Efficacy and Soil Persistence of *Fusarium solani* f. sp. *cucurbitae* for Control of Texas Gourd (*Cucurbita texana*)

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ABSTRACT

Weidemann, G. J., and Templeton, G. E. 1988. Efficacy and soil persistence of *Fusarium solani* f. sp. *cucurbitae* for control of Texas gourd (*Cucurbita texana*). Plant Disease 72: 36-38.

Fusarium solani f. sp. *cucurbitae* (*F. s.* f. sp. *cucurbitae*) effectively controlled Texas gourd (*Cucurbita texana*) when applied as preplant-incorporated conidial suspensions or as preemergence applications of *F. s.* f. sp. *cucurbitae*-infested sodium alginate granules amended with nutritional substrates. Suspensions of either microconidia or macroconidia at 3×10^{13}/ha, alone or in combination with trifluralin at 1 kg a.i./ha, provided significant control of sequentially planted Texas gourd up to 12 wk after application. *F. s.* f. sp. *cucurbitae*-infested sodium alginate granules amended with 2% (w/v) soy flour or 2% (w/v) ground oatmeal at 110 or 220 kg/ha provided greater control for a longer period than did conidial applications. Initial control levels exceeding 80% declined to 50–70% within 12 wk at the highest application rates. Soil populations of *F. s.* f. sp. *cucurbitae* declined more than 90% within 6 wk when applied as conidial suspensions. Soil populations from granular applications increased for the first 6 wk, followed by rapid declines.

Additional key words: biological weed control, mycoherbicide

Texas gourd (*Cucurbita texana* A. Gray) is a persistent, localized weed problem in soybean (*Glycine max* (L.) Merr.) and cotton (*Gossypium hirsutum* L.) fields in the Red River and Arkansas River floodplains. The weed causes yield reductions and interferes with cultivation and harvesting (9).

Texas gourd can be controlled with several preemergence and postemergence chemical herbicides, including acifluorfen, lactofen, imazaquin, and oxyfluorfen or metribuzin + 2,4-DB, but control has been inconsistent over several years (9). Effective preemergence control depends on soil moisture after application. Postemergence control depends on plant age at application and generally requires repeat applications (9). Effective chemical control also has been complicated by continual emergence of Texas gourd under favorable environmental conditions throughout the growing season.

In 1978, an endemic, soilborne fungal pathogen causing postemergence root and collar decay was isolated from Texas gourd seedlings (2,3). The pathogen was identified as *Fusarium solani* (Mart.) Appel. & Wr. f. sp. *cucurbitae* (*F. s.* f. sp. *cucurbitae*) race 1 and confirmed by mating experiments (3,11).

Greenhouse studies confirmed pathogenicity to Texas gourd and several other wild and cultivated cucurbits, including cultivated gourd (*C. pepo* L. var. *ovifera*), pumpkin (*C. pepo*), and squash (*C. pepo* var. *malopepo*) (2,3). Applications of microconidial suspensions or cornmeal-sand mixtures demonstrated effective preemergence and postemergence control in field studies. *F. s.* f. sp. *cucurbitae* soil populations declined rapidly in all soils tested (3). Other studies (20,21) demonstrated increased disease incidence and severity when *F. s.* f. sp. *cucurbitae* was applied sequentially or in a tank mix with trifluralin (Treflan EC). In addition to the environmental benefits of low toxicity and high target specificity, an advantage of *F. s.* f. sp. *cucurbitae* might include persistence in the soil after preemergence herbicides have dissipated and its ability to enhance existing chemical herbicide treatments in cotton and soybeans.

These studies were conducted to establish effective rates of several formulations of *F. s.* f. sp. *cucurbitae* and to determine the ability of *F. s.* f. sp. *cucurbitae* to persist in the soil environment at population levels sufficient to provide residual control of Texas gourd. Portions of this work have been reported previously (19).

Accepted for publication 8 September 1987 (submitted for electronic processing).

MATERIALS AND METHODS

Inoculum production. Microconidia of *F. s.* f. sp. *cucurbitae* were produced in modified Richard's solution (25 g glucose, 10 g KNO_3, 5 g KH_2PO_4, 2.5 g $MgSO_4 \cdot 7H_2O$, 0.02 g $FeCl_3$, 150 ml V-8 juice, and distilled water to 1 L) in a 14-L laboratory fermenter (28 C, 400 rpm, 1 L/L medium per minute of aeration). After 48–72 hr, conidia were separated from hyphae by filtration through a 150-mesh vibrating screen, centrifuged, and resuspended in distilled water.

Macroconidia were produced on 25–30 ml of oatmeal agar (16) in 250-ml Erlenmeyer flasks inoculated with mycelial plugs removed from the margins of actively growing colonies. The flasks were incubated on a laboratory bench with 12 hr of supplemental, cool-white fluorescent lighting at ambient air temperature (25 C). After 10–14 days, macroconidia were washed from the culture flasks with distilled water, passed through a 100-mesh screen to remove hyphal fragments, centrifuged, and resuspended in distilled water. All conidial treatments were adjusted to the desired concentrations with a hemacytometer.

Sodium alginate granules were prepared using modified methods of Walker and Connick (18). Fungal biomass, composed of mycelium and microconidia produced in a laboratory fermenter, was centrifuged, resuspended in an equal volume of distilled water, and comminuted for 10 sec in a blender to give a final concentration of $1\text{–}5 \times 10^8$ colony-forming units per milliliter. The fungal suspension was diluted 1:3 (v/v) with 1% (w/v) sodium alginate amended with 7.5% (w/v) kaolin and either 2% (w/v) ground soy flour or 2% (w/v) ground oatmeal. The mixture was dripped into 0.25 M $CaCl_2$, harvested, and air-dried on fiberglass screens. The dried granules were passed through a 3-mm-mesh screen to separate adhering granules and stored at 4 C until use.

Field studies. Field studies were conducted on Nixa cherty silt loam at the University of Arkansas Agricultural Experiment Station Farm at Fayetteville in 1985 and 1986. Applications were made on 14 June 1985 and 15 June 1986. Treatment plots measured 1 × 2 m separated by 1.5-m borders. The test was arranged as a split-plot design with treatments as the main plot and biweekly planting of Texas gourd as the subplots. Each treatment was replicated four times.

Conidial applications were preplant-incorporated at 1×10^6, 1×10^7, or 1×10^8 conidia per milliliter in 280 L/ha with a CO_2 sprayer adjusted to 140 kPa, corresponding to 3×10^{11}, 3×10^{12}, and 3×10^{13} conidia per hectare, respectively. Trifluralin alone or tank-mixed with *F. s.* f. sp. *cucurbitae* was applied at 1 kg a.i./ha. All spray applications were incorporated 3–5 cm deep with a rake. Granular applications at 110 or 220 kg/ha were broadcast by hand immediately after planting but were not incorporated.

Thirty-five Texas gourd seeds were planted in a single 1-m row across each plot after initial infestation or immediately before granular applications. Subsequent rows were planted 30 cm apart at 2-wk intervals for 6 wk. Overhead irrigation was applied after each planting and as needed. Seedling stand counts were made at weekly intervals for 6 wk. Only plants permanently wilted or killed were counted as controlled.

Soil samples were taken from plots with the highest application rate of each *F. s.* f. sp. *cucurbitae* treatment and from the control plots. Soil cores 3–5 cm deep were obtained with a 2.5-cm-diameter soil-sampling tube from five to seven random locations within each main plot. The samples were stored in plastic bags at 4 C for 24–48 hr. Each sample was thoroughly mixed by hand, and a 10-g subsample was removed and serially diluted 10^{-3} in 0.15% water agar. Separate 1-ml aliquots were pipetted onto eight PCNB (7) plates modified by substituting 50 mg of chlortetracycline HCl for neomycin. The suspensions were thoroughly distributed across each plate with a glass rod. Plates were incubated on a laboratory bench at ambient air temperature for 5–7 days, then characteristic *F. s.* f. sp. *cucurbitae* colonies (3) were counted. An additional 10-g subsample was oven-dried at 90 C for 24 hr, and colony counts were converted to a dry weight basis.

All data were subjected to analysis of variance. Treatment means from both years were combined for comparison testing of means.

RESULTS AND DISCUSSION

During the 2 yr of the study, there were significant effects for treatments, week of planting, and treatment × year interactions at $P \leqslant 0.0001$. Although the treatment × year interaction was significant, differences between years were small in nearly all treatments. However, macroconidial applications at 3×10^{11} and 3×10^{12} conidia/ha gave greater control in 1986. Also, soy flour-amended granules provided less control in 1986.

F. s. f. sp. *cucurbitae* provided >80% control of the initial Texas gourd planting when applied as aqueous conidial suspensions of 3×10^{13} conidia per hectare or as nutrient-amended sodium alginate granules applied at 110 or 220 kg/ha (Table 1). Control was comparable to that obtained with several preemergence and postemergence chemical herbicides (9) or with postemergence applications of acifluorfen (G. J. Weidemann, *unpublished*). Applications of microconidia or macroconidia at 3×10^{11} and 3×10^{12} conidia/ha resulted in lower levels of weed control.

Control of Texas gourd diminished over time in subsequent plantings but continued to provide significant residual control of emerging seedlings throughout the test. After 12 wk, control was generally better with the granular formulations than with the aqueous conidial applications.

Tank mixtures of trifluralin and *F. s.* f. sp. *cucurbitae* did not appreciably influence disease severity in the study. Previous laboratory and greenhouse studies (20,21) have demonstrated that trifluralin could enhance disease incidence and severity of *F. s.* f. sp. *cucurbitae* under some conditions, as was previously demonstrated for other soilborne pathogens (8,13). Trifluralin alone did not control Texas gourd as previously reported (20,21). An important consideration for the development of a mycoherbicide is the ability to integrate it with existing crop management systems (10,14). The compatibility of *F. s.* f. sp. *cucurbitae* with trifluralin and perhaps other herbicides (2) would be an advantage for commercial development (1,14) by extending the spectrum of weed control within a crop or by enhancing the activity of selected herbicides. Other herbicides recommended for use in cotton and soybeans should be tested for compatibility with *F. s.* f. sp. *cucurbitae*.

Populations of *F. s.* f. sp. *cucurbitae* rapidly declined in the soil when applied as preplant-incorporated conidial applications (Fig. 1). Trifluralin treatments in combination with *F. s.* f. sp. *cucurbitae* did not significantly affect soil populations (data not shown). Within 2 wk of application, the initial *F. s.* f. sp. *cucurbitae* populations had declined 40–70%. After 6 wk, <10% of the initial populations remained. However, *F. s.* f. sp. *cucurbitae* was still recovered from all treatments sampled 14 wk after infestation. Although populations declined rapidly, sufficient propagules remained except at the lowest application rates to infect significant numbers of seedlings during subsequent replants up to 6 wk after infestation.

Previous studies by Boyette et al (3) demonstrated a rapid decline of *F. s.* f. sp. *cucurbitae* in field soils when applied as conidial suspensions or as a cornmeal-sand mixture. *F. s.* f. sp. *cucurbitae* populations survived <12 mo and declined >70% within 4 wk of application. Other investigators (4,12,15) have also reported rapid declines of *F. s.* f. sp. *cucurbitae* in the soil environment compared with other formae speciales of *F. solani*. Nash and Alexander (6) attributed the rapid decline of *F. s.* f. sp. *cucurbitae* to differences in chlamydospore wall structure, but this was not confirmed by Van Eck (17). Differences in propagule nutritional levels have also been suggested as a reason for poor persistence (17).

In contrast to conidial applications, *F. s.* f. sp. *cucurbitae* populations from the granular formulations continued to increase for up to 6 wk after application and then rapidly declined (Fig. 1). No differences in persistence were noted between soy flour or oatmeal as a nutritional amendment. However, nutri-

Table 1. Control of successive plantings of Texas gourd with preemergence applications of conidial suspensions or sodium alginate granules of *Fusarium solani* f. sp. *cucurbitae*

Treatment[a]	Rate (conidia/ha or kg a.i./ha)	Texas gourd control (%)[b] at various weeks after infestation[c,d]			
		6	8	10	12
Untreated check	···	15	8	7	16
Trifluralin	1	36	16	18	28
Microconidia	3×10^{11}	46	32	20	39
	3×10^{12}	59	38	49	46
	3×10^{13}	91	65	60	52
Microconidia	$3 \times 10^{11} + 1$	40	31	22	33
+ trifluralin	$3 \times 10^{12} + 1$	72	32	32	50
	$3 \times 10^{13} + 1$	82	68	65	57
Macroconidia	3×10^{11}	58	40	27	46
	3×10^{12}	71	50	47	44
	3×10^{13}	84	73	60	60
Macroconidia	$3 \times 10^{11} + 1$	72	20	30	34
+ trifluralin	$3 \times 10^{12} + 1$	60	34	42	36
	$3 \times 10^{13} + 1$	89	61	57	61
Alginate granules	110	82	73	70	63
+ soy flour	220	87	75	67	72
Alginate granules	110	97	70	59	67
+ oatmeal	220	92	76	73	71

[a] Microconidia or macroconidia of *Fusarium solani* applied at 1×10^6, 1×10^7, or 1×10^8 conidia per milliliter in 280 L/ha, alone or in combination with trifluralin; granules composed of conidia and hyphae of *Fusarium solani* (1–5×10^8 cfu/ml) diluted 1:3 (v/v) in 1% (w/v) sodium alginate amended with 7.5% kaolin and either 2% (w/v) soy flour or ground oatmeal.

[b] Control based on final seedling stand counts 6 wk after planting. Plants permanently wilted or killed were counted as controlled. Percent control = (1 − surviving plants/total emerged plants) × 100.

[c] Persistence of control based on sequential planting of Texas gourd at biweekly intervals for 6 wk.

[d] Each value represents the combined means with four replicates over 2 yr; LSD ($P = 0.05$) = 22.

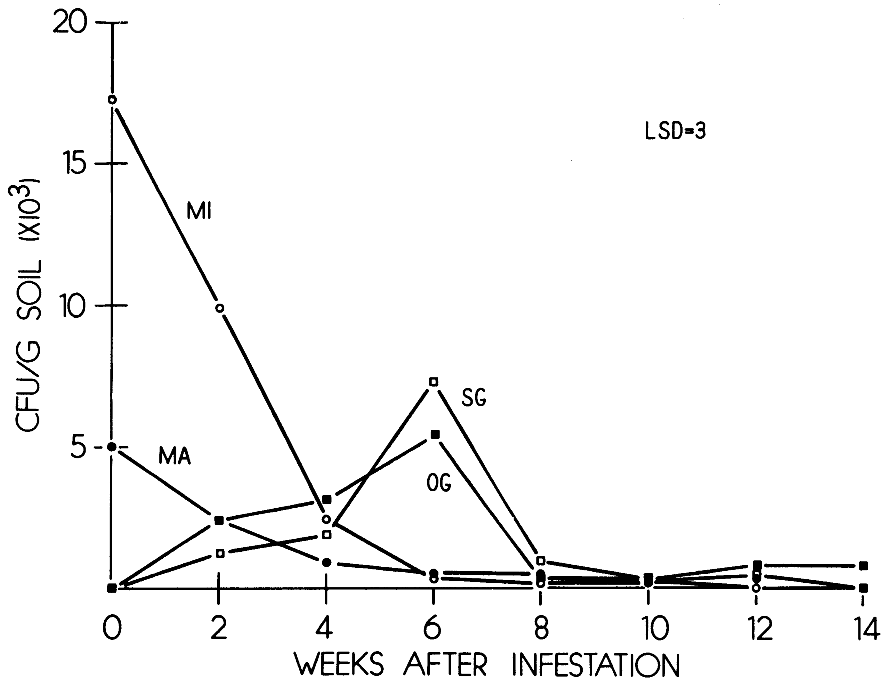

Fig. 1. Recovery of *Fusarium solani* f. sp. *cucurbitae* from field test plots when applied as microconidia (MI), macroconidia (MA), or sodium alginate granules amended with soy flour (SG) or oatmeal (OG).

tional substrates have been shown to influence sporulation and resulting soil populations of *F. s.* f. sp. *cucurbitae* (G. J. Weidemann, *unpublished*) and other fungi (5) produced in sodium alginate granules. *F. s.* f. sp. *cucurbitae* continually sporulated on the surfaces of soil-applied granules from which conidia could be washed by irrigation or rainfall. Once the granules were completely solubilized, populations declined at rates similar to those obtained with conidial applications. Modification of granular formulations could be used to either limit or extend the period of conidial production and resulting soil populations of biocontrol fungi.

This study demonstrates that conidial suspensions preplant-incorporated alone, or tank-mixed with trifluralin, and preemergence applications of granular formulations can provide effective control of Texas gourd. Although *F. s.* f. sp. *cucurbitae* is pathogenic to several cultivated cucurbits (3), they are not grown extensively where Texas gourd is endemic. Potential problems could be resolved readily with appropriate label restrictions and use.

Microconidial and macroconidial applications would be expected to result in sufficiently high soil populations to adequately control emerging seedlings for up to 4 wk after application. Granular formulations could extend the period of control up to 8 wk after application. A split application composed of a preplant-incorporated treatment of a conidial suspension followed by a granular formulation 2–4 wk later would be expected to provide sufficiently high soil populations of *F. s.* f. sp. *cucurbitae* to control Texas gourd for much of the growing season.

ACKNOWLEDGMENTS

Work was supported in part by USDA Competitive Research Grant No. 7859-2051-0-1-215-1. Appreciation is extended to D. B. Marx and staff for statistical assistance. Published with the approval of the director of the Arkansas Agricultural Experiment Station.

LITERATURE CITED

1. Bowers, R. C. 1982. Commercialization of microbial biological control agents. Pages 157-173 in: Biological Control of Weeds with Plant Pathogens. R. Charudattan and H. L. Walker, eds. John Wiley & Sons, New York. 293 pp.
2. Boyette, C. D. 1982. Evaluation of *Fusarium solani* f. sp. *cucurbitae* as a potential bioherbicide for controlling Texas gourd. Ph.D. thesis. University of Arkansas, Fayetteville. 53 pp.
3. Boyette, C. D., Templeton, G. E., and Oliver, L. R. 1984. Texas gourd (*Cucurbita texana*) control with *Fusarium solani* f. sp. *cucurbitae*. Weed Sci. 32:649-655.
4. Conroy, R. J. 1953. Fusarium root rot of cucurbits in New South Wales. J. Aust. Inst. Agric. Sci. 19:106-108.
5. Lewis, J. A., and Papavizas, C. G. 1985. Characteristics of alginate pellets formulated with *Trichoderma* and *Gliocladium* and their effect on the proliferation of the fungi in soil. Plant Pathol. 34:571-577.
6. Nash, S. M., and Alexander, J. V. 1965. Comparative survival of *Fusarium solani* f. *cucurbitae* and *F. solani* f. *phaseoli* in soil. Phytopathology 55:963-966.
7. Nash, S. M., and Snyder, W. C. 1963. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
8. Neubauer, R., and Avizohar-Hershenson, Z. 1973. Effect of the herbicide, trifluralin, on *Rhizoctonia* disease in cotton. Phytopathology 63:651-652.
9. Oliver, L. R., Harrison, S. A., and McClelland, M. 1983. Germination of Texas gourd (*Cucurbita texana*) and its control in soybeans (*Glycine max*). Weed Sci. 31:700-706.
10. Smith, R. J., Jr. 1983. Integration of microbial herbicides with existing pest management programs. Pages 189-203 in: Biological Control of Weeds with Plant Pathogens. R. Charudattan and H. L. Walker, eds. John Wiley & Sons, New York. 293 pp.
11. Snyder, W. C., and Hansen, H. N. 1941. The species concept in *Fusarium* with reference to section martiella. Am. J. Bot. 28:738-742.
12. Sumner, D. R. 1976. Etiology and control of root rot of summer squash in Georgia. Plant Dis. Rep. 60:923-927.
13. Tang, A., Curl, E. A., and Rodriguez-Kabana, R. 1970. Effect of trifluralin on inoculum density and spore germination of *Fusarium oxysporum* f. sp. *vasinfectum* in soil. Phytopathology 60:1082-1085.
14. Templeton, G. E. 1986. Mycoherbicide research at the University of Arkansas—past, present and future. Weed Sci. 34 (Suppl. 1):35-37.
15. Toussoun, T. A., and Snyder, W. C. 1961. The pathogenicity, distribution and control of two races of *Fusarium* (*Hypomyces*) *solani* f. sp. *cucurbitae*. Phytopathology 51:17-22.
16. Tuite, J. 1969. Plant Pathological Methods: Fungi and Bacteria. Burgess Publishing, Minneapolis, MN. 239 pp.
17. Van Eck, W. H. 1976. Ultrastructure of forming and dormant chlamydospores of *Fusarium solani* in soil. Can. J. Microbiol. 22:1634-1642.
18. Walker, H. L., and Connick, W. J., Jr. 1983. Sodium alginate for production and formulation of mycoherbicides. Weed Sci. 31:333-338.
19. Weidemann, G. J. 1986. Efficacy and soil persistence of *Fusarium solani* f. sp. *cucurbitae* for control of Texas gourd. (Abstr.) Annu. Meet. Weed Sci. Soc. 26:50.
20. Yu, S. M. 1984. Relationship of trifluralin to Texas gourd collar rot caused by *Fusarium solani* f. sp. *cucurbitae*. Ph.D. thesis. University of Arkansas, Fayetteville. 73 pp.
21. Yu, S. M., and Templeton, G. E. 1983. The relationship of trifluralin to collar rot of Texas gourd caused by *Fusarium solani* f. sp. *cucurbitae*. (Abstr.) Phytopathology 73:823.