

# Effect of Organic Amendments on Sclerotial Germination, Mycelial Growth, and *Sclerotium rolfsii*-Induced Diseases

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## ABSTRACT

The addition of organic residues to soil is an option to control some soil-borne diseases. Benzaldehyde and powders of kudzu (*Pueraria lobata*), velvetbean (*Mucuna deeringiana*), and pine-bark (*Pinus elliottii* and *P. taeda*) added to soil could reduce certain soil-borne diseases. This study evaluated the effects of benzaldehyde and the dried powders of kudzu, velvetbean, and pine-bark as soil amendments on germination and formation of sclerotia, on mycelial growth of *Sclerotium rolfsii*, on plant survival, and disease incidence. The data showed that high amounts of benzaldehyde (0.4 ml kg<sup>-1</sup> of soil) and velvetbean (100 g kg<sup>-1</sup>) inhibited *S. rolfsii* mycelial growth and sclerotium germination. However, low amounts of benzaldehyde (0.1 ml kg<sup>-1</sup>), kudzu (25 g kg<sup>-1</sup>), and pine-bark (25 g kg<sup>-1</sup>) stimulated mycelial growth and sclerotium germination. Kudzu (25-100 g kg<sup>-1</sup>) and velvetbean (25-100 g kg<sup>-1</sup>) inhibited the formation of sclerotia.

Nevertheless, benzaldehyde at 0.2 and 0.4 ml kg<sup>-1</sup> stimulated the formation of sclerotia. Kudzu (50 and 100 g kg<sup>-1</sup>) and pine-bark (50 g kg<sup>-1</sup>) favored the colonization of sclerotia by *Trichoderma* sp. The numbers of soybean (*Glycine max*) plants were higher and diseased plants were lower than the non-amend soil in the following treatments: kudzu (50 and 100 g kg<sup>-1</sup>), velvetbean (50 and 100 g kg<sup>-1</sup>), and pine-bark (50 g kg<sup>-1</sup>). Disease severity on tomato (*Lycopersicon esculentum*) plants was low in soil treated with kudzu or velvetbean (30 and 35 g kg<sup>-1</sup>) and pine-bark (35 g kg<sup>-1</sup>). Dried powders of kudzu, velvetbean, or pine-bark added to soil can reduce disease by reducing pathogen inoculum.

**Additional keywords:** *Glycine max*, *Lycopersicon esculentum*, *Mucuna deeringiana*, *Pueraria lobata*, velvetbean, biocontrol, and suppressive soils.

## RESUMO

**Efeitos de resíduos orgânicos no solo na germinação de esclerócios, no crescimento micelial e na ocorrência de doenças induzidas por *Sclerotium rolfsii***

A adição ao solo de resíduos orgânicos é uma opção para o manejo de doenças causadas por patógenos de solo. Benzaldeído e resíduos moídos de puerária (*Pueraria lobata*), mucuna (*Mucuna deeringiana*) e casca de *Pinus* (*Pinus elliottii* e *P. taeda*) podem reduzir certas doenças propagadas pelo solo. Este estudo avaliou os efeitos do benzaldeído e de pós-secos de puerária, mucuna e casca de *Pinus* sp. como resíduos de solo na germinação e formação de esclerócios, no crescimento micelial de *Sclerotium rolfsii*, na sobrevivência de plantas, na incidência de doença e na atividade enzimática do solo. Concentrações mais altas de benzaldeído (0,4 ml kg<sup>-1</sup> de solo) e mucuna (100 g kg<sup>-1</sup>) inibiram o crescimento micelial e a germinação de esclerócios.

Todavia, concentrações mais baixas de benzaldeído (0,1 g kg<sup>-1</sup>), puerária (25 g kg<sup>-1</sup>), e casca de *Pinus* sp. (25 g kg<sup>-1</sup>) estimularam o crescimento micelial e a germinação de esclerócios. Puerária (25-100 g kg<sup>-1</sup>) e mucuna (25-100 g kg<sup>-1</sup>) inibiram a formação de esclerócios. Contudo, benzaldeído a 0,2 e 0,4 ml kg<sup>-1</sup> estimulou a formação de esclerócios. Puerária (50 e 100 g kg<sup>-1</sup>) e casca de *Pinus* sp. (50 g kg<sup>-1</sup>) favoreceram a colonização dos esclerócios por *Trichoderma* sp. O número de plantas de soja (*Glycine max*) foi maior e o número de plantas doentes foi mais baixo em solos com puerária (50 e 100 g kg<sup>-1</sup>), mucuna (50 e 100 g kg<sup>-1</sup>) e casca de *Pinus* sp. (50 g kg<sup>-1</sup>) do que em solos sem estes resíduos. A severidade de doença em tomateiro (*Lycopersicon esculentum*) foi baixa em solo com puerária ou mucuna (30 e 35 g kg<sup>-1</sup>) e casca de *Pinus* sp. (35 g kg<sup>-1</sup>). Pós-secos de puerária, mucuna ou casca de *Pinus* sp. incorporados ao solo podem reduzir a doença devido à redução do inóculo do patógeno.

## INTRODUCTION

The management of soil-borne plant pathogens is particularly complex because these organisms live in or near the dynamic environment of the rhizosphere, and can frequently survive long periods in soil through the formation of resistant survival structures. Soil-borne pathogens are responsible for crop yield losses in many plants.

*Sclerotium rolfsii* Sacc. [*Athelia rolfsii* (Curzi) Tu & Kimbrough] causes the disease known as southern blight in a

wide variety of crops. *Sclerotium rolfsii* forms brownish sclerotia that can survive in soil for long periods, frequently tolerating biological and chemical degradation due to the presence of melanin in the outer membrane (Chet, 1975). Among the methods employed to manage *S. rolfsii* are the following: fungicide applications, solarization, use of antagonistic microorganisms, deep plowing, crop rotation, and incorporation of organic and inorganic residues (Punja, 1985). Organic matter added to soil may increase soil fertility, stimulate growth of crops, and suppress plant pathogens. When properly used,

amendments can not only reduce populations of soil-borne pathogens but also induce soil suppressiveness by stimulating activities of antagonists in soil. Enhancing numbers of antagonists in soil generally leads to a decrease in viable pathogen inoculum and a reduced need for fungicides (Linderman, 1989; Gamliel & Stapleton, 1993).

Some amendments suppress and others stimulate sclerotium germination in soil (Beute & Rodríguez-Kábana, 1979). Germinated sclerotia are much more susceptible to antagonists than viable non-germinated ones (Smith, 1972). Non-germinated sclerotia can have their viability decreased by some amendments, thus becoming more sensitive to antagonistic action by other microorganisms (Hadar & Gorodecki, 1991).

In this study dried powders of velvetbean [*Mucuna deeringiana* (Bort) Small], kudzu [*Pueraria lobata* (Willd.) Ohwi], and pine (*Pinus* spp.) and purified benzaldehyde [aromatic compound, extracted from bitter almond (*Prunus amygdalus* Batsch.)] were evaluated as amendments to soil. These amendments can result in the suppression of nematodes and other soil-borne pathogens (Rodríguez-Kábana *et al.*, 1992; Kokalis-Burelle & Rodríguez-Kábana, 1994). Used at low rates, benzaldehyde can act as a spore germination-inducing agent, can stimulate and select for biological control microorganisms against *S. rolfii*, and can act as a fungicidal or fungistatic compound when used at high concentrations (Soler-Serratos *et al.*, 1996).

Some research has been done on the effects of temperature, moisture, pH, salinity, fungicides, amendments, and volatile compounds on the germination of *S. rolfii* sclerotia (Beute & Rodríguez-Kábana, 1981; Maiti & Sen, 1988; Canullo *et al.*, 1992). However, there is limited information available on the effect of benzaldehyde, kudzu, velvetbean, and pine bark on the sclerotial germination and mycelium growth of *S. rolfii* on soil.

The objective of this study was to evaluate the effect of benzaldehyde, kudzu, velvetbean, and pine bark powder as organic amendments on the germination of *S. rolfii* sclerotia and on *S. rolfii* inoculi colonization by *Trichoderma* spp., on mycelial growth and formation of new sclerotia, on plant survival and disease incidence, and on shifts of soil microbial activities by means of soil enzyme activity measurements.

## MATERIALS AND METHODS

*Sclerotium rolfii* was isolated from diseased tomato (*Lycopersicon esculentum* Mill.) plants. The fungus was maintained at room temperature (20-25 °C) in test tubes with malt extract agar medium. *Sclerotium rolfii* colonized oat (*Avena sativa* L.) seeds were used as inoculum for soil infestation and production of soil sclerotia (Punja & Rahe, 1992).

The soil used for laboratory and greenhouse experiments was a sieved (2 mm) sandy-soil mixed with siliceous river sand (50:50 w/w). The soil was collected from a peanut (*Arachis hypogaea* L.) field at the Auburn University campus experimental farm in Auburn, Alabama. The elemental

composition and other characteristics of the soil is presented here (Auburn University Soil Testing Laboratory): Marvin sandy loam (Typic kanhapludults), CEC < 4.6 cmol kg<sup>-1</sup>, pH 6.8, C 0.65%, N 0.04%, P 19.5 mg kg<sup>-1</sup>, K 38.0 mg kg<sup>-1</sup>, Mg 65.0 mg kg<sup>-1</sup>, and Ca 300.0 mg kg<sup>-1</sup>.

Commercially available pine bark nuggets (mixture of *Pinus elliotii* Englem. and *P. taeda* L. - ProCare Earth Pak Inc.) and approximately three month old kudzu and velvetbean plants (leaves and stems) were used in the experiments. These materials were dried for two weeks on greenhouse benches (25-35 °C), and ground (<500 µm) with a Straub Model 4E Grinding Mill. The ground amendments were maintained in plastic boxes at room temperature until used. The elemental composition of the materials is presented on table 1. Benzaldehyde (C<sub>6</sub>H<sub>5</sub>CHO - FW 106.13 - specific gravity 1.04) was obtained from Aldrich Chemical Co.

### Effect of amendmets on sclerotial germination, mycelial growth, and colonization of *S. rolfii* inoculum by *Trichoderma* spp.

Two tests with soil-produced sclerotia and two with *S. rolfii*-colonized oat seeds were performed to evaluate the direct effect of amendments on surface mycelium growth and on sclerotial germination. Treatments consisted of non-amended soil (control), benzaldehyde (0.1, 0.2, and 0.4 ml kg<sup>-1</sup>), kudzu, velvetbean, and pine bark powders (25, 50, and 100 g kg<sup>-1</sup>), and an extra control with sterile soil. Materials were thoroughly mixed with soil in polyethylene plastic bags (20 x 15 cm) and then five replications of 100 g samples of each soil mixture were poured into 150 ml-plastic cups (Solo Cup Co.). After application of the amendments in soil, all experimental units received 20 ml of sterile water and were thoroughly mixed again. Determinations of sclerotial germination and mycelium growth were made 24 to 72 h after inoculum deposition on soil. The number of new sclerotia and *Trichoderma* spp.-colonized sclerotia or oat seeds was assessed three to ten days after inoculation.

Twenty sclerotia were spread 1 cm apart on the soil surface of each cup. Cups in experiments with *S. rolfii* colonized oat seeds, received five 1-cm-apart seeds on soil surface / replication (Rodríguez-Kábana *et al.*, 1975a). After the inoculum deposition, the cups were closed with a perforated (2 mm diameter) plastic lid to allow gas exchange. The cups were incubated at 27° C (Precision Scientific incubator - GCA Corporation). Cups with soil amended with benzaldehyde were placed in separate plastic boxes with lid covers to avoid gas-contamination cups with other amendments with benzaldehyde vapor. Mycelial growth was evaluated for each sclerotium or seed, 24, 48, and 72 h after inoculation according to a subjective scale (1- < 1mm growth from the sclerotium or seed; 2- 1-3mm; 3- >3mm).

### Effect of amendments on soybean (*Soybean max* L.) and tomato survival

A set of three experiments was performed under greenhouse conditions to evaluate the efficacy of benzaldehyde (0.1, 0.2, and 0.4 ml kg<sup>-1</sup>), kudzu (25, 50, and 100 g kg<sup>-1</sup>),

velvetbean (25, 50, and 100 g kg<sup>-1</sup>) and pine bark powder (25, 50, and 100 g kg<sup>-1</sup>) on soybean ('Davis') survival. One day before application of the amendments to soil, 5 g of *S. rolfssii* colonized oat seeds were mixed with 1 kg of natural soil contained in 1 liter capacity 10-cm-diameter cylindrical PVC pots (experimental unit). Treatments with sterile and natural soil, with and without the pathogen, were also included. Experiments were arranged in a completely randomized design, with 16 treatments and five or six replications of ten plants.

At the time of soil infestation and application of the amendments in soil the contents of each pot was poured into polyethylene plastic bags (30 x 20 cm) and thoroughly mixed. The only difference in procedures among experiments was in the time of sowing. Seeds were sown ten, 20 and 40 days after application of the amendments to soil, in the first, second, and third experiments, respectively. These differences in times of sow were used, in order to detect any toxic effects on seed germination due to amendments. The number of plants in each experiment was determined from seven to 21 days after sowing. Each of these tests was done twice.

Experiments with different rates of benzaldehyde, kudzu, velvetbean, and pine bark powder were performed to evaluate the effect of these amendments on tomato ('Rutgers') survival and growth. Ten rates of kudzu, velvet bean and, pine bark powder (0, 2.5, 5, 7.5, 10, 15, 20, 25, 30, and 35 g kg<sup>-1</sup> of soil), and ten rates of belzaldehyde were applied (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, and 1.2 ml kg<sup>-1</sup> of soil). Soil infestation and application of the amendments to soil were as described for the soybean experiments.

Each treatment was replicated six times and arranged in a randomized complete block design. Two tomato transplants were made, one 30 days and the other 120 days after application of the soil amendments. In the transplantings two 30-day-old tomato plants were used. Enumeration of sclerotia on the soil surface was made 14 days after application of the soil amendments, and disease severity and plant growth evaluations were made at the end of each planting cycle.

#### Effect of amendments on soil enzyme activity

For the tomato experiments, samples were collected 30 and 120 days after application of the soil amendments, sieved (2 mm), and kept at -10°C. Measurement of esterase activities was performed by a modification (Soler-Serratos *et al.*, 1996) of the method described by Schnürer & Rosswall (1982), based on fluorescein diacetate hydrolysis. Readings were expressed in terms of µg of fluorescein produced h<sup>-1</sup> g<sup>-1</sup> soil, based in a previously prepared calibration curve with Fluorescein Na salt (Aldrich Chemical Co. Inc.). Catalase (E.C.1.11.1.6) activity was determined according to Rodríguez-Kábana & Truelove (1970). Catalase activity was expressed in meq H<sub>2</sub>O<sub>2</sub> decomposed h<sup>-1</sup> g<sup>-1</sup> of soil. Controls with and without sterile soil were taken into account for non-biological H<sub>2</sub>O<sub>2</sub> decomposition

Soil pH was determined with demineralized water [McLean (1982) modified]. After pH measurement the soil supernatant from each sample was used to determine conductivity (µS cm<sup>-1</sup>) with a conductivity meter (TDS WD-

**TABLE 1** - Elemental composition of kudzu (*Pueraria lobata*), velvetbean (*Mucuna deeringiana*), and pine bark (*Pinus spp.*) powders

Elemental composition	Kudzu	Velvetbean	Pine Bark
C (%)	44.31	45.04	51.76
N (%)	1.38	2.60	0.22
C:N ratio	32.11	17.32	235.27
P (%)	0.06	0.26	0.02
K (%)	0.80	1.55	0.08
Ca (%)	1.08	1.23	0.21
Mg (%)	0.29	0.35	0.03
Cu (mg kg <sup>-1</sup> )	1.45	20.02	0.00
Fe (mg kg <sup>-1</sup> )	308.54	498.42	94.94
Mn (mg kg <sup>-1</sup> )	49.48	219.43	73.43
Zn (mg kg <sup>-1</sup> )	11.25	20.42	12.61
B (mg kg <sup>-1</sup> )	24.16	30.01	7.42
Al (mg kg <sup>-1</sup> )	435.45	998.73	683.81
Ba (mg kg <sup>-1</sup> )	49.86	138.42	13.06
Co (mg kg <sup>-1</sup> )	0.13	2.50	1.62
Cr (mg kg <sup>-1</sup> )	1.19	2.61	0.16
Pb (mg kg <sup>-1</sup> )	0.00	0.25	0.00
Na (mg kg <sup>-1</sup> )	97.38	185.13	7.17
Mo (mg kg <sup>-1</sup> )	1.52	2.04	0.86

Analyses made by: Soil Testing Laboratory at Auburn University, Auburn, AL

35661-30, Oakton Co.) [Rhoades (1982) modified].

Treatments in the experiments were arranged in a randomized complete block. The data were analyzed by two-way or three-way ANOVA, depending on the objective of the experiment. Differences among averages were evaluated for significance according to Fisher's PLSD test ( $P < 0.05$ ). Regression or correlation analyses were performed following standard procedures. SigmaStat v. 2 (SPSS / Jandel Scientific Software) and StatView v. 4.53 (Abacus Concepts) were used for processing the data.

## RESULTS

### Mycelial growth

Soil treatments with benzaldehyde at 0.1 and 0.2 ml kg<sup>-1</sup>, kudzu and pine bark at all rates, and velvet bean at 25 g kg<sup>-1</sup> stimulated mycelial growth after 24 h of incubation (Figure 1A). Benzaldehyde at 0.4 ml kg<sup>-1</sup> and velvetbean at 100 g kg<sup>-1</sup> reduced the mycelial growth (Figure 1A and B). After 48 h, velvet bean (50- 100 g kg<sup>-1</sup>), benzaldehyde (0.4 ml kg<sup>-1</sup>), and kudzu (50-100 g kg<sup>-1</sup>) inhibited mycelial growth. When colonized oat seeds where used as inoculum, benzaldehyde (0.4 ml kg<sup>-1</sup>) and velvetbean (100 g kg<sup>-1</sup>) once more reduced mycelial growth for 48 h, while kudzu (100 g kg<sup>-1</sup>) and pine bark (100 g kg<sup>-1</sup>) both reduced it for at least 24 h after incubation (Figure 1C and D).

### Sclerotium germination

Sclerotium germination was stimulated by benzaldehyde (0.1-0.2 ml kg<sup>-1</sup>), 24 h after incubation (Figure 1E). Kudzu and velvetbean (25-50 g kg<sup>-1</sup>) and pine bark (25-100 g kg<sup>-1</sup>) stimulated

germination (Figure 1E). Benzaldehyde (0.4 ml kg<sup>-1</sup>), velvetbean (100 g kg<sup>-1</sup>) reduced germination 24 h after incubation in both experiments (Figure 1E). Velvetbean (100 g kg<sup>-1</sup>) partially inhibited sclerotial germination even 48 h after incubation (Figure 1F).

#### Sclerotium formation

Sclerotium formation from older ones increased in soil amended with benzaldehyde (0.4 ml kg<sup>-1</sup>), kudzu (100 g kg<sup>-1</sup>), and pine bark (25 g kg<sup>-1</sup>) (Figure 1G). Sclerotium production was different when oat seeds were used as inoculum, where soil amended with benzaldehyde generally induced sclerotium formation, and treatments with kudzu, velvetbean, and pine bark reduced production of sclerotia (Figure 1H).

#### *Sclerotium rolfisii* inoculum colonization by *Trichoderma* spp.

Numbers of *Trichoderma* spp. colonies were highest in soils treated with kudzu (50-100 g kg<sup>-1</sup>) and pine bark (50 g kg<sup>-1</sup>), from three to ten days after incubation. Velvetbean amendments had no effect on the number of these fungi on *S. rolfisii* inoculum (Figure 1I).

#### Survival of soybean and tomato plants

Figure 2 presents data on the percentages of plants and diseased plants, respectively, in soil infested with *S. rolfisii* and sowed with soybean. The percentage of emerged plants was higher in soil amended with kudzu, velvetbean and pine bark (Figure 2A). Numbers of diseased plants (not considering non-germinated seeds) were lower in kudzu, velvetbean, and pine bark amended soil than in non-amended soil (Figure 2B). The percentage of diseased plants was low in treatments with benzaldehyde (0.4 ml kg<sup>-1</sup>), kudzu (25 to 100 g kg<sup>-1</sup>), velvetbean (25-100 g kg<sup>-1</sup>), and pine bark (25-100 g kg<sup>-1</sup>). Depending on the time of sowing (Figure 2C and D), amendments resulted in different effects, but in general treatments with kudzu, velvetbean and pine bark increased the percentage of emerged plants (Figure 2A), decreased the percentage of diseased plants (Figure 2B), and increased shoot and root weight (Table 2), when planting was done at 40 days after application of the amendments in soil.

#### Effects of amendments on disease severity, tomato growth, soil enzymatic activities, soil pH, and conductivity

Eighty days after planting, increasing rates of velvetbean, pine bark, and kudzu tended to reduce disease severity (Figure 3 and Table 2). Benzaldehyde amended soils had few effect on disease severity.

Plant height and weight (shoots and roots) were increased in response to increased rates of applications of velvetbean and kudzu at both planting times, but pine bark and benzaldehyde applications had no effect on these variables (Table 2). Catalase and fluorescein diacetate hydrolysis (FDA) activities at 30 days after application of the amendments increased with increasing amounts of velvetbean, pine bark, kudzu, and benzaldehyde (FDA activities only) (Table 2). At 120 days after application of the amendments enzymatic

activities were slightly higher than as the values in 30 days for treatments with velvet bean (for 30 g kg<sup>-1</sup>, the catalase activity increased from 0.327 to 0.334 meq H<sub>2</sub>O<sub>2</sub> decomposed h<sup>-1</sup> g<sup>-1</sup> of soil, and the FDA activities from 4.147 to 6.410 μg of fluorescein produced h<sup>-1</sup> g<sup>-1</sup> soil). Pine bark amendments resulted in increased catalase and FDA activities. Data on catalase activity for kudzu amendments 120 days (for 30 g kg<sup>-1</sup> = 0.345 meq H<sub>2</sub>O<sub>2</sub> decomposed h<sup>-1</sup> g<sup>-1</sup> of soil) after application of the amendments indicated a similar response to the one at 30 days (for 30 g kg<sup>-1</sup> = 0.347 meq H<sub>2</sub>O<sub>2</sub> decomposed h<sup>-1</sup> g<sup>-1</sup> of soil), but FDA activity values were significantly (P < 0.05) higher (for 30 g/kg = 4.559 μg of fluorescein produced h<sup>-1</sup> g<sup>-1</sup> soil) than the control (1.369 μg of fluorescein produced h<sup>-1</sup> g<sup>-1</sup> soil). Catalase activities, in treatments with benzaldehyde (0.1, 0.4, 0.8 to 1.2 ml kg<sup>-1</sup>), were different (for 0.1 ml/kg = 0.206 meq H<sub>2</sub>O<sub>2</sub> decomposed h<sup>-1</sup> g<sup>-1</sup> of soil) from the control (0.111 meq H<sub>2</sub>O<sub>2</sub> decomposed h<sup>-1</sup> g<sup>-1</sup> of soil) at 30 days after application of the amendments.

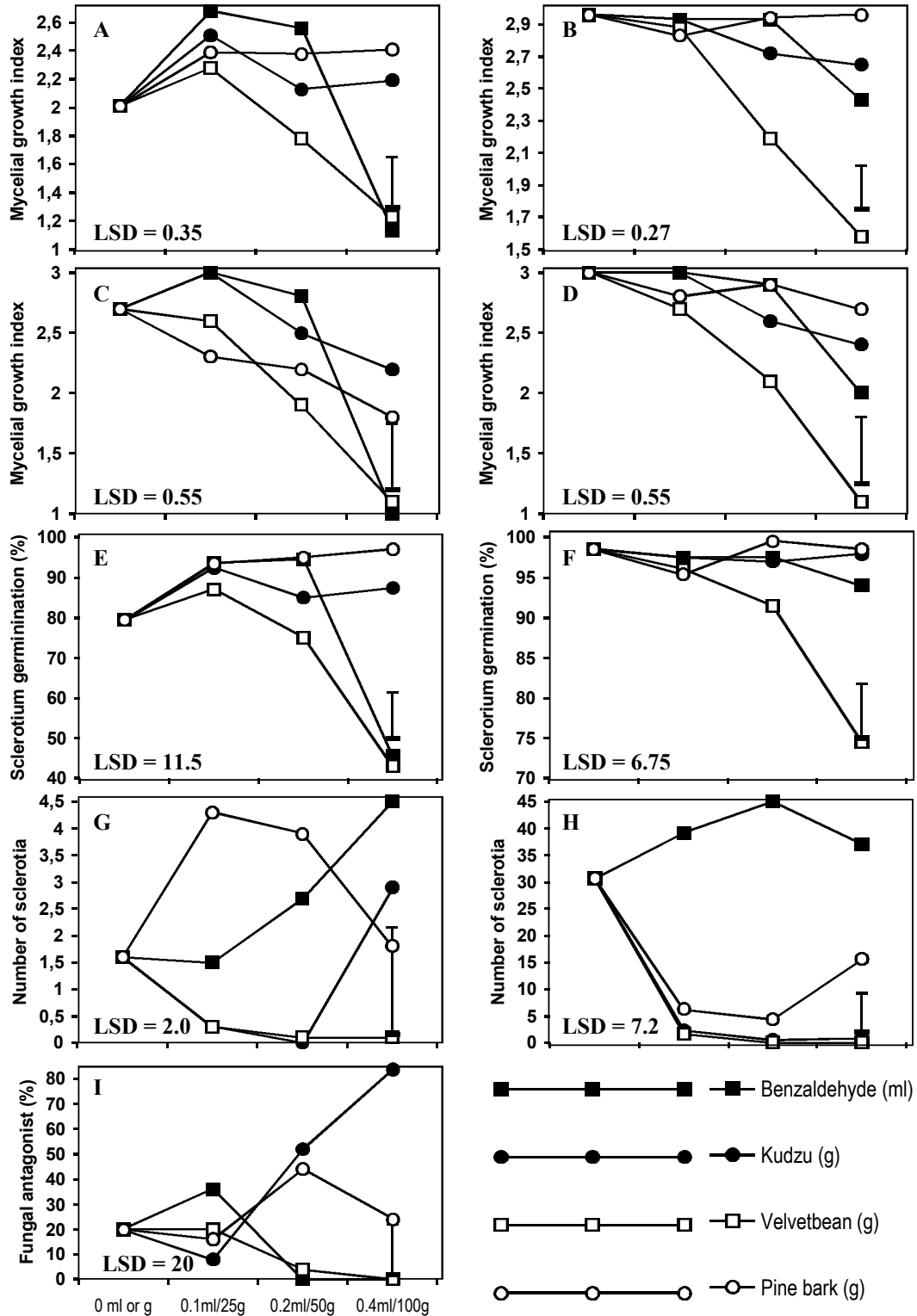
Soil pH tended to increase with the rate of amendment used in the case of velvetbean, kudzu, and benzaldehyde, while the opposite was observed with pine bark amendments (Table 2). For velvetbean and kudzu, conductivity tended to increase in proportion with the rate of amendment applied (Table 2).

Benzaldehyde dosages were positively correlated with plant height and shoot weight, and negatively correlated with FDA activity at 30 days after application of the amendments. Kudzu rates were positively correlated with plant height, shoot weight, root weight, catalase and FDA activities, and soil pH and conductivity, but negatively correlated to disease severity. Velvetbean rates were negatively correlated with disease severity and number of surface sclerotia, but positively correlated with plant height, shoot weight, root weight, catalase and FDA activities, soil pH and conductivity. Thirty days after application, pine bark rate was negatively correlated with disease severity, number of surface sclerotia, and soil pH, but positively correlated with root weight (2nd planting), catalase and FDA activities, and conductivity (Table 2).

## DISCUSSION

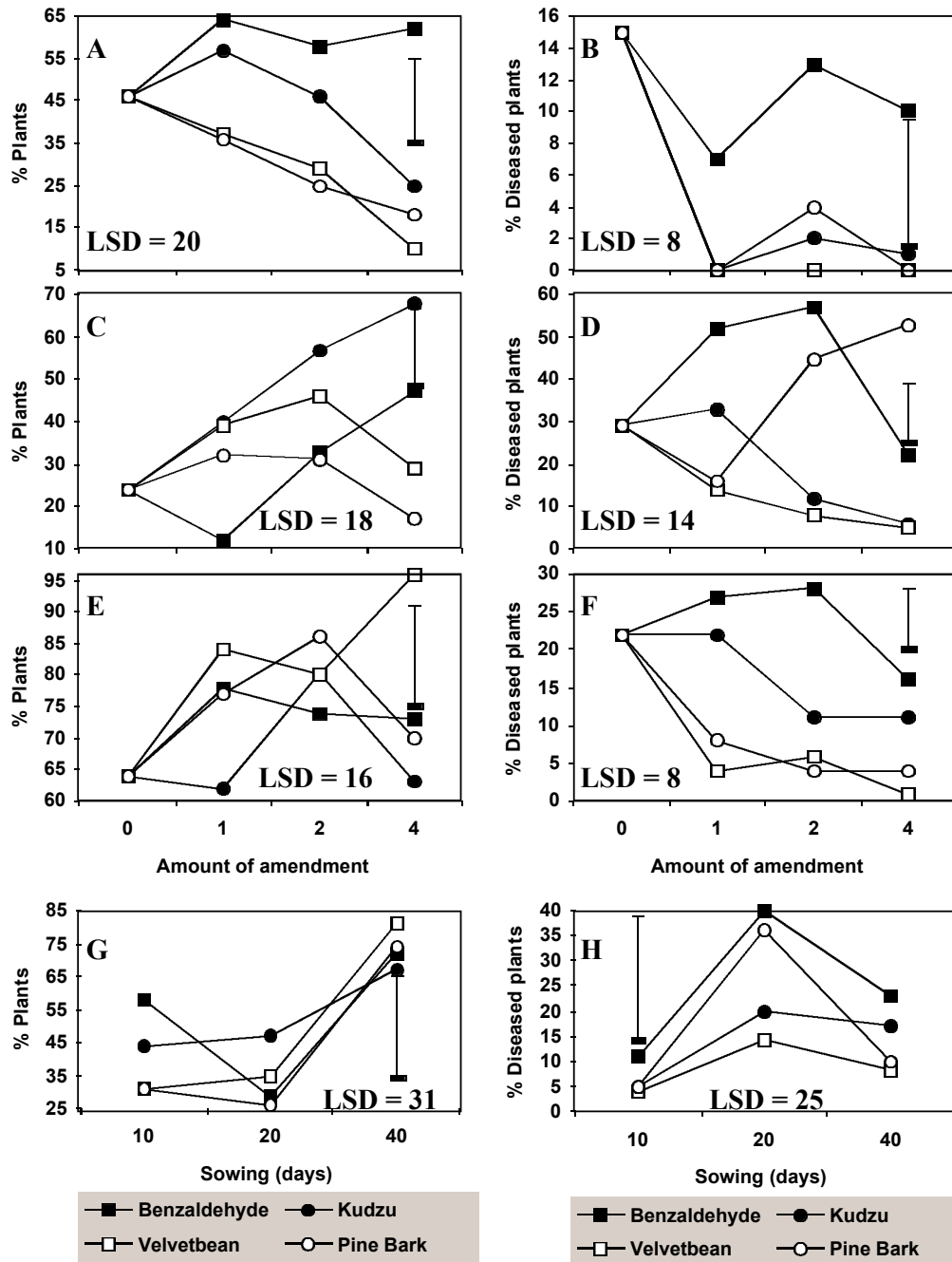
It is necessary to study the effects of amendments on sclerotial germination and mycelial growth of *S. rolfisii*. This knowledge helps identify some of the reasons for success or failure of amendments to control this fungus. Our results clearly indicate that stimulation of mycelial growth by *S. rolfisii* varies with the type of inoculum (colonized seeds or sclerotia). Previous studies (Beute & Rodríguez-Kábana, 1979) showed the stimulation of mycelial growth induced by kudzu residues. These authors concluded that stimulation was due to the liberation of volatile compounds released by decomposition of kudzu fragments. In our studies, germination of sclerotia was similarly stimulated by the lowest rates of amendments.

The highest rates of benzaldehyde and velvetbean inhibited mycelial growth and germination of sclerotia of *S. rolfisii* for at least 24 h after inoculum incubation. Flor (1926) found that benzaldehyde at high rates was fungistatic or fungicidal. Sclerotial germination is a critical stage of the *S.*



**FIG. 1** - Effect of amounts [in ml/kg for benzaldehyde and g/kg for kudzu (*Pueraria labata*), velvetbean (*Mucuna deeringiana*) or pine bark (*Pinus* spp.)] of amendments on *Sclerotium rolfii* mycelial growth from germinated sclerotia (A and B) and from oat seed inoculum (C and D), on sclerotial germination (E and F), 24 (A, C and E) and 48 h (B, D and F) after incubation, on formation of new sclerotia from older ones (G) and from oat seed inoculum (H), and on formation of colonies (I) of *Trichoderma* spp. on *S. rolfii* inoculum. Data represent an average of two tests. Bar = LSD = Fisher's least significant difference (P = 5%).

Effect of soil organic amendments on sclerotial germination, mycelial growth...



**FIG. 2** (A-H) - Percentage of survival and diseased soybean (*Glycine max*) plants, 14 days after sowing, in soil infested with *Sclerotium rolfsii* and treated with organic amendments. In (A) through (F) the axis values correspond to amount of soil organic amendments [0, 1, 2, and 4 represent for benzaldehyde 0, 0.1, 0.2, and 0.4 ml/kg of soil, and for kudzu (*Pueraria labata*), velvetbean (*Mucuna deeringiana*) or pine bark (*Pinus* spp.) 0, 25, 50, and 100 g/kg of soil]. In (G) and (H) the axis values correspond to time of sowing after soil treatment. Figures (A) and (B) represent data for sowing time of ten days after soil treatment. Figures (C) and (D) represent data for sowing 20 days after soil treatment. Figures (E) and (F) represent data for sowing 40 days after soil treatment. Data represent an average of two experiments. Bar = LSD = Fisher's least significant difference ( $P = 5\%$ ).

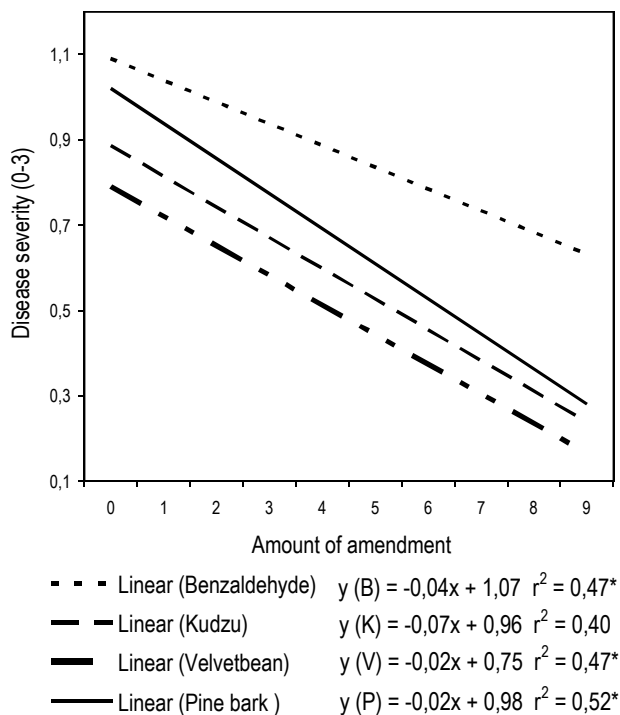
*rolfsii* cycle. Delay or inhibition of sclerotial germination and reduction of sclerotial viability by antagonists, decreases both the inoculum potential of the fungus and disease incidence.

Rodríguez-Kábana *et al.* (1987) demonstrated reduction of sclerotial germination with increasing rates of chitin in soil, and attributed, in part, this reduction to the action of

**TABLE 2** - Correlation matrixes for amendment rates and parameters measured in soil infested with *Sclerotium rolfsii* and planted with tomato (*Lycopersicon esculentum*) cv. Rutgers

Amendment rate x	Benzaldehyde	Kudzu	Velvetbean	Pine bark
Disease Severity 80 d <sup>1</sup>	n.s. <sup>2</sup>	-0.351** <sup>3</sup>	-0.295* <sup>3</sup>	-0.332**
Disease Severity 100 d <sup>1</sup>	n.s.	-0.365**	-0.342*	-0.328*
No. Surface Sclerotium <sup>4</sup>	n.s.	n.s.	-0.514*** <sup>3</sup>	-0.411***
Plant height 1 <sup>st</sup> pl.	n.s.	0.324*	0.331**	n.s.
Shoot weight 1 <sup>st</sup> pl.	n.s.	0.514***	0.722***	n.s.
Root weight. 1 <sup>st</sup> pl.	n.s.	0.406***	0.554***	n.s.
Plant height 2 <sup>nd</sup> pl.	0.284*	0.761***	0.609***	n.s.
Shoot weight 2 <sup>nd</sup> pl.	0.294*	0.767***	0.790***	n.s.
Root weight 2 <sup>nd</sup> pl.	n.s.	0.632***	0.628***	0.256*
Catalase (30 d) <sup>5</sup>	n.s.	0.828***	0.811***	n.s.
Catalase (120 d)	n.s.	0.922***	0.915***	0.630***
FDA = Esterase (30 d) <sup>6</sup>	-0.517***	0.568***	0.830***	0.489***
FDA (120 d)	0.273*	0.700***	0.899***	0.575***
Conductivity (30 d)	n.s.	0.913***	0.933***	0.301*
Conductivity (120d)	n.s.	0.827***	0.881***	n.s.
pH (30 d)	-0.659***	0.942***	0.843***	-0.912***
pH (120 d)	n.s.	0.961***	0.963***	-0.883***

<sup>1</sup> Disease severity (80 and 100 d after tomato second planting); <sup>2</sup> n.s.= no statistical difference; <sup>3</sup> \* significance at 5.0%; \*\* at 1.0%; and \*\*\* at 0.1%; <sup>4</sup> No. Surface Sclerot. = Number of surface sclerotium formed 14 d after soil treatment; <sup>5</sup> 30 and 120 d = 30 and 120 d after soil treatment; <sup>6</sup> Esterase like enzymes expressed by fluorescein diacetate hydrolysis (FDA) activities.



**FIG. 3** - Effect of amount of soil organic amendments [0, 1, 2, 3, 4, 5, 6, 7, 8, 9 represent for benzaldehyde 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, and 1.2 ml kg<sup>-1</sup> of soil, and for kudzu (*Pueraria labata*), velvetbean (*Mucuna deeringiana*) or pine bark (*Pinus* spp.) 0, 2.5, 5, 7.5, 10, 15, 20, 25, 30, and 35 g kg<sup>-1</sup> of soil] on disease severity, 80 days after transplanting of tomato (*Lycopersicon esculentum*) plants cv. Rutgers in soil infested with *Sclerotium rolfsii* (average of two plantings). \*Significance at 5.0% on regression analyses. B = benzaldehyde, K = kudzu, V = velvetbean, and P = pine bark).

chitinolytic microorganisms. Hadar & Gorodecki (1991) found an inhibitory effect of grape marc compost on sclerotial germination and viability, and associated this effect with high numbers of *Penicillium* isolated from sclerotia.

In our studies, the most common fungi growing on the surface of sclerotia were *Trichoderma* spp. (mainly *T. harzianum* Rifai) and occasionally *Gliocladium virens* Miller *et al.* (Figure 1I). Mukherjee *et al.* (1995) compared one isolate of *G. virens* and one isolate of *T. harzianum* in relation to their antagonistic properties on *Rhizoctonia solani* Kühn and *S. rolfsii*. These authors concluded that only *T. harzianum* parasitized the hyphae of *S. rolfsii*, and both parasitized the hyphae of *R. solani*. Both antagonists were equally comparable in terms of antibiosis, and *G. virens* was most aggressive in destroying sclerotia of *S. rolfsii* (Mukherjee *et al.*, 1995).

Lack of stimulation in formation of new sclerotia in treatments with kudzu or velvetbean might be associated with the presence of antagonists in the case of kudzu and the lowest rate of velvetbean, or with toxicity in the case of high rates of velvet bean. Liberation of toxic or fungistatic compounds could be associated with reduced formation of sclerotia in soil with kudzu and mucuna. Pine bark developed more new sclerotia than kudzu and velvetbean, but less than the non-treated control. However, benzaldehyde generally induced formation of significantly more sclerotia than the non-amended control. Nevertheless, the high numbers of sclerotia formed in response to high rates of benzaldehyde could be associated with an inducing effect of this volatile aromatic compound for hyphal tip curling and hyphal agglomeration, as well as reduction on hyphal growth. These are important phases in sclerotial formation (Zarini & Christias, 1997).

Our results indicated that there is an optimal period of

time for planting after soil amendment. If sowing is implemented too soon after, application of amendments there can be a problem. Soybean germination in treatments with velvet bean and pine bark was low when sowing occurred prior to 20 days after application of the amendments in soil. A phytotoxic effect was noticed when these amendments were applied in high rates ( $> 25 \text{ g kg}^{-1}$ ) and planting made prior to 20 days after application of the soil amendments. On the other hand, amendments increased the number of plants when planting was done 40 days after application of soil amendments (Figure 2B). Not only should rates of amendments be investigated but also when sowing or planting should be carried out. This might explain, in part, why some soil amendments work in one situation and do not work in another. Mian & Rodríguez-Kábana (1982) reported the phytotoxic effect of kudzu ( $100 \text{ g kg}^{-1}$ ) in terms of the reduction of germination of squash (*Cucurbita pepo* L.) seeds, and reported that at 1% ( $10 \text{ g/kg}$ ) kudzu hay was not toxic to the plant and reduced severity of disease on squash caused by *Meloidogyne arenaria* (Neal) Chitwood. Most organic amendments are initially detrimental to plants at rates over 5% ( $50 \text{ g kg}^{-1}$ ) or when their C:N ratio is lower than 12 (Rodríguez-Kábana *et al.*, 1987). The best plant development was found when planting was done 40 days after the application of the amendments, this might also be associated with the availability of nutrients for the plants due to the decomposition of kudzu and velvetbean.

Pine bark amended soil generally has reduced disease incidence or severity and increased plant emergence. Kokalis-Burelle & Rodríguez-Kábana (1994) reported that fresh or composted pine bark at a rate of  $100 \text{ g kg}^{-1}$  improved the emergence of lentil (*Lens culinaris* Medik.) in soil infested with *R. solani* or *S. rolfssii*. Also, these authors correlated the improvement of seedlings emergence with the increased numbers of *Penicillium chrysogenum* Thom isolated from soils treated with pine bark.

Soil catalase and FDA activities increased positively with the rate of kudzu, velvet bean or pine bark added to soil, reflecting increased microbial activity. Previous studies showed positive correlation between amendments and microbial activity (Kokalis-Burelle & Rodríguez-Kábana, 1994; Soler-Serratosa *et al.*, 1996).

Results from this study indicate that organic amendments can be used to manage *S. rolfssii* and disease caused by this pathogen. The mode of action of these amendments against the pathogen is complex and unknown. Certainly stimulation of microbial activities, determined by physical and chemical properties of materials used for amendments has an important role. Each amendment was associated with an optimal rate to decrease disease incidence and severity, and increase plant survival and growth.

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