981, 10 individual soil samples were collected along the diagonals from plot A and from a fifth plot (E, 180 × 25 m), previously planted three consecutive times (fall, spring, and fall crops) with *E. lathyris*. Each soil core was individually assayed for *M. phaseolina*.

To determine the longevity of sclerotia in the field, 12 nylon packets (about 6 × 7 threads/cm^2 or 14 × 18 mesh/in.^{2}) measuring 15 × 15 cm were buried 15 cm deep in uncultivated, unirrigated soil (adjacent to plot A) in January 1981. Each packet contained 5 g of dried stems of *E. lathyris* that were infected with *M. phaseolina*. These tissues contained 2,000 ± 240 sclerotia per gram, based on plate assays of comparable material ground to pass through a 40-mesh screen (about 2.5-mm^2 openings). Twelve nylon packets (14 × 18 mesh) measuring 10 × 10 cm were similarly buried. Within each of these packets was a nylon bag (about 39 × 78 threads/cm^2 or 100 × 200 mesh) holding a mixture of 2 g of ground (40 mesh) plant tissue containing 400 ± 48 sclerotia and 2 g of field soil with 2 ± 1 sclerotia per gram of soil. The contents of one packet from each set were retrieved each month (over a 12-mo period) for assaying. Infected stems (in varying stages of disintegration) were first ground to pass through a 40-mesh screen. Dilution-plate (mPDA) assays were made of three subsamples of material from a given packet. Colony counts were made and means determined after plates had been incubated for 5 days in the dark at 34 C.

Inoculation studies. Four-month-old transplants of *E. lathyris* (about 40 cm tall), raised in dry-heat-pasteurized potting soil (1 field soil:1 peat:1 sand, v/v) were used in two unrepeated studies involving different amounts and sources of inocula and postinoculation incubation conditions. Before inoculation, plants were uprooted and rinsed well in tap water to remove adhering soil particles.

In the first study, the *E. lathyris* were transplanted into 15-cm pots (one plant per pot) containing a 3:1:1 (v/v) steam-sterilized mixture of field soil:vermiculite: sand. This was adjusted to contain 1, 5, 25, or 50 sclerotia of *M. phaseolina* per gram of soil by thoroughly mixing into the potting medium an appropriate amount of dried, infected stems of *E. lathyris* ground to pass through a 40-mesh screen. There were five replicates per concentration. Control plants were grown in uninfested soil. The pots were suspended in a wooden box (internally heated to 34 C) in the greenhouse and incubated for 6 wk.

In the second test, naturally infested field soil (50 sclerotia per gram) was diluted with dry-heat-pasteurized potting soil (1 field soil:1 peat:1 sand) to achieve 1, 5, or 25 sclerotia per gram of soil. Test plants (10 replicates per concentration) were placed individually in 10-cm pots containing either field soil or a dilution. Control plants were grown in pasteurized soil. All plants were incubated for 8 wk in a growth chamber with 14 hr of light at 34 C and 10 hr of darkness at 26 C.

Plants were checked each day but watered only when the top 2 cm of soil was dry. This varied between pots in the two controlled-temperature chambers but usually was once every 2–3 days. All plants were assayed for infection, either at the time of aboveground symptom expression or, if the plant appeared healthy, at the end of the experiment. Infection was determined by plating root segments on mPDA as described previously.

RESULTS

Disease development and incidence. As determined by isolations, 69 and 100%, respectively, of the apparently healthy *E. lathyris* plants collected in May and June 1981 (from the October 1980 planting in plot A) were infected by *M. phaseolina*. None of these plants had root lesions or vascular browning. The percentages of *M. phaseolina* isolations from roots occurring 0–7, 8–14, 15–21, and 22–28 cm below the soil surface in May and June were 38 and 50, 25 and 37, 6 and 69, and 19 and 43, respectively. In May, 18% of the plants from which *M. phaseolina* was isolated were infected at two or more soil depths. By June, 31% of the plants had root infections at several depths. Ninety-one percent of the infected but symptomless plants collected in May had infections on lateral and feeder roots only; the remaining infected plants also had taproot infections. The situation had changed in June, when 50% of the infected, symptomless plants had infections in taproots and lateral and feeder root infections only. Mortality in plots A, B, C, and D ranged from 90 to 98% by early September 1981.

Symptomless weed hosts from which *M. phaseolina* was isolated included *Amaranthus palmeri* Wats., *Euphorbia hyssopifolia* L., *E. prostrata* Aiton, *Ipomea coccinea* L., *Sonchus oleraceus* L., and *Tidestromia languinosa* (Nutt.) Standl. *M. phaseolina* was not isolated from *Amaranthus graecizans* L., *Ambrosia confertiflora* DC., *Boerhaavia coulteria* (Hook. f.) Wats., *Echinochloa colonum* (L.) Link., *Hymenothrix wislizeni* Gray, *Leptochloa filiformis* (Lam.) Beauv., *Physalis wrightii* Gray, or *Solanum elaeagnifolium* Cav.

In the October 1981 planting of *E. lathyris* (plot A), progressively more roots were found infected with *M. phaseolina* after plant emergence. In November, 15% of the symptomless plants were infected with this fungus, whereas by December, 90% were infected. Again, there were no lesions on roots or indications of vascular discoloration. Aboveground symptoms were first evident in May 1982.

Sclerotial populations of *M. phaseolina* in soil. The number of viable sclerotia per gram of soil in plot A generally remained low from February (1.1 ± 1.6) through September 1981 (2.3 ± 3.2), although 25% of the plants showed symptoms by July and 90% were dead by early September 1981. The number of sclerotia increased to 4.70 ± 7.2/g of soil after the plant residue was plowed into the soil in October and continued to increase in November (80.0 ± 31.7) and December (246.0 ± 44.0) but decreased during January 1982 to 69.6 ± 16.0.

Sclerotia in the 10 separate samples collected in February 1981 from plots A (safflower) and E (*E. lathyris*) varied from 0 to 5 and 1 to 88 sclerotia per gram of soil, respectively. Sclerotia were detected in the field as deep as 22–28 cm in April (2 sclerotia per gram soil) and May 1981 (0.1 sclerotium per gram soil).

Viable sclerotia were recovered throughout the 12-mo sampling period from the buried packets. The number of sclerotia in the ground stems dropped from the original January 1981 level (2,000 ± 240 sclerotia per gram) to 1,310 ± 52 in February 1981. The number of sclerotia in subsequent samplings always exceeded the original inoculum level but varied, increasing to a peak of 75,000 ± 6,999 in May, then decreasing with variable numbers through the following January. Lowest numbers (3,300 ± 577) were in November. Sclerotia recovered from the soil-plant residue mixture always exceeded the original inoculum level (400 ± 48) but fluctuated between

559 ± 68 and 560 ± 96 (February and April) and 3,900 ± 220 (September). Sclerotial numbers in the plant residue and in the soil-plant residue mix did not fluctuate concomitantly.

Inoculum densities in the composite soil samples taken in plots B, C, and D before the spring 1981 planting of *E. lathyris* were 0.7 ± 0.5, 0.2 ± 0.2, and 11.5 ± 2.9 sclerotia per gram of soil, respectively. Although the initial sclerotial populations following soybeans (plot D) were more than 15 times the number in soils following crops of cotton or safflower, 0.2 sclerotium per gram of soil (plot C) was sufficient to cause 90% crop loss of *E. lathyris*. Populations of sclerotia per gram in plots B, C, and D, respectively, fluctuated within the following ranges during the summer growing season: 0.7–3.0, 0.2–4.3, and 11.5–28.3.

Assays also detected 0.7 ± 0.6 sclerotia per gram of soil in a composite soil sample from plot A (safflower) collected in the field in January 1981 and 0.6 ± 0.6 sclerotia per gram 1 yr later, following storage at room temperature.

Inoculation studies. Regardless of inoculation procedures or incubation conditions, at least 17 days elapsed before stem and/or foliar symptoms were noted. Generally, sclerotia were observed on plants after death. Of the 30 plants inoculated in the two procedures with at least 25 sclerotia per gram of soil, 23 developed typical symptoms and died by the end of the experiment (60 days). The remaining plants had varying degrees of root-stem discoloration/disintegration. *M. phaseolina* was recovered from 27 plants. Only three of 15 plants survived inoculations with five sclerotia per gram of soil. The fungus was only recovered from the dead plants. Mortality rates after inoculations with one sclerotium per gram of soil varied with the procedure used. All plants were infected and four died when the inoculum was incorporated into sterilized potting soil. The fungus could not be recovered from any plant when the inoculum was combined with field soil alone.

DISCUSSION

The populations of *M. phaseolina* propagules reported in this paper did not vary greatly from those in other agricultural crops and soils (2,13), keeping in mind that time of sampling, isolation technique, and prior cropping history may influence the number of sclerotia detected in soil. For *E. lathyris*, population differences were of little consequence because an initial concentration of 0.2 sclerotium per gram of soil could result in a 90% reduction in stand in the field.

Our data show that *M. phaseolina* can be recovered from symptomless plants of *E. lathyris* during the winter in southern Arizona. Although it is not known whether or not hyphae are quiescent once the plant has been invaded, no cortical or vascular discolorations of infected roots were noted. Although infection by *M. phaseolina* is frequently associated with water stress and high temperatures (6), such conditions are not required for infection of *E. lathyris*, but rather, relate to symptom induction. The fact that infection can occur months before symptoms are evident affects not only the timing of applications of protective, nonsystemic chemicals but the need for repeated applications, depending on the longevity of fungicidal effect. Such expenses could be prohibitive, requiring development of other control procedures. This concern also would apply to other crops that also have prolonged delays between infection by *M. phaseolina* and expression of symptoms.

Control of *M. phaseolina* by fallow is confounded by the degree of moisture in the soil. Dhingra and Sinclair (3) recovered at least 95% of the original inoculum buried in dry soil for 7 wk. Our data show that propagules can survive in untilled soils for 1 yr at 15-cm depths and for at least 1 yr in dry soil under laboratory conditions. It has been shown, however, that the longevity of sclerotia in soil is reduced at a water potential of −0.01 bars (11) or at 40–100% moisture-holding capacity (3).

Use of wet fallow to reduce populations of *M. phaseolina* propagules in soil (3) may be complicated by the occurrence of weed hosts. There are three reports of weeds as hosts of this fungus (1,7,18); we specify six new host species. The role that infected weeds play in contributing to inoculum levels in our soils is not known; sclerotia were not detected in the infected roots at the time of our isolations. However, the fact that *M. phaseolina* can rapidly form sclerotia in or on dead roots of other hosts (4) indicates that sclerotia should be found on the roots of these weeds in time. Whether or not sclerotia are formed, weed hosts do provide for limited survival and persistence of *M. phaseolina*.

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LITERATURE CITED

1. Bruton, B. D. 1982. Horse purslane, *Trianthema portulacastrum*, as a host of *Macrophomina phaseolina*. (Abstr.) Phytopathology 72:355.
2. Cottingham, C. 1981. Numbers and distribution of sclerotia of *Macrophomina phaseolina* in soils of South Carolina. Plant Dis. 65:355-356.
3. Dhingra, O. D., and Sinclair, J. B. 1974. Effect of soil moisture and carbon:nitrogen ratio on survival of *Macrophomina phaseolina* in soybean stems in soil. Plant Dis. Rep. 58:1034-1037.
4. Dhingra, O. D., and Sinclair, J. B. 1978. Biology and pathology of *Macrophomina phaseolina*. Imprensa Universitaria, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. 166 pp.
5. Edmunds, L. K. 1964. Combined relation of plant maturity, temperature, and soil moisture to charcoal stalk rot development in grain sorghum. Phytopathology 54:514-517.
6. Ghaffar, A., and Erwin, D. C. 1969. Effect of soil water stress on root rot of cotton caused by *Macrophomina phaseoli*. Phytopathology 59:795-797.
7. Ghaffar, A., and Zentmyer, G. A. 1968. *Macrophomina phaseolina* on some new weed hosts in California. Plant Dis. Rep. 52:223.
8. Johnson, J. J., and Hinman, C. W. 1980. Oils and rubber from arid land plants. Science 208:460-464.
9. Mihail, J. D., and Alcorn, S. M. 1982. Quantitative recovery of *Macrophomina phaseolina* sclerotia from soil. Plant Dis. 66:662-663.
10. Peoples, T. R., Alcorn, S. M., Bloss, H. E., Clay, W. F., Flug, F., Hoffman, J. J., Lee, C. W., Luna, S., McLaughlin, S. P., Steinberg, M., and Young, D. 1981. *Euphorbia lathyris* L.: A future source of extractable liquid fuels. Biosources Dig. 3:117-123.
11. Shokes, F. M., Lyda, S. D., and Jordans, W. R. 1977. Effect of water potential on the growth and survival of *Macrophomina phaseolina*. Phytopathology 67:239-241.
12. Short, G. E., Wyllie, T. D., and Ammon, V. D. 1978. Quantitative enumeration of *Macrophomina phaseolina* in soybean tissues. Phytopathology 68:736-741.
13. Short, G. E., Wyllie, T. D., and Bristow, G. R. 1980. Survival of *Macrophomina phaseolina* in soil and in residue of soybean. Phytopathology 70:13-17.
14. Vanderplank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York. 349 pp.
15. Verhoeff, K. 1974. Latent infections by fungi. Annu. Rev. Phytopathol. 12:87-98.
16. Young, D. J., and Alcorn, S. M. 1982. Soilborne pathogens of *Euphorbia lathyris*: *Macrophomina phaseolina*, *Pythium aphanidermatum*, and *Rhizoctonia solani*. Plant Dis. 66:236-238.
17. Young, D. J., Gilbertson, R. L., and Alcorn, S. M. 1982. A new record for longevity of *Macrophomina phaseolina* sclerotia. Mycologia 74:504-505.
18. Young, P. A. 1949. Charcoal rot of plants in east Texas. Texas Agric. Exp. Stn. Bull. 712. 33 pp.