Formulation of a Soil Amendment to Control Damping-off of Slash Pine Seedlings

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Portion of a Ph.D. dissertation submitted by the first author to the Graduate School, University of Georgia, Athens.

This research was supported in part by a grant from the Georgia Forestry Commission; the first author was supported by a fellowship from the National Science Council of the Republic of China.

Accepted for publication 2 August 1990.

ABSTRACT

Huang, J. W., and Kuhlman, E. G. 1991. Formulation of a soil amendment to control damping-off of slash pine seedlings. Phytopathology 81:163-170.

Seventeen chemical amendments were added (1%, w/w) individually to sandy soil artificially infested with *Rhizoctonia solani* (AG-4) or a binucleate species of *Rhizoctonia* (CAG-3). Alum, $Al_2(SO_4)_3$, $CaCl_2$, $Ca(NO_3)_2$, CaO, glycerine, K_2HPO_4, KCl, K_2SO_4, NH_4NO_3, $(NH_4)_2SO_4$, urea, and triple superphosphate inhibited colonization of pine stem segments by *R. solani* and the binucleate *Rhizoctonia* to varied degrees. In 0.1% (w/v) water solution, these chemicals inhibited sporulation of *Pythium aphanidermatum*. Ammonium sulfate suppressed the incidence of damping-off by 20–35% as compared with other N-fertilizers; ammonium nitrate enhanced damping-off by *R. solani*. A growth medium of 100% milled pine bark almost completely controlled damping-off by *Pythium* and reduced damping-off caused by *R. solani* by 50%, but it did not protect seedlings from infection by the binucleate *Rhizoctonia*. A soil amendment (SF-21) was formulated from 750 g of milled pine bark, 35 g of $(NH_4)_2SO_4$, 10 g of triple superphosphate, 30 g of $CaCl_2$, 25 g of KCl, 150 g of $Al_2(SO_4)_3$, and 750 ml of 10% glycerine. Added at 1% (w/w) to soil, SF-21 controlled more than 50% of damping-off of slash pine seedlings caused by *P. aphanidermatum*, *R. solani*, and binucleate *Rhizoctonia* in fumigated or nonfumigated soils in the greenhouse. In fumigated and nonfumigated soils in field microplots with or without a damping-off pathogen and amended at the rate of 2,400 kg/ha before sowing slash pine seeds, SF-21 significantly reduced postemergence damping-off caused by *R. solani*, *P. aphanidermatum*, and *Fusarium moniliforme* var. *subglutinans* by 36–38, 25–28, and 12–22%, respectively, in two tests. When added after the first appearance of damping-off, the SF-21 mixture also reduced losses to those fungi, with the exception of *F. m. subglutinans* in the first test. SF-21 treatments also increased the number of healthy seedlings produced per unit of area and the height of slash pine seedlings. Populations of *R. solani* and *P. aphanidermatum* were reduced by 50–90% 4 wk after treatment of the microplots. In soil amended with SF-21, the predominant fungi, *Trichoderma harzianum, T. aureoviride, Penicillium oxalicum, P. funiculosum, Gliocladium deliquescens,* and *G. fimbriatum* were stimulated, and the colony-forming units increased from 0.9×10^5 to 3.8×10^6/g of dry soil and remained high for more than 50 days.

Additional keywords: biocontrol, pine bark mulch.

Damping-off is a continuing problem in forest tree nurseries. Common fungi associated with damping-off of slash pine (*Pinus elliottii* Engelm. var. *elliottii*) in Georgia include *Rhizoctonia solani* Kühn, binucleate *Rhizoctonia* spp., *Pythium aphanidermatum* (Edson) Fitzp., and *Fusarium* spp. (14). Soil fumigation with methyl bromide is recommended to control damping-off of pine seedlings; however, Huang and Kuhlman (13) found that *R. solani* and *P. aphanidermatum* may be disseminated to fumigated nursery soils in unsterile pine bark mulch.

Organic amendments that intensify microbiological activity and enhance competition among soil microorganisms can result in lysis of inoculum structures of pathogenic fungi and favor the growth of plants (3,23). Therefore, by using organic amendments, it may be possible to render fumigated nursery soils suppressive to plant pathogens. For effective control, however, organic amendments must be applied at rates of 13.6–27.5 t of dry materials per hectare (15,23). In mechanized farming, these rates are not economically feasible (23).

The effects of inorganic amendments on the plant and pathogen are either direct, without the mediation of soil microorganisms, or indirect through biological transformation (10). Inorganic amendments can be applied at lower rates than organic amendments, but continuous and heavy application of chemical fertilizers has resulted in loss of organic matter and accumulation of toxic elements in fertile soils (1). To avoid undesirable effects of both organic and inorganic amendments and to take advantage of their beneficial effects, combined applications of inorganic and organic materials should be considered for controlling plant diseases. The objective of our study was to screen inorganic and organic materials individually, and then use a mixture of materials for control of damping-off of slash pine seedlings in greenhouse trays and field microplots.

MATERIALS AND METHODS

Soil and inoculum. Soil, collected from a forest site in Clarke County, GA, had a pH of 5.8, an inorganic composition of 95.0% sand, 3.4% clay, and 1.6% silt, and a 1.0% organic matter content. Isolate BB-08 (AG-4) of *R. solani* was recovered from pine bark mulch. Isolate WH-10 (CAG-3) of a binucleate *Rhizoctonia* sp. was recovered from a seedling of loblolly pine (*Pinus taeda* L.) with symptoms of damping-off. Isolates VW-06 and VWB-01 of *P. aphanidermatum* were from a diseased seedling of slash pine and pine bark mulch, respectively. Isolate FMS-05 of *Fusarium moniliforme* Sheld. var. *subglutinans* (Wollenw. & Reinking) (≡*F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas), was from a loblolly pine seed. All isolates were maintained on potato-dextrose agar (PDA) or V-8 juice agar (V-8). For soil infestation, chopped potato-soil inoculum of *R. solani* (16) and chopped eggplant-soil inoculum of *P. aphanidermatum* (14) were prepared. Soils were infested by mixing inoculum and soil fumigated with methyl bromide (280 kg/ha) in ratios of 1:1 and 1:4.

Evaluation of soil amendments. Soils infested with *R. solani* and binucleate *Rhizoctonia* sp. were amended with 17 chemicals applied singly at the rate of 1% of the air-dry weight of the soil. Test chemicals were: dextrose, glycerine, K_2HPO_4, KCl, K_2SO_4, $MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$, NH_4NO_3, $(NH_4)_2SO_4$, urea (Baker, Inc., Phillipsburg, NJ), $CaCl_2$, $CaCO_3$, $Ca(NO_3)_2$ (Fisher Co.,

Fairlawn, NJ), $Al_2(SO_4)_3$, triple superphosphate (Hoffman Co., Greencastle, IN), alum ($Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$) (Wang Derm, Inc., Atlanta, GA), CaO (Pfaltz & Bauer, Inc., Waterbury, CT), and starch (Merck Co., Darmstadt, Germany). Control soil did not receive an amendment. Two-hundred-gram lots of amended or nonamended soils were placed separately in polyethylene bags, and soil moisture was adjusted to 12% (w/w) with sterile distilled water. After 3 wk, survival of inoculum in soils was estimated by a pine-stem segment-colonization method (13). Results were recorded as the percentage of stem segments yielding *R. solani* or binucleate *Rhizoctonia* on 2% water agar containing 300 μg/ml of streptomycin sulfate (13).

Effects of the amendments on *P. aphanidermatum* were tested in solutions. Solutions were prepared by dissolving each (0.1%, w/v) of the 17 chemicals in deionized water. Deionized water alone was the control. Zoospore production by isolates of *P. aphanidermatum* was obtained from 5-day-old cultures by cutting three 10-mm-diameter disks of V-8 agar with a cork borer from the colony margin and placing these in a 55-mm-diameter petri dish containing 10 ml of chemical solution at 25 C (26). After 22 hr, the solution was carefully decanted and the population of zoospores was estimated with a Coulter Particle Counter, Model ZBl (Coulter Electronics, Inc., Hialeah, FL) and a hemacytometer.

To compare the suppressive ability of $Al_2(SO_4)_3$ and alum, 0, 0.1, 0.25, 0.5, 0.75, and 1.0% (w/w) of the chemicals were added separately to soils infested with *R. solani* and binucleate *Rhizoctonia*. After 1 wk, the amount of inoculum surviving was evaluated by the stem-segment colonization method. Also, the effect of 0, 10, 50, 100, 500, and 1,000 μg/ml of each chemical in solution on zoospore production by *P. aphanidermatum* was compared.

Greenhouse test. Soils were infested separately with *R. solani*, binucleate *Rhizoctonia*, and *P. aphanidermatum* to assess the effects of nitrogen sources on damping-off incidence among slash pine seedlings in a greenhouse. Inoculum densities of the pathogens were determined by the stem-segment colonization method for *Rhizoctonia* spp. and with Burr and Stanghellini's medium (2) for *P. aphanidermatum*. Inoculum density for *Rhizoctonia* spp. was adjusted to 25% colonization of pine stem segments, and that of *P. aphanidermatum* to 60 colony-forming units (cfu) per gram of dry soil by adding fumigated soil. Infested soils were amended with 125 μg/g of P and 350 μg/g of K by adding triple superphosphate and KCl. A concentration of 350 μg/g of N was obtained with $Ca(NO_3)_2$, NH_4NO_3, $(NH_4)_2SO_4$, and urea. Nitrogen fertilizer was not applied in the control. Germinating pine seed with approximately 3-mm-long hypocotyls were sown in plastic flats (15 × 12 × 6.5 cm; 10 seeds per flat) of fertilized soil infested with one of the test fungi. There were four replicate flats per fungus. The percentage of seedlings with damping-off was recorded 5 wk after planting.

Milled pine bark (60% *P. taeda* and 40% *Pinus echinata* Mill), with a pH of 4.4 and a bulk density of 0.23 g/cm^3 that had been composted for more than 9 mo, was obtained from Fernacre Farms, Washington, GA. Five combinations of pine bark and sandy soil were prepared at the rates of 0:100, 1:99, 10:90, 50:50, and 100:0 (w/w), respectively. Each combination was placed in 16 flats. Each flat was infested with 5 g of vermiculite inoculum of isolate BB-08 of *R. solani*, WH-10 of *Rhizoctonia* spp., or VW-06 or VWB-01 of *P. aphanidermatum*. The pathogens were grown in a chopped potato-vermiculite mixture (250 g of vermiculite, 450 g of chopped potatoes, and 300 ml of distilled water) for 20 days at 25 C; the vermiculite inoculum was air-dried and screened to yield 1–2 mm^2 pieces of inoculum. Flats with infested media were maintained in the greenhouse for 7 days before 10 germinated seeds of slash pine were sown in each flat. Percentage of seedlings with damping-off was recorded 5 wk after sowing.

From the evaluation of chemical amendments and N-fertilizers, $Al_2(SO_4)_3$, KCl, $CaCl_2$, triple superphosphate, $(NH_4)_2SO_4$, glycerine, and milled pine bark were selected as candidates for the soil amendment formulation. In the laboratory, 25 combinations of these ingredients were bioassayed by stem-segment colonization and zoospore-production methods. Two mixtures, SF-21 and SF-22, emerged as the best combinations for inhibition of *Rhizoctonia* spp. and *P. aphanidermatum*. Therefore, they were used to control damping-off of slash pine seedlings in the greenhouse. The SF-21 mixture consists of 150 g of $Al_2(SO_4)_3$, 25 g of KCl, 30 g of $CaCl_2$, 10 g of triple superphosphate, 35 g of $(NH_4)_2SO_4$, 750 g of milled pine bark, and 750 ml of 10% glycerine. In SF-22, 35 g of urea replaced $(NH_4)_2SO_4$ of the SF-21 mixture. Soils infested with *R. solani* (BB-08) or *P. aphanidermatum* (VW-06) were mixed separately with fumigated and nonfumigated soils. The inoculum density of *R. solani* was 50% colonization of pine stem segments and that of *P. aphanidermatum* was 100 cfu/g of dry soil at the time amendments were added. One percent (w/w) of air-dried SF-21 or SF-22 mixture and 0.75% (w/w) of milled pine bark were added to infested soils. Soil without amendments was used as a control. After 7 days, 10 germinated seeds per flat of soil were sown. The percentage of seedlings with damping-off was recorded 5 wk after planting.

Microplot test. Split-split-plot experiments with randomized complete block designs were conducted in 108 microplots (60 × 60 × 45 cm of plywood) in Clarke County, GA. Isolates of *R. solani* (BB-08), *P. aphanidermatum* (VW-06), and *F. m. subglutinans* (FMS-05) were used separately in three blocks (main plots) of 12 microplots each. Soils were fumigated with methyl bromide or were nonfumigated. For *R. solani* and *P. aphanidermatum*, subplots were infested and noninfested soils. For the seedborne *F. m. subglutinans*, inoculated and uninoculated seeds were subplots. Soils amended with SF-21 mixture before planting, after planting, and a control (nonamended), were sub-subplots.

Each microplot was filled to within 15 cm of the top with fumigated or nonfumigated soil. All fumigation was done with Brom-o-gas (methyl bromide 98% + chloropicrin 2%; Great Lakes Chem. Co., West Lafayette, IN) at 280 kg/ha under polyethylene plastic. Soil infested with *R. solani* or *P. aphanidermatum* was broadcast evenly over the soil at a rate of 2.25 kg/m^2 of soil surface in the appropriate microplots and then mixed into the upper 10 cm of soil. Half of the 12 microplots in each block were selected for infestation, and half were not infested (control). The SF-21 mixture was applied before or after sowing at the rate of 2,400 kg/ha. For the before-sowing treatments, amendments were broadcast evenly over the soil surface in microplots and raked into the upper 6 cm of the soil. For the after-sowing treatment, amendments were broadcast evenly over the soil surface in microplots and watered by an overhead sprinkler after the first symptoms of damping-off appeared.

Seeds of slash pine were surface-disinfested with 1% NaOCl-2%H_2O_2 (14) and then soaked in water for 2 days. For inoculation of *F. m. subglutinans*, air-dried surface-disinfested seeds were immersed in a conidial suspension of the fungus (10^6 conidia per milliter) in 0.1% (w/v) water agar (Difco Laboratories, Detroit, MI) and air-dried again for 3 hr. Control seeds were soaked in 0.1% water agar solution only. In each microplot, 220 seeds were distributed evenly in five rows, 60-cm long spaced 10 cm apart, and covered with 1 cm of soil. However, each Fusarium plot in the first test contained only 170 seeds. Pine bark (Pennington Enterprises, Inc., Madison, GA) was placed 1 cm deep over the soil as a mulch. Black shade cloth (4-mm-mesh) was stretched over the microplots to keep out rodents, birds, and deer.

The microplot experiment was conducted twice in 1989. In the first test, soil was amended 2 days before seed was sown on 3 May. Symptoms of damping-off were first observed 17 May, and the SF-21 mixture was added to after-planting plots. In the second test, soil was amended 8 days before the 28 August sowing date. Symptoms of damping-off appeared 15 September, and the SF-21 mixture was added to after-planting plots. Seedlings were watered by overhead sprinklers as needed. On 30 May and 12 September, 10% carbaryl dust was used to control nursery pine sawflies (*Diprion frutetorum* Fabricius). Emerging seedlings and final stands were counted in the central 35 × 35-cm area of each microplot. An emergence percentage in the infested plots was calculated from the ratio of emergence in infested versus noninfested soil. All dead seedlings were removed weekly. The causal agents were confirmed by isolations from surface-disinfested,

symptomatic segments of seedlings on 2% water agar. Heights of 10 randomly selected seedlings per microplot were measured 6 wk after sowing. Cumulative postemergence damping-off was determined after 8 wk.

Composite soil samples from microplots amended before planting or nonamended in Rhizoctonia and Pythium blocks were collected for microbial analyses five times at 8- to 14-day intervals during each test. The first sampling was made just after amendments were made. Six random soil samples from each microplot were composited, mixed, air-dried, and sifted through a 2-mm-mesh screen. Population densities of microorganisms other than *R. solani* and *P. aphanidermatum* were estimated by soil dilutions on selective media (15). Dilutions were plated in quadruplicate on nutrient agar for bacteria, on arginine-glycerol-salt agar for actinomycetes (15), and on peptone-dextrose-rose bengal agar (15) alone and supplemented with pentachloronitrobenzene at 100 μg/g (18) for fungi. Inoculum densities of *R. solani* and *P. aphanidermatum* were determined by pine-stem segment-colonization method (13) and Burr and Stanghellini's medium (2), respectively.

Statistical analyses. In all soil amendment studies, a randomized complete block design was used with four replicates of each treatment or concentration. For the greenhouse tests, experimental flats were assigned in a completely randomized design with four replicates (flats) per treatment. All experiments were repeated at least twice, with similar results. Data from the second trial are presented. Data were evaluated by analysis of variance (ANOVA) and general linear model statistical procedures with the SAS/STAT system for personal computers (SAS Institute, Inc., Cary, NC). The general linear model was used to analyze the factors of fumigation, infestation, and soil amendment in split-split-plot designs. Comparisons among treatment means were made with Duncan's multiple range test ($P = 0.05$).

RESULTS

Effect of chemical amendments on suppressiveness to the pathogens. $Al_2(SO_4)_3$, alum, $CaCl_2$, $Ca(NO_3)_2$, CaO, glycerine, K_2HPO_4, KCl, NH_4NO_3, $(NH_4)_2SO_4$, and urea greatly inhibited

TABLE 1. Effect of various chemicals on pine stem colonization by *Rhizoctonia solani* AG-4 (BB-08) and a binucleate *Rhizoctonia* CAG-3 (WH-10), and on zoospore production of *Pythium aphanidermatum* (VW-06 and VWB-01)

Chemicals[w]	Stem segment colonization (%)[x]		Zoospores produced[y] (10^4/ml)	
	BB-08	WH-10	VW-06	VWB-01
$Al_2(SO_4)_3$	0 h[z]	0 f	0 e	0 h
Alum	4 h	0 f	0 e	0 h
$CaCl_2$	0 h	0 f	0.3 e	1.3 ef
$CaCO_3$	88 bc	100 a	1.7 d	5.4 b
$Ca(NO_3)_2$	4 h	4 f	0.3 e	1.8 de
CaO	0 h	0 f	0 e	0 h
Dextrose	86 cd	88 b	0.2 e	1.6 de
Glycerine	25 g	0 f	1.9 d	3.2 c
K_2HPO_4	0 h	0 f	2.7 c	2.1 d
KCl	0 h	16 e	0.4 e	0.6 gh
K_2SO_4	54 f	38 d	0.5 e	1.2 efg
$MgCO_3 \cdot Mg(OH)_2$	79 d	100 a	0 e	0 h
NH_4NO_3	0 h	4 f	0.2 e	0.8 fg
$(NH_4)_2SO_4$	4 h	0 f	0 e	0 h
Starch	100 a	88 b	3.7 b	5.1 b
Urea	0 h	0 f	0 e	0 h
TSP	65 e	58 c	2.9 c	1.4 ef
None	94 ab	96 a	4.4 a	8.1 a

[w] For *Rhizoctonia*, 1% (w/w) chemical was added to infested soil with 12% water content; for *P. aphanidermatum*, 0.1% (w/v) chemical was dissolved in deionized water, Alum = $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$. TSP = triple superphosphate.

[x] Three weeks after soil treatment.

[y] Twenty-two hours after first flooding at 25 C.

[z] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

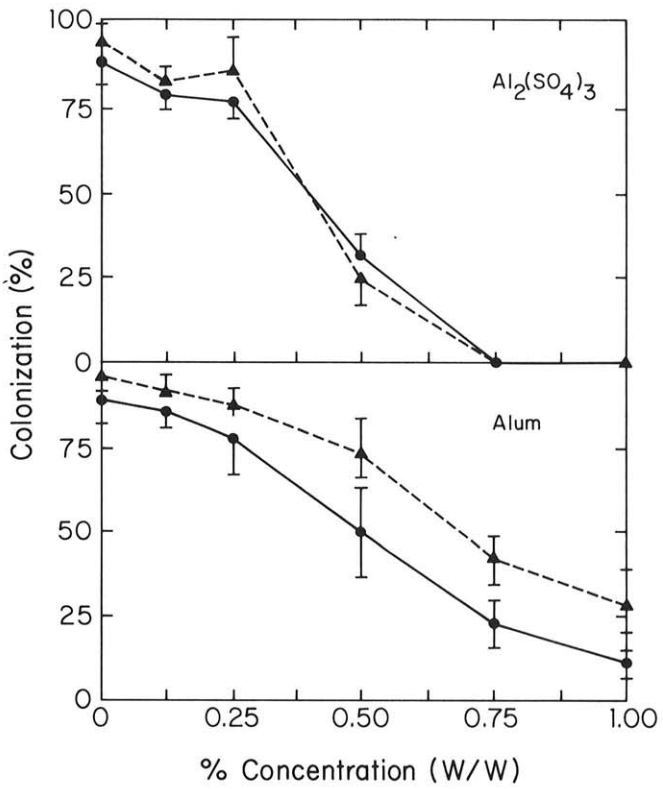

Fig. 1. Effect of different $Al_2(SO_4)_3$ and alum concentrations on pine stem-segment colonization by *Rhizoctonia solani* AG-4 (BB-08) (solid line) and a binucleate *Rhizoctonia* sp. CAG-3 (WH-10) (broken line). Vertical bars represent standard deviation ($n = 4$).

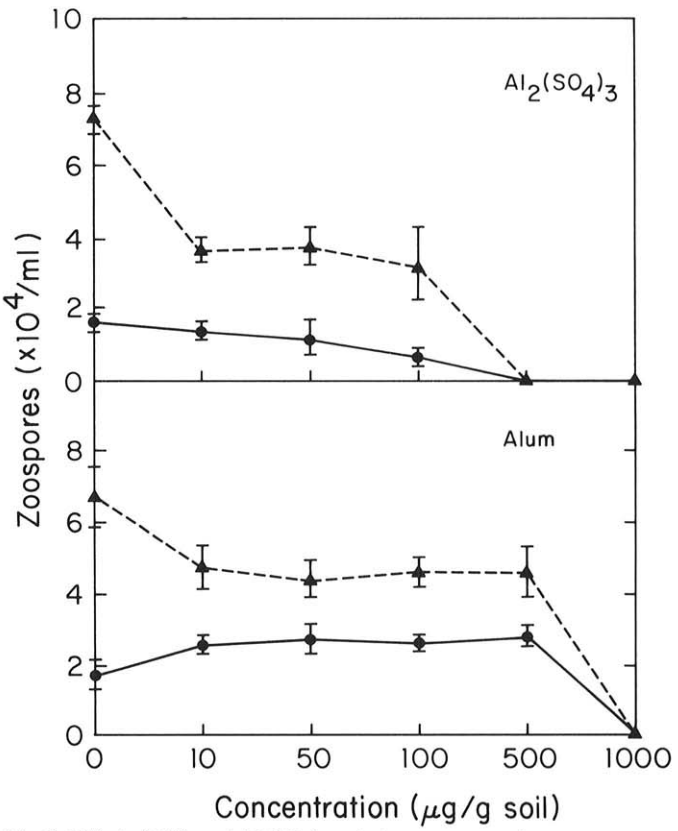

Fig. 2. Effect of different $Al_2(SO_4)_3$ and alum concentrations on zoospore production by *Pythium aphanidermatum* (isolates VWB-01 [broken line] and VW-06 [solid line]) 22 hr after first flooding at 25 C. Vertical bars represent standard deviation ($n = 4$).

stem-segment colonization by *R. solani* and the binucleate *Rhizoctonia* (Table 1). K_2SO_4 and triple superphosphate reduced colonization by these pathogens. However, CaO, $CaCO_3$, K_2HPO_4, $MgCO_3 \cdot Mg(OH)_2$, and urea discolored pine stem segments, which we interpreted to be phytotoxicity. All chemicals inhibited zoospore production by *P. aphanidermatum* (Table 1). Hyphal growth from V-8 agar disks into the water solution was observed in all chemical treatments, but $Al_2(SO_4)_3$, alum, and CaO inhibited sporangial formation by *P. aphanidermatum*. Zoospore release was inhibited by $MgCO_3 \cdot Mg(OH)_2$, $(NH_4)_2SO_4$, and urea, even though sporangia formed in these solutions.

Alum versus aluminum sulfate. $Al_2(SO_4)_3$, at the 0.75% rate, completely inhibited stem segment colonization by isolates BB-08 and WH-10 of *Rhizoctonia* spp. 7 days after amendment; 16–29% colonization by *Rhizoctonia* spp. occurred from infested soil amended with 1% alum (Fig. 1). $Al_2(SO_4)_3$ also was more effective than alum for inhibiting zoospore production by *P. aphanidermatum* at 500 μg/ml (Fig. 2).

Effect of N-fertilizers on damping-off. Damping-off of slash pine seedlings by *R. solani* (BB-08), binucleate *Rhizoctonia* (WH-10), and *P. aphanidermatum* (VWB-01) was inhibited 20–35% when $(NH_4)_2SO_4$ was applied to infested soil 1 wk before planting (Table 2). However, isolate VW-06 of *P. aphanidermatum* was unaffected by the N source. Urea also reduced damping-off of slash pine caused by the binucleate *Rhizoctonia*, but not that caused by *P. aphanidermatum* or *R. solani*.

TABLE 2. Effect of nitrogen sources on incidence of damping-off of slash pine seedlings for 5 wk in fumigated soil infested with *Rhizoctonia solani* AG-4 (BB-08) and a binucleate *Rhizoctonia* CAG-3 (WH-10), or *Pythium aphanidermatum* (VWB-01, VW-06) in the greenhouse

	Damping-off[y] (%)			
	Rhizoctonia spp.		*P. aphanidermatum*	
N-fertilizers[x]	BB-08	WH-10	VWB-01	VW-06
$Ca(NO_3)_2$	63 b[z]	78 ab	70 a	65 a
NH_4NO_3	100 a	90 a	75 a	78 a
$(NH_4)_2SO_4$	68 b	50 c	43 b	65 a
Urea	70 b	60 bc	80 a	58 a
None	88 ab	85 a	78 a	60 a

[x] N-P-K (350-125-350 μg/g of soil) was mixed with infested soil 7 days before planting. Sources of P and K were triple superphosphate and potassium chloride, respectively. None was a combination without nitrogen as a control.

[y] Damping-off included pre- and postemergence damping-off.

[z] Means ($n = 4$) in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

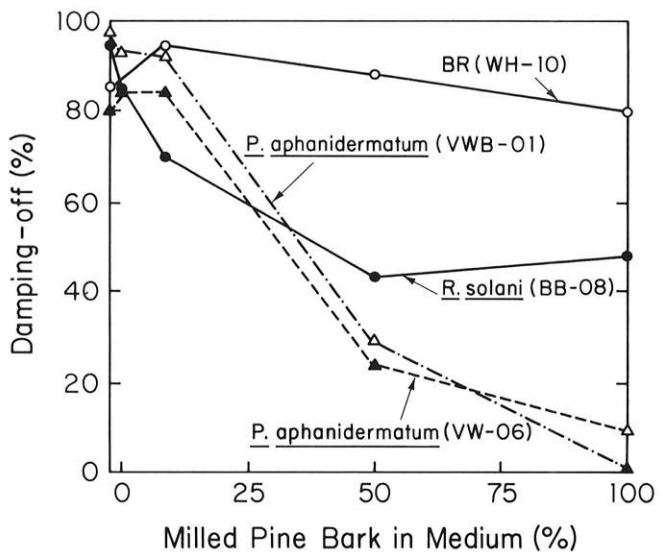

Fig. 3. Effect of milled pine bark-sandy soil media on incidence of damping-off of slash pine seedlings by *Rhizoctonia solani* AG-4, a binucleate *Rhizoctonia* sp. CAG-3 (BR), and *Pythium aphanidermatum* in the greenhouse for 5 wk. Media were infested with vermiculite inocula of the pathogens 7 days before planting.

Effect of milled pine bark on disease occurrence. Damping-off caused by *R. solani* AG-4 (BB-08) and *P. aphanidermatum* (VW-06 and VWB-01) was significantly inhibited by adding increasing proportions of milled pine bark between 10 and 100% of soil volume (Fig. 3). Proportions of 1–10% milled pine bark in sandy soil did not significantly suppress damping-off. A 100% milled pine bark medium almost completely protected slash pine seedlings from infection by *P. aphanidermatum*, but roots were shorter and darker brown than those in sandy soil 5 wk after planting. Milled pine bark did not inhibit incidence of damping-off caused by binucleate *Rhizoctonia* CAG-3 (WH-10).

Disease incidence in greenhouse tests. SF-21 and SF-22 in fumigated and nonfumigated soils infested with *R. solani* AG-4 (BB-08) or *P. aphanidermatum* (VW-06) reduced damping-off incidence (Table 3). The SF-21 mixture was more effective than the SF-22 mixture for control of Rhizoctonia damping-off; however, there was no significant difference between SF-21 and SF-22 mixtures for control of Pythium damping-off. The same amount of milled pine bark in SF-21 and SF-22 mixtures did not reduce the incidence of damping-off caused by either fungus.

Microplots. SF-21 reduced the soil pH from 5.8 to 4.6. By 8 days after amendment, the soil surface was green due to the growth of green molds. SF-21 applied before planting suppressed postemergence damping-off of slash pine seedlings by *R. solani* AG-4, *P. aphanidermatum*, and *F. m. subglutinans* in field microplots (Table 4). In all noninfested treatments, seedling emergence was excellent and no postemergence damping-off occurred (data not shown). In both tests, amendment of infested soils with SF-21 before sowing increased seedling emergence by 5–20%. The increase in emerged seedlings in the Pythium and Fusarium microplots was statistically significant only in the second test. Low temperatures from 3 to 10 May 1989 slowed emergence and postemergence damping-off. Moreover, applications of SF-21 mixture before sowing in both tests significantly suppressed postemergence damping-off caused by *R. solani*, *P. aphanidermatum*, and *F. m. subglutinans* by 36–38, 25–28, and 12–22%, respectively. Finally, in both tests, amendments before planting significantly increased the final number of seedlings per 1,225 cm^2 in plots with those three pathogens. When SF-21 mixture was added after damping-off first appeared, it suppressed postemergence damping-off caused by the pathogens, except there were no differences in losses caused by *F. m. subglutinans* in the first test (Table 4).

Slash pine seedlings in microplots amended before planting were taller than those in nonamended plots regardless of pathogen (Table 5). When SF-21 mixture was applied after symptoms developed, seedling height differences were not as great or were not significantly different from the nonamended treatment. An ANOVA indicated that soil fumigation before pathogen infesta-

TABLE 3. Effect of soil amendments on damping-off of slash pine seedlings by *Rhizoctonia solani* AG-4 (BB-08) and *Pythium aphanidermatum* (VW-06) in fumigated or nonfumigated soil for 5 wk in greenhouse

	Damping-off (%)			
	Fumigated soil		Nonfumigated soil	
Treatment[x]	BB-08	VW-06	BB-08	VW-06
SF-21	38 c[y]	33 b	23 c	30 b
SF-22	55 b	45 b	43 b	50 b
Pine bark	95 a	70 a	70 a	78 a
None	100 a	78 a	70 a	85 a

[x] 1.0% of dry SF-21 or SF-22 and 0.75% (w/w) of milled pine bark were mixed with infested soils. SF-21 consisted of 150 g of $Al_2(SO_4)_3$, 25 g of KCl, 30 g of $CaCl_2$, 10 g of triple superphosphate, 35 g of $(NH_4)_2SO_4$, 750 g of milled pine bark, and 750 ml of 10% glycerine. SF-22 was similar to SF-21, except it had 35 g of urea instead of $(NH_4)_2SO_4$.

[y] Means within columns followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

tion did not significantly affect seedling emergence, postemergence damping-off, height of seedlings, or final stand in the two tests.

Effect of formulated soil amendments on pathogen survival. Propagules of *R. solani* decreased more rapidly in amended than in nonamended treatments in both fumigated and nonfumigated soils (Fig. 4). Stem-segment colonization by *R. solani* was reduced significantly 8–14 days after SF-21 was applied.

When fumigated soil was infested with *P. aphanidermatum*, its population density increased rapidly for 8–14 days (Fig. 5). No such increase occurred in fumigated or nonfumigated soil when SF-21 was applied. After 28 days in amended soil, propagules of *P. aphanidermatum* were reduced 50–90%.

Other microorganisms associated with formulated soil amendments. Attempts were made to determine whether certain microbial populations were associated with disease suppression in amended soils. Population densities of bacteria and actinomycetes were not significantly affected by SF-21 application (data not shown); however, population densities of fungi in fumigated and nonfumigated soils amended with SF-21 mixture were much higher than in nonamended control soils 8 and 14 days after treatment in tests 2 and 1, respectively (Figs. 4 and 5). Fungi were stimulated by SF-21, and their population density increased from 0.9×10^5 to 3.8×10^6 cfu/gm of dry soil in the amended soil. Some decrease was observed after 50 days, but the density remained higher than in the nonamended soil.

Trichoderma harzianum Rifai, *T. aureoviride* Rifai, *Pencillium oxalicum* Currie & Thom, *P. funiculosum* Thom, *Gliocladium deliquescens* Sopp., and *G. fimbriatum* Gilman and Abbott were the predominant fungi in amended soils. Treatment-induced increases were especially apparent for *Trichoderma* spp. and *Penicillium* spp. In amended microplots, the population density of *Trichoderma* spp. was much higher in fumigated soil than in nonfumigated soil, whereas the population density of *Penicillium* spp. was much higher in nonfumigated soil than in fumigated soil. Therefore, the total population density of *Trichoderma* spp. and *Penicillium* spp. was not significantly different (data not shown).

DISCUSSION

SF-21, a formulated soil amendment, significantly reduced damping-off of slash pine seedlings caused by *R. solani, P. aphanidermatum,* and *F. m. subglutinans* (Tables 3 and 4), promoted seedling growth (Table 5), and increased the final seedling stands (Table 4). Further, it reduced the inoculum density of *R. solani* and *P. aphanidermatum* and stimulated proliferation of *Trichoderma* spp., *Penicillium* spp., and *Gliocladium* spp. (Figs. 4 and 5). SF-21 includes two major ingredients: pine bark and aluminum sulfate. The use of pine bark as mulch in pine nursery beds is common in the United States (20). Other researchers have reported that pine bark suppresses Pythium and Phytophthora root rot (12,25). A linear increase in fresh root weight of Helleri holly with an increasing percentage of pine bark in the medium, regardless of high population densities of *Pythium irregulare* Buisman, resulted in a reduction in pathogenesis (8). In our research, a medium of 100% milled pine bark almost completely suppressed damping-off of slash pine seedlings by *P. aphanidermatum*, but media containing 1–10% of milled pine bark did not suppress *P. aphanidermatum* and *R. solani* (Fig. 3). The smaller concentrations are similar to the amounts of pine bark mulch typically applied to nursery beds.

The types of microorganisms we isolated from composted pine bark were similar to those found by others studying biocontrol in container media (12). Additionally, inhibitors with fungicidal activity have been found in composted pine bark (11). Efforts to exploit the use of pine bark by multiplying populations of indigenous and introduced antagonists to achieve effective biocontrol of plant pathogens led us to evaluate chemical additives

TABLE 4. Effect of SF-21 amendment on emergence, postemergence damping-off, and final stands of slash pine seedlings in microplots infested with *Rhizoctonia solani* AG-4 (BBH08) and *Pythium aphanidermatum* (VWH06) or seeds treated with *Fusarium moniliforme* var. *subglutinans* (FMSH05) in 1989

Treatment[w]	Seedlings emerged (%)[x]			Postemergence damping-off (%)			Final stand (per 1,225 cm^2)[y]		
	BB-08	VW-06	FMS-05	BB-08	VW-06	FMS-05	BB-08	VW-06	FMS-05
First test (May–July)									
Amended before planting	58 a	93 a	89 a	20 c	4 b	14 b	47 a	89 a	55 a
Amended after planting	41 b	88 a	81 a	44 b	10 b	24 a	24 b	81 a	43 b
Nonamended	38 b	86 a	82 a	58 a	32 a	26 a	16 b	60 b	43 b
Second test (Aug–Oct)									
Amended before planting	64 a	95 a	89 a	14 c	3 c	10 c	53 a	92 a	81 a
Amended after planting	53 b	86 b	88 a	39 b	10 b	19 b	32 b	77 b	72 b
Nonamended	54 b	77 c	84 b	50 a	28 a	32 a	28 b	56 c	56 c

[w] Amended before planting: 2 days in the first test, 8 days in the second test. Amended after planting when disease symptoms first appeared: 14 days after planting in first test, 7 days after planting in second test.

[x] % emerged = seedlings in infested plot ÷ seedlings in noninfested plot × 100.

[y] Final stands per 1,225 cm^2 were counted 8 wk after planting. Initially, 220 seeds per plot (3,600 cm^2) were sown, except FMS-05 plots in first test had 170 seeds per plot.

[z] Means within columns within tests followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

TABLE 5. Effect of SF-21 amendment on height of 6-wk-old slash pine seedlings in microplots infested with *Rhizoctonia solani* AG-4 (BB-08) and *Pythium aphanidermatum* (VW-06) or seeds treated with *Fusarium moniliforme* var. *subglutinans* (FMS-05) in 1989[w]

Treatment[x]	Height of seedlings (cm)[y]					
	First test (May–July)			Second test (Aug–Oct)		
	BB-08	VW-06	FMS-05	BB-08	VW-06	FMS-05
Amended before planting	7.9 a[z]	8.1 a	7.7 a	7.7 a	7.5 a	7.4 a
Amended after planting	7.4 b	7.7 ab	7.2 b	7.3 b	7.3 b	7.1 b
Nonamended	6.9 c	7.5 b	6.9 b	6.6 c	6.9 c	7.0 b

[w] Analysis of variance indicated no effect of fumigation or infestation on seedling height at 6 wk.

[x] Amended before planting: 2 days in the first test, 8 days in the second test. Amended after planting when disease symptoms first appeared: 14 days after planting in the first test, 7 days after planting in the second test.

[y] Height of seedlings was obtained from average of 10 plants in central 1,225 cm^2 area per plot.

[z] Means within columns followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

to pine bark.

Aluminum sulfate, an acidifying agent, controls damping-off of pine seedlings (9,31). Weindling and Fawcett (29) also used it to control damping-off of citrus seedlings. Naturally occurring soil aluminum reduced pathogenesis of the sunflower pathogens *Verticillium albo-atrum* Reinke & Berthier, and *Whetzelinia sclerotiorum* (Lib.) Korf & Dumont (22). In our study, colonization by *Rhizoctonia* spp. and zoospore production by *P. aphanidermatum* were significantly reduced by $Al_2(SO_4)_3$, KCl, $CaCl_2$, and glycerine (Table 1). These chemicals also stimulated *Trichoderma* spp. to colonize pine bark segments and grow on the soil surface (unpublished data). *Trichoderma* spp. in acid soils have been reported to prevent *Rhizoctonia* from killing citrus seedlings (29), and fungistasis towards *Trichoderma* is more pronounced in alkaline than in acid soils (5,19). In our study, pine stem segments were colonized by *Trichoderma* spp. when the pH in soils infested with *Rhizoctonia* spp. was lowered with $Al_2(SO_4)_3$.

The best N-fertilizer for suppressing damping-off of slash pine in fumigated soil was $(NH_4)_2SO_4$ (Table 2). For growth requirements of *Trichoderma* spp. and slash pine seedlings, we selected and formulated $Al_2(SO_4)_3$, $CaCl_2$, KCl, triple superphosphate, glycerine, and $(NH_4)_2SO_4$ with milled pine bark for suppression of damping-off of slash pine seedlings in nursery beds.

Wells et al (30) added inocula of *T. harzianum* with its food base to soil for biocontrol of *Sclerotium rolfsii* Sacc. Papavizas (24) mentioned that a potential problem in the use of a readily available substrate with *Trichoderma* or *Gliocladium* is the possible stimulation of a pathogen and, hence, an increase in disease. With the SF-21 amendment, the indigenous population of *Trichoderma* spp. and other green molds rapidly colonized milled pine bark and suppressed *Rhizoctonia* and *Pythium*. The SF-21 mixture was the best formulated organic mixture for controlling damping-off of slash pine seedlings of 25 experimental formulas we tested.

In Taiwan, S-H mixture, a combination of bagasse, rice husks, oyster shells, mineral ash, and fertilizers controls many soilborne diseases in horticultural and agronomic crops (28). Control of Fusarium wilt diseases by amendment of soil with S-H mixture is the result of a combination of biotic and abiotic factors (27,28). Davey and Papavizas (6) reported that the suppression of *R. solani* in soil by composted organic amendment and supplementary nitrogen was related to the general microbial activity in the soil. AG-4 types of *R. solani* attacking the host at or near the ground line are sensitive to CO_2 concentration, which varies with the rate of microbial activity and soil type (7).

A different mechanism probably explains SF-21 control of damping-off of slash pine. *Trichoderma* is a well-known antagonist of soilborne plant pathogens. Propagules of *Trichoderma* generally formed less than 3% of the total fungal propagules in a variety of forest soils in the southeastern United States (4). The recolonization of a fumigated soil is accomplished mainly by survivors that have the shortest response times and the fastest growth rates. Among fungi, species of *Trichoderma* and *Mucor* usually are first to colonize fumigated soil (3), with the predominant *Trichoderma* spp. being *T. hamatum* (Bonord.) Bainier, *T. harzianum, T. koningii* Oudem., and *T. viride* Pers.:Fr. (21). In our study, the population density of *Trichoderma* spp. was significantly higher in fumigated than in nonfumigated soil when

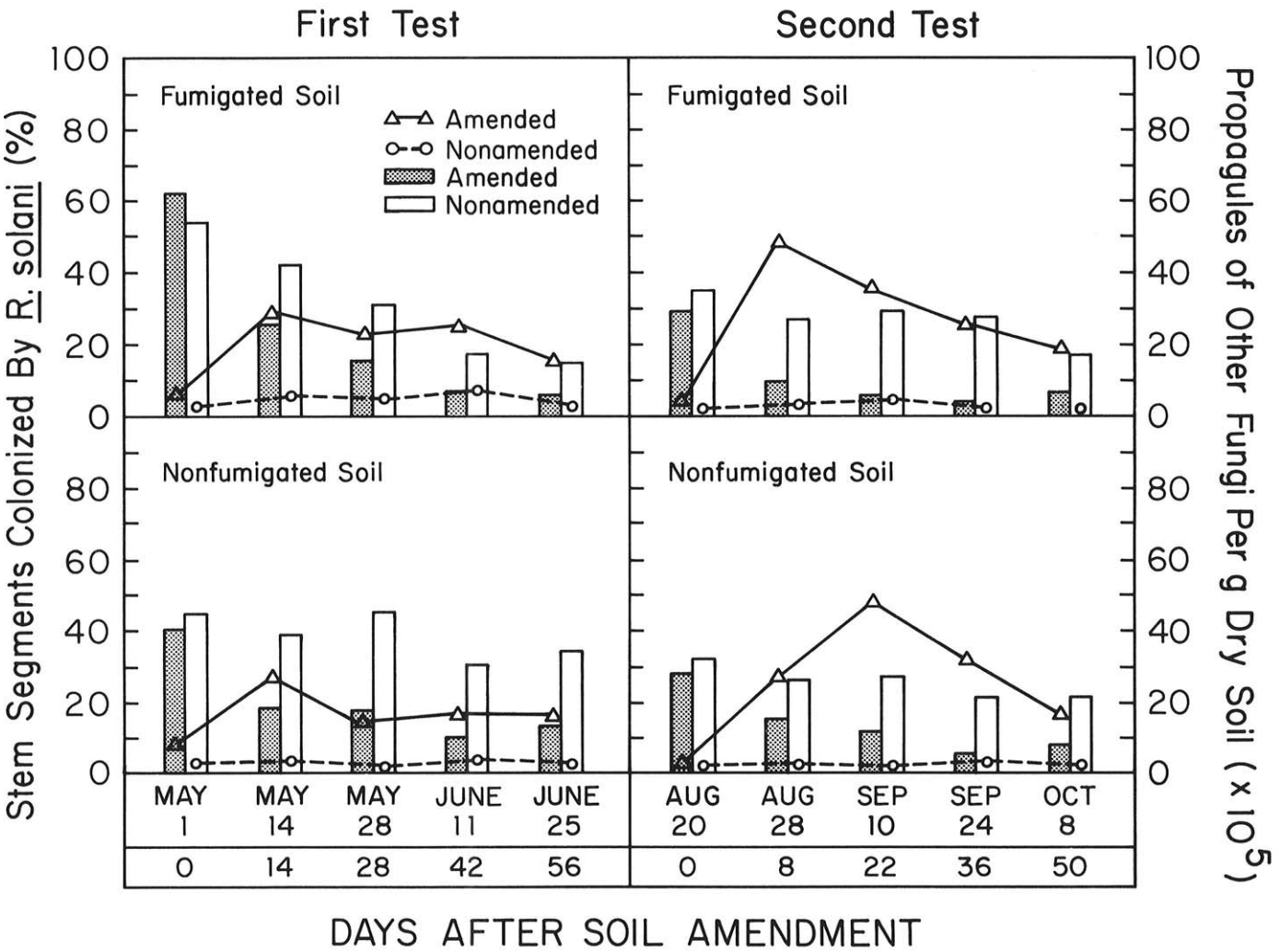

Fig. 4. Effect of SF-21 amendment on population densities of *Rhizoctonia solani* AG-4 (bars) and other fungi (lines) in fumigated and nonfumigated soils planted with slash pine seeds in field microplots in Clarke County, GA, on 3 May and 28 August 1989.

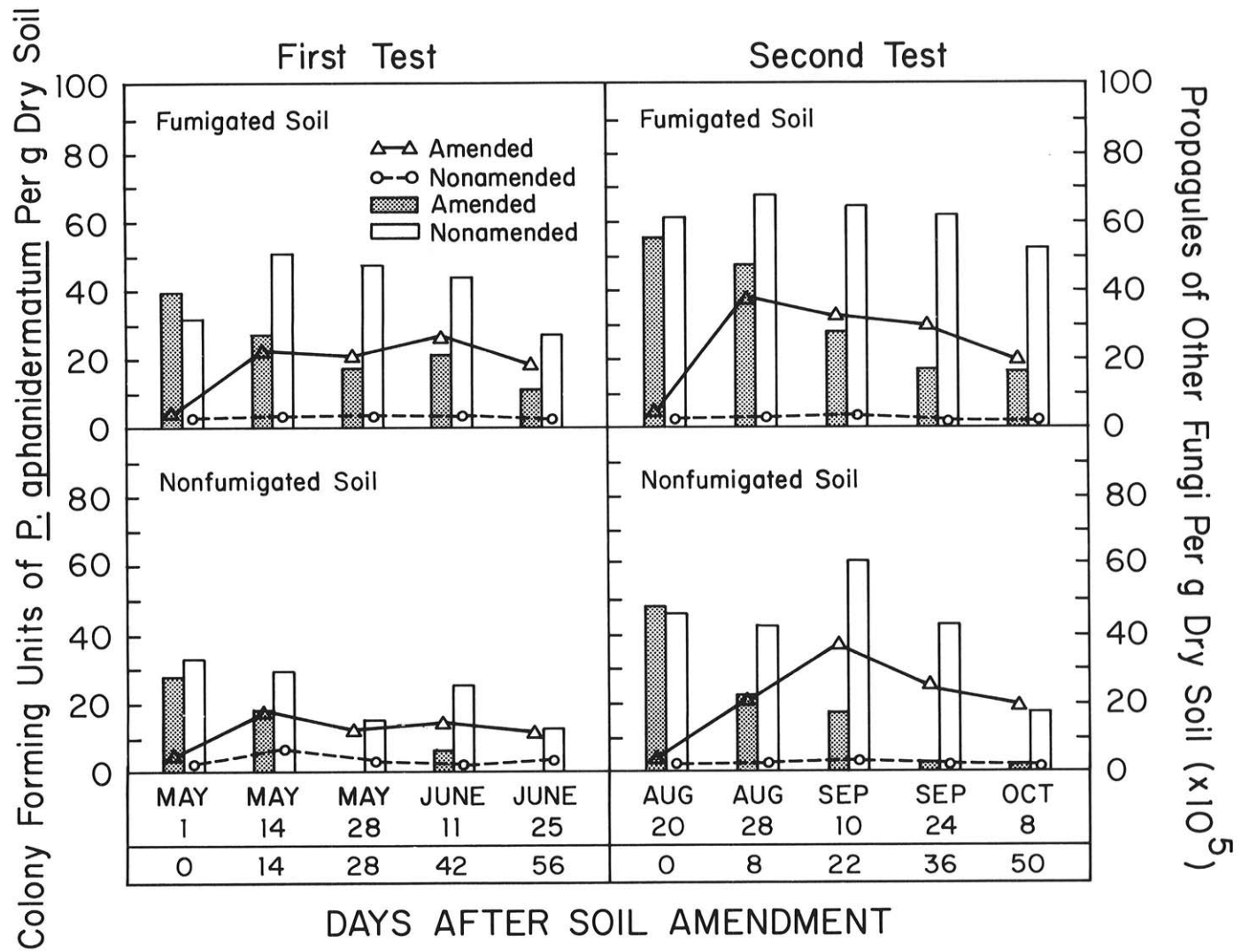

Fig. 5. Effect of SF-21 amendment on population densities of *Pythium aphanidermatum* (bars) and other fungi (lines) in fumigated and nonfumigated soils planted with slash pine seeds in field microplots in Clarke County, GA, on 3 May and 28 August 1989.

SF-21 was applied. Among plant root pathogens, *Pythium* spp. rapidly colonize fumigated soil (3). *P. aphanidermatum* multiplied in fumigated soil without soil amendment, whereas SF-21 inhibited and reduced population densities of *P. aphanidermatum* in fumigated and nonfumigated soil (Fig. 5).

Kommedahl and Windels (17) first identified *P. oxalicum* as an antagonist and demonstrated its effectiveness in controlling seedling blight and seed rot of pea caused by a soilborne complex that included *Pythium* spp. (32). *Penicillium* spp. were more common in nonfumigated than in fumigated soil after application of SF-21. Therefore, although the fungi involved differ, the effects of SF-21 on pathogens are similar in fumigated and nonfumigated soils. We have shown that SF-21 is able to kill or weaken *R. solani*, *P. aphanidermatum*, and *F. m. subglutinans* by making them more vulnerable to antagonists in the expanded associated microflora.

The future development, application, and success of biocontrol with SF-21 mixture will require further research efforts to discover how widely applicable this treatment is in different nurseries, to understand how and if it stimulates other antagonists in soils with higher pHs, to learn how persistent the control is, and to learn to use them effectively and economically.

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