Effects of Nutritional Amendments on Conidial Production of *Fusarium solani* f. sp. *cucurbitae* on Sodium Alginate Granules and on Control of Texas Gourd

G. J. WEIDEMANN, Assistant Professor, Department of Plant Pathology, University of Arkansas, Fayetteville 72701

ABSTRACT

Weidemann, G. J. 1988. Effects of nutritional amendments on conidial production of *Fusarium solani* f. sp. *cucurbitae* on sodium alginate granules and on control of Texas gourd. Plant Disease 72: 757-759.

Production of conidia by the fungus *Fusarium solani* f. sp. *cucurbitae* on sodium alginate granules was enhanced by the addition of nutritional amendments. The addition of 2% (w/v) ground oatmeal, cornmeal, or soy flour significantly increased conidial production on the surface of granules and resulted in higher soil populations than granular treatments without amendments or granules amended with carboxymethyl cellulose. More macroconidia were produced than microconidia with all treatments. Chlamydospores formed from hyphae and macroconidia for all treatments within 2 wk of soil application. Soil populations exceeded 2×10^5 cfu/g of soil when granules were amended with cornmeal, oatmeal, or soy flour. Texas gourd control was greater than 80% when soy flour- or oatmeal-amended granules were applied preemergence at 220 or 440 kg/ha in greenhouse tests. In field studies, oatmeal- and soy flour-amended granules gave greater than 80% control of Texas gourd within 6 wk when applied preemergence at 220 kg/ha.

Additional keywords: biological control, *Cucurbita texana*, mycoherbicide

Fusarium solani (Mart.) Appel. & Wr. f. sp. *cucurbitae* is a soilborne pathogen of several wild and cultivated cucurbits including Texas gourd (*Cucurbita texana* A. Gray), an escaped ornamental that has become a problem weed in cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* (L.) Merr.) fields in the Arkansas and Red River floodplains (11). Although several currently registered herbicides can provide effective control in soybeans, control has been inconsistent in some years and has been further complicated by continued weed emergence throughout the growing season (11). Few effective chemicals are available for use in cotton.

Fusarium solani f. sp. *cucurbitae* has been shown to provide effective control of Texas gourd (2,16) and could serve as an alternative to existing chemical controls, or could enhance existing herbicide treatments. Previous studies have demonstrated preemergence and postemergence control with soil applications of aqueous suspensions of microconidia or macroconidia, or as preplant incorporated cornmeal-sand mixtures (2,16).

Formulation of fungi in sodium alginate granules has been shown to be an effective means for conidial production (3,14) and for application of biocontrol agents (4,5,9,12,13,16) comparable to that obtained with other carriers (1,6–8,15). Lewis and Papavizas (9) demonstrated increased soil populations of *Trichoderma* and *Gliocladium* when wheat bran was added to alginate granules as a nutritional amendment. However, little information is available on the effects of other nutrients on conidial production and resulting soil populations of biocontrol fungi.

The objectives of this study were 1) to determine the effects of several nutritional amendments on conidial production of *F. s.* f. sp. *cucurbitae* on the surface of sodium alginate granules, 2) to determine soil populations of *F. s.* f. sp. *cucurbitae* after application of various granular treatments, and 3) to determine control of Texas gourd after preemergence application of *F. s.* f. sp. *cucurbitae*-infested sodium alginate granules in greenhouse and field studies. Limited portions of this work have been previously published (16,17).

Accepted for publication 25 April 1988 (submitted for electronic processing).

MATERIALS AND METHODS

Inoculum production. Sodium alginate granules were prepared using modified methods of Walker and Connick (14). Microconidia and hyphae of *F. s.* f. sp. *cucurbitae* were produced in modified Richard's solution (25 g of glucose, 10 g of KNO_3, 5 g of KH_2PO_4, 2.5 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of $FeCl_3$, 150 ml of V-8 juice, and distilled water to 1 L) in 500-ml Erlenmeyer flasks on a rotary shaker (28 C, 250 rpm). After 10–14 days, the cultures were centrifuged at low speed and the pellet was resuspended in an equal volume of distilled water and comminuted for 10 sec in a blender to give a final concentration of $1\text{–}5 \times 10^8$ cfu/ml. The unwashed treatment was handled similarly, except that the culture fluid was not centrifuged and resuspended.

Fungal suspensions were diluted 1:3 (v/v) with 1% (w/v) sodium alginate amended with 7.5% (w/v) kaolin. Nutritional amendments consisted of 2% (w/v) ground cornmeal, ground oatmeal, soy flour, or carboxymethyl cellulose added to the fungus-alginate mixture. The control granules consisted of sodium alginate and kaolin without amendment or fungus. The mixtures were dripped into 0.25 M of $CaCl_2$, harvested, and air-dried on fiberglass screens for 24–48 hr. The dried granules were passed through a 3-mm-mesh screen to separate adhering granules and were stored at 4 C until used. Recovery of *F. s.* f. sp. *cucurbitae* from granules was determined by plating 10–15 granules of each treatment on PCNB medium (10) modified by substituting 50 mg of chlortetracycline HCl for neomycin.

Conidial production. Conidial production was determined by adding 25–30 granules to the surface of 50 g of an air-dried sandy loam soil in 16-cm-diameter petri dishes. Soil moisture was adjusted to 15 mbar. The plates were sealed with plastic wrap and incubated at 28 C with 12 hr of cool-white fluorescent lighting. After 7 and 14 days, 10 granules were removed with a forceps, suspended in 10 ml of distilled water, agitated for 10 sec on a vortex mixer, and allowed to stand for 5 sec. Conidial concentrations were determined with a hemacytometer.

To determine the percentage of each spore type, two drops of conidial suspension were mixed with one drop of lactophenol-cotton blue on a microscope slide, covered with a cover slip, and examined under a light microscope at 250×. Percentages were based on counts of 50–100 conidia.

Soil populations. To determine *F. s.* f. sp. *cucurbitae* soil populations, 10 granules were placed on the surface of 20 g of the sandy loam soil used previously in 6-cm-diameter plastic petri dishes. Soil moisture was adjusted to 15 mbar and the plates were sealed with plastic wrap and incubated as before. After 7 and 14 days, the contents of each plate were transferred to bottles containing 100 ml of 0.15% water agar. The bottles were vigorously shaken and the contents serially diluted in 0.15% water agar to a final dilution of 10^{-4}. From the final dilution, 1 ml was pipetted to each of eight PCNB plates. Characteristic *F. s.* f. sp. *cucurbitae* colonies were enumerated after 5–7 days.

Greenhouse studies. Texas gourd control was determined by planting 10 pregerminated Texas gourd seeds into 13 × 18 cm styrofoam flats containing a sandy loam soil. Immediately following planting, 0.5 or 1.0 g of each of the treatment granules, equivalent to 220 or 440 kg/ha, respectively, was added to the soil surface of each flat. The flats were arranged in a randomized complete block and maintained in the greenhouse with 15 hr of supplemental fluorescent lighting. Seedling stand counts were made at weekly intervals for 4 wk.

Field studies. Field studies were conducted on Nixa cherty silt loam at the University of Arkansas Agricultural Experiment Station Farm at Fayetteville. Treatment plots measured 1 × 2 m separated by 1.5 m borders, and were arranged as a randomized complete block. Applications of granules amended with soy flour or oatmeal were broadcast by hand on 14 June, 1985 and 15 June, 1986 at 110 or 220 kg/ha immediately after planting 35 Texas gourd seeds across each plot. Controls consisted of plots without granular applications. Overhead irrigation was applied after planting, and as needed thereafter. Seedling stand counts were made weekly for 6 wk.

Treatments for all studies were replicated four times and all experiments were repeated twice. All data were subjected to analysis of variance. Treatment means were separated using Duncan's multiple range test at $P = 0.05$.

RESULTS AND DISCUSSION

All amendments, except carboxymethyl cellulose, significantly increased conidial production of *F. s.* f. sp. *cucurbitae* on the surface of sodium alginate granules within 14 days of soil application (Table 1). Conidial production on cornmeal-amended granules was delayed, with maximum production occurring 2 wk after soil application. Conidial production on the surface of soy flour-amended granules declined during the incubation period. Inclusion of the fermentation broth in the granules did not increase conidial production over that obtained with washed conidia and hyphae. In contrast to several other fungi (3,13,14), *F. s.* f. sp. *cucurbitae* required added nutrients to promote abundant sporulation on sodium alginate granules. Walker (13), Walker and Connick (14), and Boyette and Walker (3) obtained abundant conidia of several fungi without added nutrients. However, Lewis and Papavizas (9) and Papavizas et al (12) showed increased conidial production and higher soil populations of several biocontrol fungi with the addition of wheat bran to granules.

More macroconidia than microconidia were produced on the surface of the granules with all treatments following application to soil (Table 1). After 14 days, a small number of chlamydospores had formed from hyphae and macroconidia. Previous studies with *F. s.* f. sp. *cucurbitae* (16,17) suggest that macroconidia give better control of Texas gourd than microconidia at equivalent concentrations and persist for longer periods in the soil environment. Therefore, amendments that promote the production of abundant macroconidia would be preferred.

The addition of nutritional amendments also resulted in higher soil populations (Table 2) and increased levels of Texas gourd control (Table 3) over that obtained with unamended treatments. Highest soil populations of 7×10^5 cfu/g were obtained with soy flour-amended granules 2 wk after soil application. An apparent difference was noted between the high soil population of *F. s.* f. sp. *cucurbitae* recovered from soy flour-amended granules and somewhat lower conidial production on the surface of the granules after 14 days in comparison with other treatments. The difference may be related, in part, to differences in colonization of the granules by microbial competitors as well as from differences in soil colonization from hyphae radiating from the granules. Papavizas et al (12) showed higher soil populations of *Talaromyces flavus* (Klocker) Stolk & Samson for 24 wk with wheat bran-amended granules than with unamended granules. The addition of bran also supported higher soil populations of *Trichoderma* for up to 9 wk (9). Field studies with *F. s.* f. sp. *cucurbitae* (17) demonstrated increasing soil populations for 6 wk following application of soy flour- or oatmeal-amended granules followed by a rapid decline after the granules were solubilized. Less soluble formulations could be developed to extend the period of conidial production and thus increase the period of effective control.

Preemergence applications of granules amended with cornmeal, oatmeal, or soy flour effectively controlled Texas gourd at both 220 and 440 kg/ha in greenhouse tests (Table 3). Boyette and Walker (4) obtained similarly high levels of control of velvetleaf (*Abutilon theophrasti* Medik.) and prickly sida (*Sida spinosa* L.) with a granular formulation of *Fusarium lateritium* Nees ex Fr. applied at 1,120 kg/ha. Field tests of preemergence applications of soy flour- or oatmeal-amended granules in 1985 (Table 4) provided greater than 90% control of Texas gourd within 6 wk when applied at 110 or 220 kg/ha. In contrast, untreated controls had a 14% mortality over the same 6-wk period. In 1986, Texas gourd control was slightly reduced with preemergence applications of both soy flour- or oatmeal-amended granules, but exceeded 80% when applied at 220 kg/ha.

Table 1. Conidial production of *Fusarium solani* f. sp *cucurbitae* on sodium alginate granules 14 days after soil application in petri plate tests

Treatment[x]	Conidia produced[y,z] ($\times 10^4$)	Macroconidia (%)	Microconidia (%)	Chlamydospores (%)
Unwashed biomass	1	43	52	5
Washed biomass	2	21	72	6
Cornmeal	37	29	62	8
Oatmeal	45	8	87	4
Carboxymethyl cellulose	3	27	71	2
Soy flour	20	16	80	4

[x]Conidia produced on sodium alginate granules composed of fermenter biomass ($1–5 \times 10^8$ cfu/ml) diluted 1:3 (v/v) in 1% (w/v) sodium menter alginate amended with 7.5% (w/v) kaolin. Nutritional amendments were added to washed biomass at 2% (w/v). Values represent the means of four replications.

[y]Average number of conidia produced per granule.

[z]Treatments were significant at $P = 0.0001$ using analysis of variance (LSD = 8.3).

Table 2. Soil population of *Fusarium solani* f. sp. *cucurbitae* treated with sodium alginate granules in petri plate tests

Treatment[y]	Population of *F. s.* f. sp. *cucurbitae* per gram of soil ($\times 10^4$)[z]
Unwashed biomass	13 b
Washed biomass	13 b
Cornmeal	23 cd
Oatmeal	24 d
Carboxymethyl cellulose	15 bc
Soy flour	68 e
Control	0 a

[y]Conidia produced on sodium alginate granules composed of fermenter biomass ($1–5 \times 10^8$ cfu/ml) diluted 1:3 (v/v) in 1% (w/v) sodium alginate amended with 7.5% (w/v) kaolin. Nutritional amendments were added to washed biomass at 2% (w/v).

[z]Numbers in this column are the means for the combined observations at 7 and 14 days for each treatment with four replications. Numbers followed by the same letter do not differ significantly at $P = 0.05$, according to Duncan's multiple range test.

The use of sodium alginate granules containing *F. s.* f. sp. *cucurbitae* would have several advantages over other possible conidial formulations. Although microconidia are readily produced in liquid fermentation, spore-drying technology is not well developed and remains a formulation problem with many fungi. Alternatively, the production of sodium alginate granules is simple and can produce a uniform product with a long shelf life. Although the granules were generally used within 60 days, recovery was still nearly 100% 1 year after production when stored at 4 C. Other studies have also demonstrated successful long-term storage of fungi in sodium alginate granules. Lewis and Papavizas (9) and Papavizas et al (12) demonstrated better survival of soil biocontrol agents at 5 C than at 25 C. Fravel et al (5) found declines in propagule viability of several fungi encapsulated in sodium alginate and stored at 25 C but could still recover viable propagules after 12 wk, particularly when calcium gluconate was substituted for calcium chloride. Walker and Connick (14) successfully stored several pelletized fungi for 6–8 mo at both 4 and 25 C.

In contrast to previous granular mixtures (2,8) or diatomaceous earth (1), sodium alginate granules have the advantage of light weight and uniform particle size that could be applied with existing equipment. The use of nutritional amendments to increase sporulation and provide higher soil populations suggests that lower application rates can be used to achieve acceptable levels of control and extend the period of control over that obtained with unamended formulations.

Granules offer several potential advantages over that obtained with spray applications of soilborne fungi. The granule serves as a site for continued conidial production, while spores applied as a liquid suspension may require several applications. In previous field studies with *F. s.* f. sp. *cucurbitae* (16,17), granular formulations could extend the period of Texas gourd control up to 8 wk or more after application while spray applications of microconidia or macroconidia provided adequate control for 4 wk. Granular formulations could also be modified to favor the fungal propagule most desired or control its persistence in the soil environment. Granules could be formulated with other biological or chemical pesticides to minimize the influence of microbial competitors or to complement existing pest control practices.

The formulation of soilborne biocontrol agents in sodium alginate granules offers a high degree of versatility that should have wide application in plant pathology.

Table 3. Control of Texas gourd with preemergence applications of sodium alginate granules of *Fusarium solani* f. sp. *cucurbitae* in greenhouse experiments

Treatment[w]	Texas gourd control (%)[x,y] 220 kg/ha	440 kg/ha	Mean[z]
Unwashed biomass	14	28	21 a
Washed biomass	3	11	7 a
Cornmeal	65	83	73 b
Oatmeal	85	94	90 b
Carboxymethyl cellulose	30	12	21 a
Soy flour	85	85	78 b
Control	16	10	13 a

[w] Conidia produced on sodium alginate granules composed of fermenter biomass ($1–5 \times 10^8$ cfu/ml) diluted 1:3 (v/v) in 1% (w/v) sodium alginate amended with 7.5% (w/v) kaolin. Nutritional amendments were added to washed biomass at 2% (w/v).
[x] Each value represents the combined means of four replications.
[y] Control based on final seedling stand counts 6 wk after planting. Percent control = (1 − surviving plants/total emerged plants) × 100.
[z] The treatment × rate interaction was not significant using analysis of variance. Numbers in this column are the means for the combined observations at 220 and 440 kg/ha for each treatment. Numbers followed by the same letter do not differ significantly at $P = 0.05$, according to Duncan's multiple range test.

Table 4. Control of Texas gourd with preemergence field applications of sodium alginate granules of *Fusarium solani* f. sp. *cucurbitae* in 1985 and 1986

	Texas gourd control (%)[y,z]			
	1985		1986	
Treatment	110 kg/ha	220 kg/ha	110 kg/ha	220 kg/ha
Oatmeal granules	98	93	78	84
Soy flour granules	96	93	68	81
Untreated	14	···	15	···

[y] Control based on final seedling stand counts 6 wk after planting.
[z] Each value represents the mean of four replications.

ACKNOWLEDGMENTS

Work was supported in part by U.S. Department of Agriculture Competitive Research Grant No. 7859-2051-0-1-215-1. Appreciation is extended to K. A. Joyce and A. S. Mintz for technical assistance, and to D. B. Marx for statistical assistance. Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

LITERATURE CITED

1. Backman, P. A., and Rodriguez-Kabana, R. 1975. A system for the growth and delivery of biological control agents to the soil. Phytopathology 65:819-821.
2. 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.
3. Boyette, C. D., and Walker, H. L. 1985. Production and storage of inoculum of *Cercospora kikuchii* for field studies. Phytopathology 75:183-185.
4. Boyette, C. D., and Walker, H. L. 1986. Evaluation of *Fusarium lateritium* as a biological herbicide for controlling velvetleaf (*Abutilon theophrasti*) and prickly sida (*Sida spinosa*). Weed Sci. 34:106-109.
5. Fravel, D. R., Marois, J. J., Lumsden, R. D., and Connick, W. J., Jr. 1985. Encapsulation of potential biocontrol agents in an alginate-clay matrix. Phytopathology 75:774-777.
6. Jones, R. W., Pettit, R. E., and Taber, R. A. 1984. Lignite and stillage: Carrier and substrate for application of fungal biocontrol agents to soil. Phytopathology 74:1167-1170.
7. Kelley, W. D. 1976. Evaluation of *Trichoderma harzianum*-impregnated clay granules as a biocontrol for *Phytophthora cinnamomi* causing damping-off of pine seedlings. Phytopathology 66:1023-1027.
8. Lewis, J. A., and Papavizas, G. C. 1984. A new approach to stimulate population proliferation of *Trichoderma* species and other potential biocontrol fungi introduced into natural soils. Phytopathology 74:1240-1244.
9. Lewis, J. A., and Papavizas, G. C. 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.
10. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
11. 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.
12. Papavizas, G. C., Fravel, D. R., and Lewis, J. A. 1987. Proliferation of *Talaromyces flavus* in soil and survival in alginate pellets. Phytopathology 77:131-136.
13. Walker, H. L. 1981. Granular formulation of *Alternaria macrospora* for control of spurred anoda (*Anoda cristata*). Weed Sci. 29:342-345.
14. Walker, H. L., and Connick, W. J., Jr. 1983. Sodium alginate for production and formulation of mycoherbicides. Weed Sci. 31:333-338.
15. Walker, H. L., and Riley, J. A. 1982. Evaluation of *Alternaria cassiae* for the biocontrol of sicklepod (*Cassia obtusifolia*). Weed Sci. 30:651-654.
16. 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.
17. 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 Dis. 72:36-38.