Rhizosphere Effect of Herbicide-Stressed Sicklepod (*Cassia obtusifolia*) on Chlamydospores of *Fusarium oxysporum* f. sp. *vasinfectum*

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ABSTRACT

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Equal concentrations of sterile seed + root exudates from eight weed-plant species promoted higher levels of chlamydospore germination of *Fusarium oxysporum* f. sp. *vasinfectum* and induced more rapid germ-tube extension than occurred in sterile, demineralized water. Germination ranged from 91% in spurred anoda (*Anoda cristata*) exudate to 49% in goosegrass (*Eleusine indica*) exudate and 24% in water. Germination in sicklepod (*Cassia obtusifolia*) exudate was 83%. Linuron-treated sicklepod seedlings cultured in a system of water and glass beads liberated 19.8% more exudate than untreated plants, but chlamydospore germination in exudates from these two sources did not differ significantly, nor was germination different in rhizosphere soil collected from treated and untreated plants growing in a sterile system. Spore germination, however, was significantly suppressed in rhizosphere soil from roots of herbicide-treated sicklepod growing in natural (nonsterilized) soil. Populations of *Fusarium* spp. and the general fungal flora of the rhizosphere also were reduced compared with populations in the rhizosphere of untreated plants. These results suggest a foliar-herbicide-induced influence on soil fungistasis in the rhizosphere of declining target plants after treatment.

Additional key words: microbial populations

Plant pathogen behavior and root diseases are distinctly related to certain rhizosphere phenomena (6). The environment of the root-soil interface is created in part by the interactions between chemical substances (exudates) released into the soil by living roots and the microorganisms that use these substances as nutrient sources or are inhibited by them. Foliar-applied chemicals can affect root exudation by altering the physiological processes of a plant or by downward translocation and release from roots into the soil, either intact or as metabolic by-products (10).

Research has shown that soil-applied herbicides affect the activities of pathogenic fungi and the occurrence or severity of root disease (2,4,12). No information is available, however, on the response of plant pathogens to the root environment of target (weed) plants after foliar application of herbicides. Because nonsusceptible plants are important in the perpetuation of some root pathogens, such as *Fusarium* spp., any alteration of the rhizosphere of such plants by herbicides may affect the pathogen's life cycle. Our study was an effort to determine the quantitative change in root exudation during the stress period between postemergence herbicide application and the death of weed plants and to assess the subsequent effect on chlamydospores of *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. & Hans. and on microbial populations in the rhizosphere of one selected target plant, *Cassia obtusifolia* L. This species was selected for further study on the basis of its intermediate effect on spore germination in preliminary tests, its importance as a cotton field weed, and its suitable growth features for experimentation.

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MATERIALS AND METHODS

Preliminary screening. Combined seed and root exudates were collected from eight species of common weed plants. Seeds were surface-disinfested for 1 hr in 50 ml of 0.52% sodium hypochlorite containing 2 ml of 95% ethanol and 0.2 ml of Tween 20 (polyoxyethylene 20 sorbitan monolaurate). Several seeds of each plant species (number depending on size) were placed in each of 25 sterile, screw-capped vials (15 × 45 mm) with enough sterile, demineralized water to cover the seeds. The vials with seeds were kept at 10 C for 24 hr to induce maximum exudation, then transferred to 27 C. After seed germination, sterile water was again added and the vials were shaken gently. The exudate solutions were tested for bacterial or fungal contamination, and nonsterile vials were discarded. Sterile solutions for each plant species were pooled in preweighed test tubes, freeze-dried, and the weights recorded.

Sterile water was added to each tube of freeze-dried exudate to make a 2-mg/ml solution. These solutions were tested for effects on germination of chlamydospores of *Fusarium oxysporum* f. sp. *vasinfectum* (ATCC 7808). The spores were produced in a liquid, soil-extract medium (5) and separated from the mycelium by sonication (sonicator cell disruptor, model W175, Heat Systems-Ultrasonics, Inc.) for 2 min at power control setting 2 and a 0.5-sec pulse rate. Seed-root exudate from each weed species was then mixed with spore suspension in shallow wells of five microscope depression slides to provide 1 mg of exudate and 50,000 chlamydospores per milliliter; five slides with spores in sterile water served as the control. At intervals of 4, 6, 8, and 10 hr, germination and germ-tube extension were determined for 50 spores per slide.

Tests with exudates from untreated and herbicide-stressed plants. A single species, sicklepod (*C. obtusifolia*), was selected for further study. Eight surface-disinfested, pregerminated seeds were placed on a glass-fiber mat over a column of sterile glass beads and demineralized water in each of 12 glass tubes (200 × 30 mm) closed with Styrofoam plugs. The resistance of glass beads to root growth is known to increase the quantity of root exudates (3). This system permitted collection of root exudates while excluding seed exudates. The tubes were placed under combined incandescent and fluorescent lights (6 klux) timed automatically for 12 hr of light and 12 hr of darkness. The temperature was constant at 25 C.

After 3 days, when roots were fully extended among the beads, the cotyledons and upper stems of plants in six of the tubes were treated with a 24-mg/ml suspension of linuron (Lorox 50W) containing a trace of Tween 20. Immediately after the death of treated plants, the root exudate solutions were collected and pooled; solutions from untreated, healthy plants also were collected at this time. The pooled solutions were dispensed into sterile, oven-dried, preweighed glass tubes. The solutions were freeze-dried and weights recorded. The residue was then diluted with sterile water to provide exudate solutions of 2 mg/ml, which, when added to depression-slide wells with a chlamydospore suspension of 50,000 spores per

milliliter, were reduced to 1 mg/ml. Six depression slides per exudate type were held in moist chambers for 10 hr, then subsamples were transferred to standard microscope slides and 50 spores (300 spores per treatment) were examined for germination and germ-tube extension. A spore was considered germinated if the germ tube length was at least equal to spore width.

Chlamydospore germination in rhizosphere soil. Chlamydospore germination was observed in rhizosphere soil from untreated or herbicide-stressed sicklepod plants that had been grown in either sterilized or nonsterilized soil. Sandy loam soil (sand 70%, silt 20%, and clay 10%) was obtained from a field plot maintained under a long-term rotation of cotton, soybean, and corn and a high-fertility status for more than 12 yr. Eighteen sterilized glass tubes (200 × 30 mm) were filled aseptically with screened, sterilized (autoclaved) soil, and 18 tubes with nonsterilized soil. Twelve tubes of each group received eight surface-sterilized, pregerminated seeds. Six of the 18 tubes received no seeds; these served as a source of nonrhizosphere soil. After 3 days, leaves and stems of plants in six tubes of either sterile or nonsterile soil were treated with linuron as before; other plants were not treated.

When treated plants showed symptoms of stress and decline, both treated and untreated plants were removed and any adhering rhizosphere soil was brushed back into the tubes. All of the soil in tubes with plants was considered rhizosphere soil, because the root systems had permeated the entire soil column. An equivalent amount of soil (nonrhizosphere) was collected from the unplanted tubes. Chlamydospores of *F. oxysporum* f. sp. *vasinfectum*, produced in the same manner as in the preliminary screening, were buried in the soil on filter membranes and processed according to the method of Adams (1) to determine germination. One milliliter of a chlamydospore suspension (100,000 spores per milliliter) was applied to 25-mm, Type HA Millipore filters (0.45-μm pore size), and the excess water was removed by vacuum. One filter with spores was then buried in soil (moisture adjusted to 9% of dry weight) in each of 12 sterile Coors No. 1 porcelain crucibles per treatment. After 10 hr at 27 C, the filters were recovered and stained for microscope observation as described by Adams (1). Germination was recorded for 50 spores per filter on 12 filter replicates per treatment.

Chlamydospore production. Spore production from mycelial fragments was determined in soil from the following six sources: 1) rhizosphere soil from untreated sicklepod plants grown in sterilized soil, 2) rhizosphere soil from herbicide-stressed plants in sterilized soil, 3) rhizosphere soil from untreated plants in nonsterilized soil, 4) rhizosphere soil from herbicide-stressed plants in nonsterilized soil, 5) sterile nonrhizosphere soil, and 6) natural nonrhizosphere soil. Methods for plant culture and treatment and for collecting rhizosphere soil were as previously described.

Soil from each source was placed into a separate plastic bag, and 10 ml of a chopped-mycelium suspension (prepared from a Czapek-Dox culture) was added and mixed thoroughly. The infested soil was then transferred to sterile, deep culture dishes (400-ml capacity) with petri dish covers and incubated at 25 C for 30 days. Three-gram samples were then added to 30 ml of water in 50-ml Erlenmeyer flasks, shaken 10 min on a mechanical shaker, stirred for 10 min on a magnetic stirrer, and allowed to settle for 12 min. A 1-ml sample, taken from the top portion of the suspension, was applied to a 25-mm Type HA Millipore filter over suction leaving small soil particles and chlamydospores on the filter membrane surface. Six filters were prepared for each of the six soil treatments; these were placed on glass slides, stained as before, and mounted in glycerin for microscope examination. The total number of chlamydospores in 10 fields of view (430×) was recorded for each filter, and means were obtained from the six replicates of each treatment.

Microbial populations. General microbial populations in the rhizosphere were determined as indicators of gross changes in the environment induced by foliar herbicide treatment. Field soil of the same type and source as used for chlamydospore tests was used to grow sicklepod plants in large glass tubes and provide rhizosphere soil from healthy and herbicide-stressed plants. Plants were cultured and treated as before, then populations of rhizosphere or nonrhizosphere bacteria and fungi were estimated according to a short dilution and plating procedure described by Curl and Rodriguez-Kabana (7).

Analysis of variance and Duncan's multiple range test were performed for all data except in the preliminary screening. Any references to significant differences in the text are based on $P = 0.05$.

RESULTS

Chlamydospore germination in seed-root exudates. After 10 hr of incubation, germination of chlamydospores ranged from 49% in goosegrass exudate to 91% in spurred anoda exudate compared with 24% in sterile water (Table 1). Germ tube growth (Table 2) after 10 hr also was least affected by goosegrass exudate and the water control. Thus, equal quantities of exudate from different plant species may confer different levels of benefit to chlamydospore germination and early hyphal growth.

Effects of sterile root exudates from herbicide-stressed sicklepod on chlamydospore germination. Herbicide stress resulted in a 19.8% increase in the quantity of freeze-dried root exudate over that obtained from untreated sicklepod plants. The average germination rate of chlamydospores in root exudate from stressed sicklepod (74.6%), however, was not significantly different from that of spores in exudate from healthy sicklepod (69%), as determined in deep-well slides containing 1 mg of freeze-

Table 1. Germination of *Fusarium oxysporum* f. sp. *vasinfectum* chlamydospores in sterile seed and root exudates of common weed plants[a]

Plant source of exudates	Germination (%)	
	6 hr	10 hr
Spurred anoda (*Anoda cristata* (L.) Schlecht.)	32	91
Velvetleaf (*Albutilon theophrasti* Medic.)	33	88
Prickly sida (*Sida spinosa* L.)	27	87
Tall morning glory (*Ipomoea purpurea* (L.) Roth.)	45	86
Sicklepod (*Cassia obtusifolia* L.)	22	83
Redroot pigweed (*Amaranthus retroflexus* L.)	40	81
Johnsongrass (*Sorghum halepense* (L.) Pers.)	29	71
Goosegrass (*Eleusine indica* (L.) Gaertn.)	15	49
Sterile water	11	24

[a] Surface-sterilized seeds were germinated in sterile demineralized water; the exudate solution was collected, freeze-dried, weighed, resuspended in sterile water, and tested in depression slides for effect on spore germination.

Table 2. Germ-tube extension of *Fusarium oxysporum* f. sp. *vasinfectum* chlamydospores in sterile seed and root exudates of common weed plants[a]

Plant source of exudates	Germ-tube length (μm)	
	6 hr	10 hr
Tall morning glory	36	80
Velvetleaf	42	76
Johnsongrass	46	64
Spurred anoda	25	64
Prickly sida	32	64
Sicklepod	21	63
Redroot pigweed	22	57
Goosegrass	22	39
Sterile water	34	34

[a] Surface-sterilized seeds were germinated in sterile demineralized water; the exudate solution was collected, freeze-dried, weighed, resuspended in sterile water, and tested in depression slides for effect on spore germination and germ-tube growth.

dried exudate per milliliter of spore suspension (100,000 spores per milliliter). The pH (7.5–7.7) of the exudates did not differ significantly.

Germination of chlamydospores in rhizosphere soil. Germination of chlamydospores on the surface of membrane filters buried in sterile rhizosphere soil from healthy (untreated) sicklepod was only 25% and not significantly different from 31.7% germination observed in sterile rhizosphere soil from herbicide-stressed plants (Table 3). Each of these values, however, was lower than that for spores in nonrhizosphere soil (49.7%). In natural (nonsterile) rhizosphere soil from healthy sicklepod plants, chlamydospore germination (29.8%) was significantly higher than in either rhizosphere soil from herbicide-stressed plants (17.5%) or nonrhizosphere soil (20%). The extent of germ-tube lysis of spores in nonsterile rhizosphere soil from herbicide-treated plants did not differ significantly from lysis in rhizosphere soil from untreated plants.

Chlamydospore production. When plants were cultured in sterilized soil and the rhizosphere soil from roots was used in spore production tests, the number of chlamydospores produced by *F. oxysporum* f. sp. *vasinfectum* in soil from roots of herbicide-stressed sicklepod was significantly lower than the number in soil from untreated plants (Table 4). Like the earlier spore germination results (Table 3), spore production in nonrhizosphere (plantfree) soil was considerably higher than in rhizosphere soil of either treatment. In nonsterilized soil, spore production was extremely low for either treatment and for nonrhizosphere soil, and no differences attributable to herbicide treatment were observed (Table 4).

Microbial populations. The highest average number of colony-forming units (cfu) of *Fusarium* spp. (38,000/g of soil) was recorded for nonrhizosphere soil and the lowest (12,300/g) for rhizosphere soil from herbicide-stressed sicklepod (Table 5); the population in rhizosphere soil of untreated sicklepod was slightly but not significantly higher than that in soil associated with treated plants. The non-*Fusarium* fungal population was significantly lower in rhizosphere soil of herbicide-treated plants than in the rhizosphere of healthy untreated plants or in nonrhizosphere soil. Relative bacterial populations ranged from 45.9 million per gram of nonrhizosphere soil to 38.4 million per gram in rhizosphere soil of herbicide-treated sicklepod.

DISCUSSION

The principal factors that determine the quantitative and qualitative nature of root exudates have been discussed by Hale and Moore (10) and by Curl and Truelove (8). Among these factors, plant species and developmental stage are considered of major importance. Quantities and kinds of amino acids, sugars, organic acids, vitamins, and a host of miscellaneous compounds can be expected to vary among the exudates of different plant species (13,15–18). It is not surprising, therefore, that sterile seed-root exudates of the eight weed-plant species tested in our initial screening program varied in their enhancement of *F. oxysporum* f. sp. *vasinfectum* chlamydospore germination compared with a low-nutrient water treatment. However, although nutrient differences probably accounted for most of the variation in spore germination, it is also recognized that exudates of some plants contain fungal growth inhibitors that may contribute to differences in spore germination (8).

Linuron-treated sicklepod seedlings growing in a system of water and glass beads liberated 19.8% more exudate than untreated plants, yet chlamydospore germination in exudates of the two sources did not differ significantly; this suggested that the qualitative nature of exudate probably was not measurably altered in water culture. Similarly, when sicklepod was cultured in sterilized sandy loam, germination of chlamydospores in rhizosphere soil taken from roots of herbicide-treated plants was not significantly different from germination in soil from roots of untreated plants. At the same time, germination in nonrhizosphere, sterilized soil was higher than in either rhizosphere soil, suggesting that sicklepod root exudate may contain fungistatic components. The percentage of spore germination was reduced to its lowest value (17.5%) in rhizosphere soil from roots of herbicide-treated plants growing in natural (nonsterilized) soil. This value, significantly lower than the 29.8% germination in rhizosphere soil from untreated sicklepod, indicated a treatment effect that resulted in either the release of spore-inhibiting substances in the root exudate or the intensification of natural soil fungistasis; root exudation of foliar-applied chemicals, or metabolic by-products, has been demonstrated (9,14). Though the presence or absence of linuron or its by-products in root exudates of treated sicklepod was not determined in this study, the inhibition of spore germination by such compounds should be considered a possibility. Generally, however, there is relatively little basipetal movement of urea herbicides when applied to leaves (11); the increased root exudation by sicklepod following foliar treatment with linuron is viewed here primarily as a physiological

Table 3. Germination of *Fusarium oxysporum* f. sp. *vasinfectum* chlamydospores in rhizosphere soil taken from roots of herbicide-treated (linuron) and untreated sicklepod seedlings cultured either in a sterile-soil system or in nonsterilized field soil

Soil source	Spore germination (%)[z]	
	Sterilized soil	Non-sterilized soil
Nonrhizosphere	49.7 a	20.0 a
Untreated-sicklepod rhizosphere	25.0 b	29.8 b
Herbicide-treated sicklepod rhizosphere	31.7 b	17.5 a

[z] Each value represents the mean percentage germination of 600 spores (50 spores × 12 replicates) per treatment (soil source); within a column, means followed by the same letter do not differ significantly at $P = 0.05$.

Table 4. Chlamydospore production by *Fusarium oxysporum* f. sp. *vasinfectum* in rhizosphere soil taken from roots of herbicide-treated (linuron) and untreated sicklepod seedlings cultured either in a sterile-soil system or in nonsterilized field soil

Soil source	Number of chlamydospores[z]	
	Sterilized soil	Non-sterilized soil
Nonrhizosphere	15.0 a	1.7 a
Untreated-sicklepod rhizosphere	7.0 b	2.0 a
Herbicide-treated sicklepod rhizosphere	2.3 c	0.8 a

[z] Each value represents the mean number of spores in 10 microscope fields of view at 430× for six replicates (soil-water suspension samples); within a column, means followed by the same letter do not differ significantly at $P = 0.05$.

Table 5. Microbial populations in rhizosphere soil of herbicide-treated (linuron) and untreated sicklepod seedlings

Soil source	Population densities (cfu/g dry soil)[z]		
	Bacteria + Streptomycetes (× 10^6)	Fungi (× 10^3)	
		Fusarium spp.	Other
Nonrhizosphere	45.9 a	38.0 a	37.0 a
Untreated-sicklepod rhizosphere	40.4 a	14.5 b	35.8 a
Herbicide-treated sicklepod rhizosphere	38.4 a	12.3 b	14.5 b

[z] Colony-forming units, determined by standard dilution and plating procedures; within a column, means followed by the same letter are not significantly different at $P = 0.05$.

response to stress as the treated plants declined.

Further indications that sicklepod rhizosphere, especially under the influence of herbicide stress, may create a fungus-suppressive (rather than stimulatory) environment are seen in the lower number of *Fusarium* chlamydospores produced and the reduced populations of the general microflora in rhizosphere soil.

Although the specific roles of root-exudate chemical components and microbial interactions in the rhizosphere have not been determined in this study, it seems evident that herbicide stress of the sicklepod plant by foliar application may alter root exudation to some degree and affect spore germination and the reproductive behavior of *F. oxysporum* f. sp. *vasinfectum*.

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