Fitopatologia Brasileira

REFERÊNCIA

BLUM, Luiz E. B.; RODRÍGUEZ-KÁBANA, Rodrigo. Powders of kudzu, velvetbean, and pine bark added to soil increase microbial population and reduce Southern blight of soybean. **Fitopatologia Brasileira**, v. 31, n. 6, p. 551-556, nov./dez. 2006. DOI: https://doi.org/10.1590/S0100-41582006000600003. Disponível em: https://www.scielo.br/j/fb/a/Ldwmp5Zt8nwwpGKrgmTwFMM/?lang=en#. Acesso em: 10 set. 2021.

Powders of Kudzu, Velvetbean, and Pine Bark Added to Soil Increase Microbial Population and Reduce Southern Blight of Soybean

Luiz E. B. Blum[1] & Rodrigo Rodríguez-Kábana[2]

[1]Universidade de Brasília, Departamento de Fitopatologia, CEP 70910-900, Brasília, DF, Brazil, e-mail: luizblum@pesquisador.cnpq.br; [2]Auburn University, Plant Pathology Department, 209 Life Sciences Building, 36849Auburn AL, USA.

(Accepted for publication 20/10/2006)

Author for correspondence: Luiz Eduardo Bassay Blum

BLUM, L.E.B. & RODRÍGUEZ-KÁBANA, R. Dried powders of kudzu, velvetbean, and pine bark added to soil increase microbial population and reduce Southern blight of soybean. Fitopatologia Brasileira 31:551-556. 2006.

ABSTRACT

Southern blight (*Sclerotium rolfsii*) of soybean (*Glycine max*) is an important disease throughout the world. Some soil amendments can reduce disease levels by improving soil microbial activity. The main goals of this study were to investigate the effects of soil amendments such as dried powders of kudzu (*Pueraria lobata*), velvetbean (*Mucuna deeringiana*), and pine bark (*Pinus taeda*), on soil microbial population and disease caused by *S. rolfsii* on soybean. Pine bark, velvetbean (mucuna) and kudzu (25 g kg^{-1}) added to soil were effective in reducing disease incidence [non-amended (NA) ~ 39%; amended (A) ~ 2 to 11%)]. *Bacillus megaterium* was the bacteria most frequently isolated in soils with velvetbean or kudzu (NA ~ log 5.7 CFU g^{-1} of dried soil; A ~ log 6.2). Soils with velvetbean and kudzu stimulated increase in population of *Enterobacter aerogenes* (NA ~ log 3; A ~ log 5.1-5.8). *Pseudomonas putida* population was higher in A than in NA (NA ~ log 4; A ~ log 5.5), and was negatively correlated (r = -0.83, P = 1%) to disease incidence. Soil amended with kudzu and pine bark stimulated increases in populations of *Trichoderma koningii* (NA ~ log 1.6; A ~ log 2.9) and *Penicillium citreonigrum* (NA ~ log 1.3; A ~ log 2.6), respectively. *Penicillium herquei* soil population increased with addition of kudzu (NA ~ log 1.2; A, ~ log 2.5). These microorganisms are antagonists of soil-borne pathogens. Powders of velvetbean, kudzu, and pine bark can increase antagonistic population in soil and reduce disease.

Additional keywords: *Sclerotium rolfsii*, stem rot, soil amendments, *Mucuna*, *Trichoderma*.

RESUMO

Pós-secos de kudzu, mucuna e casca de pinus adicionados ao solo aumentam a população microbiana e diminuem a murcha por esclerócio em soja.

A murcha (*Sclerotium rolfsii*) da soja (*Glycine max*) é uma doença mundialmente importante. Alguns resíduos orgânicos quando adicionados ao solo podem reduzir a doença devido ao aumento da atividade microbiana. Neste estudo investigaram-se os efeitos de pós-secos de kudzu (*Pueraria lobata*), mucuna (*Mucuna deeringiana*) e casca de pinus (*Pinus taeda*) adicionados ao solo, na população de microrganismos e na doença em soja. Casca de pinus (CP), mucuna e kudzu (25 g kg^{-1}) incorporados ao solo reduziram a incidência de doença [sem aditivos (SA) ~ 39%; com aditivos (CA) ~ 2 a 11%)]. *Bacillus megaterium* foi freqüentemente isolada em solo com mucuna ou kudzu (SA ~ log 5,7 UFC g^{-1} de solo seco; CA ~ log 6,2).Solos com mucuna e kudzu aumentaram a população de *Enterobacter aerogenes* (SA ~ log 3; CA ~ log 5,1-5,8). A população de *Pseudomonas putida* foi maior em solos CA do que em solos SA (SA ~ log 4; CA ~ log 5,5). Houve correlação negativa (r = -0,83; P = 0,01) entre a quantidade de doença e a população de *P. putida*. Solos com kudzu e CP aumentaram a população de *Trichoderma koningii* (SA ~ log 1,6; CA ~ log 2,9) e *Penicillium citreonigrum* (SA ~ log 1,3; CA ~ log 2,6), respectivamente. A população de *Penicillium herquei* aumentou devido a adição de kudzu (SA ~ log 1,2; CA ~ log 2,5). Estes microrganismos são antagonistas de patógenos no solo. A adição ao solo de mucuna, kudzu e CP aumentou a população de microrganismos antagonistas e reduziu a doença.

Palavras-chave adicionais: *Sclerotium rolfsii*, podridão, resíduo orgânico, mucuna, *Trichoderma*.

INTRODUCTION

Sclerotium rolfsii Sacc. [teleomorph *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.] is responsible for crop yield losses in many important plants around the world. This pathogen can cause stem or foot rot on soybean (*Glycine max* L.) and 500 other species of plants. Recommendations have been made for use of fungicides, microbial antagonists, crop rotation, soil organic or inorganic amendments, solarization, and resistant cultivars (Punja, 1988).

Cultural practices have their limitations, but can be low-cost alternatives for reduction of soil-borne pathogen populations, diseases, and improvement of crop growing (Punja, 1988). Organic amendments to soil generally increase soil fertility, growth of subsequent crops, and suppress plant pathogens (Blum & Rodriguez-Kábana, 2004).

If correctly applied, soil amendments can reduce populations of soil-borne pathogens by stimulating increases in populations of some naturally occurring antagonists. The activities and populations of some microorganisms in soil are altered when amendments are added (Blum & Rodriguez-Kábana, 2004). Studies under greenhouse conditions with benzaldehyde and pine (*Pinus* L.) bark powder (Kokalis-Burelle & Rodríguez-Kábana, 1994) showed that some selected microorganisms had their populations increased. This increase in populations of beneficial organisms can be associated with decreases in populations of some soil-borne fungi.

Blum & Rodriguez-Kábana (2004) reported a positive correlation between rates of amendments and activities of soil enzymes, indicating an increase on soil microbial activity, which could be associated to disease reduction. In addition, the availability of kudzu [*Pueraria lobata* (Willd.) Ohwi], velvetbean [*Mucuna deeringiana* (Bort) Merr.], and pine bark in the southeast U.S. and the possibility for management of *S. rolfsii* with these amendments, led us to study the effect of kudzu, velvetbean (mucuna), and pine bark powder on soil microbial population and Southern blight (*S. rolfsii*) on soybean.

MATERIAL AND METHODS

Sclerotium rolfsii inoculi from PDA (Potato dextrose agar, Difco Co.) cultures were prepared using oat kernels (Beute & Rodríguez-Kábana, 1979). After growth, these colonized seeds were dried (22°C). A sandy-loam soil was sieved and mixed with siliceous sand (50:50w/w). The elemental composition and other characteristics of the soil used for the greenhouse experiments were: Source area - Auburn, AL (USA); Previous crop - Peanut (*Arachis hypogaea* L.); Soil type - sandy loam; CEC - < 4.6 cmol.kg^{-1}; pH 6.8; P 19.5 mg kg^{-1}; K 38 mg kg^{-1}; Mg 65 mg kg^{-1}; Ca 300 mg kg^{-1}; C 0.7%; N 0.1%.

The amendments used in the experiments were dried and ground fresh pine bark, kudzu and velvetbean (Three-month-old plants - leaves and stem) as described by Blum & Rodriguez-Kábana (2004). The Soil Testing Laboratory at Auburn University, Auburn, AL, determined the elemental composition of the powdered materials. The results of the analyses of powdered amendments are presented on Table 1.

Two experiments were performed in greenhouse (25 ± 5 °C) with soil infested with *S. rolfsii* (5 g colonized oat seeds kg^{-1} of soil). For both tests soil treatments were as presented on Figure 1 [control (without amendment), kudzu, velvetbean, and pine bark powder (25 g kg^{-1}), controls with autoclave sterilized (with and without *S. rolfsii*) and non-sterilized (without *S. rolfsii*) soil were also applied, but were not used for statistical analyses]. Each treatment was replicated six times. An experimental unit in the experiments was a 1 liter pot (1 L capacity cylinder shape PVC pot) with 1 kg of soil (1kg of soil and 1.0 g of 10-10-10 N-P-K). Infestation of soil with pathogen was made 2 days prior to application of the amendments. Sowing with 'Davis' soybean (10 seeds per pot) was done 30 days after application of the amendments [Plantings in amended soil prior to 30 days after application of the amendments are not recommended due to possible phytotoxic effects on soybean seedlings induced by the organic residues (Blum & Rodríguez-Kábana, 2004)]. Evaluation of disease incidence was done 30 days after sowing. Irrigation of plots was by pouring 100 mL of water per pot when necessary.

TABLE 1 – Elemental composition of amendments (kudzu, velvetbean and pine bark) used in the experiments

Nutrient	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

* Three-month-old plants (leaves and stem).

Soil samples from each pot were collected 60 days after application of the amendments, and were used for microbial isolations and evaluation of soil moisture content. Ten grams of freshly collected soil from each plot were thoroughly mixed for 5 minutes (tray shaker) with 90 mL of sterile water, and then diluted 10 fold to 10^{-6}. For fungi, dilutions 10^{-2}-10^{-3} were used and for bacteria 10^{-5}-10^{-6}. The resulting suspensions were then spiral plated (Spiral System) in the appropriate culture media. The selective medium used to isolate fungi from soil was MEA (Malt extract agar, Difco Co., amended with 100 mg L^{-1} tetracycline hydrochloride; 50 mg L^{-1} rose Bengal). Isolations of bacteria were done in 5% TSA (Tryptic soy agar Difco Co., amended with 50 mg L^{-1} benomyl). Number of colonies per plate was determined directly two and five days after inoculation for bacteria and fungi, respectively.

Twenty bacterial isolates from each replication were randomly picked with sterile tooth picks, grown in TSA, and maintained in small tubes (1.2 mL) with 1.0 mL of sterile water. Identification of bacterial isolates was based on analysis of fatty acid methyl-esters profiles (Stead, 1988). Bacterial samples were prepared as described by Kloepper *et al.* (1992), analyzed with a Hewlett-Packard Gas Chromatography (5890 II), and identified according to Sherlock Microbial Identification System software. Twenty fungal isolates from each replication were randomly picked and maintained in tubes with MEA. Identification of fungi was based on morphology and growth on MEA, PDA and Czapek Dox Agar (Difco Co.), and consulting appropriate literature (Domsch *et al*., 1980).

Treatments in the experiments were arranged in a randomized complete block design, and data were analyzed by ANOVA. Differences among means were evaluated for significance by Tukey's test.

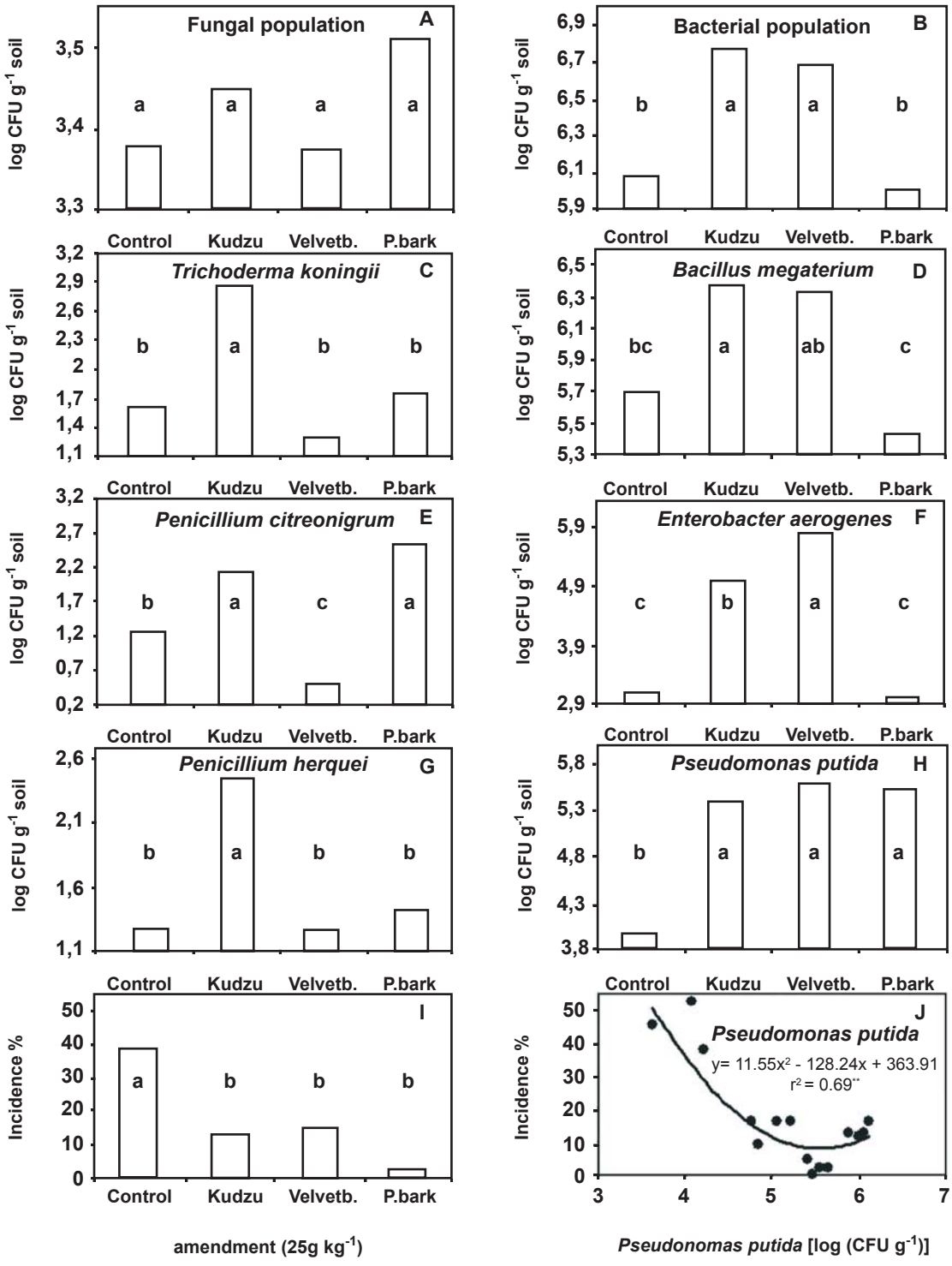

FIG. 1 - Effects of soil amendments (kudzu, velvetbean, and pine bark) on: A. fungal population (CFU = Colony forming unit g^{-1} of dried soil); **B.** bacterial population (CFU g^{-1} of dried soil); **C-H.** selected predominant potential antagonists; **I.** soybean disease (*Sclerotium rolfsii*) incidence, and; **J.** regression between disease incidence and *Pseudomonas putida* population (** coefficient of regression significant at P = 1%). Evaluation made 60 days after soil treatment (30 days after soybean sowing). Bars followed by same letters are significantly equal (Tukey, P < 5%). (Data represent an average of two experiments).

RESULTS AND DISCUSSION

In this study was shown that certain types of amendments stimulate the increase on populations of some naturally occurring antagonists and, consequently, decrease the amount of disease. Furthermore, this work adds new information and complements data presented by Blum & Rodriguez-Kábana (2004) concerning the effects of organic amendments on *S. rolfsii* sclerotial germination and mycelial growth.

Total fungal population averages (~ log 3.3-3.5 CFU g^{-1} of dried soil) did not differ among treatments (Fig. 1A). However, total bacterial populations were significantly higher in soil treated with velvetbean (~ log 6.7 CFU g^{-1}) or kudzu (~ log 6.8 CFU g^{-1}) in comparison to the non-amended (~ log 6.1 CFU g^{-1}) soil (Fig. 1B). Previously, evaluations of microbial populations were made from 0 to 70 days after soil amendment, therefore, was determined that the population of microorganisms in amended soil was higher around 60 days after soil amendment under greenhouse conditions (data not shown). Independently of the soil treatment, a total of 85 species of bacteria was identified, and the most frequent ones were *Bacillus megaterium* de Bary and *Comamonas acidovorans* (den Doren) Tamaoka *et al*. (Data not shown). *Bacillus megaterium* was the most common (~ log 6.4 CFU g^{-1}) bacteria in soils amended with kudzu (Fig. 1D). Velvetbean amended soils frequently contained *B. megaterium* (~ log 6.3 CFU g^{-1}), *Enterobacter aerogenes* Hormaech & Edwards (~ log 5.9 CFU g^{-1}), and *Pseudomonas putida* (Trevisan) Migula (~ log 5.5 CFU g^{-1}) (Fig. 1D, F, H). Pine bark treated soil had large numbers of *P. putida* (~ log 5.4 CFU g^{-1}). *Penicillium citreonigrum* Dierckx, *P. herqueri* Bainier & Sartory, and *Trichoderma koningii* Oudem. were the most common fungi isolated from soil amended with kudzu (Fig. 1C, E, G). Soils amended (~ log 2.5 CFU g^{-1}) with pine bark had more *P. citreonigrum* (Fig. 1E) than the non-amended (~ log 1.3 CFU g^{-1}) control.

Disease incidence was reduced from ~ 39% (non-amended control) to ~ 11%, ~ 12%, and ~ 2% in soils amended with kudzu, velvetbean and pine bark, respectively (Fig. 1I). Previous work has shown that amendments may reduce pathogen populations and diseases (Beute & Rodríguez-Kábana, 1979). Kokalis-Burelle & Rodríguez-Kábana (1994) reported that pine bark powder was effective in reducing mycelial growth of *S. rolfsii*, as well as in improving lentil (*Lens culinaris* Medik.) stands in greenhouse experiments. Pine bark can suppress *Fusarium oxysporum* f.sp. *chrysanthemi* Litrell, G.M. Armst. & J.K. Armstr., *F. oxysporum* f.sp. *lini* W.C. Snyder & H.N. Hansen, and *Phytophthora cinnamomi* Rands (Kokalis-Burelle & Rodríguez-Kábana,1994; Hardy & Sivasithamparam, 1991). Velvetbean (mucuna) is a useful rotation crop with many important crops for reduction of soil borne pathogens of many crops (Rodríguez-Kábana *et al*., 1992). Blum & Rodriguez-Kábana (2004) reported an increase of occurrence of *Trichoderma* Pers. spp. parasitizing sclerotia of *S. rolfsii* in soil amended with kudzu and pine-bark. These authors also reported that there was a negative correlation between the number of viable sclerotia of *S. rolfsii* and amount of velvetbean and pine bark.

Antagonistic microorganisms might be involved in disease suppression when soil amendments are used. Bacteria with potential for biocontrol of soil-borne pathogens increased in some amended soils. Velvetbean treated soils favored increase in populations (Non-amended soil, ~ log 3 CFU g^{-1}; amended soil, ~ log 5.9 CFU g^{-1}) of *E. aerogenes* (Fig. 1F). Kudzu amended soil enhanced (Non-amended soil, ~ log 5.7 CFU g^{-1}; amended soil, ~ log 6.4 CFU g^{-1}) *B. megaterium* (Fig. 1D), and pine bark was favorable (Non-amended soil, ~ log 4.1 CFU g^{-1}; amended soil, ~ log 5.5 CFU g^{-1}) to *P. putida* (Fig. 1H). All these bacteria are recognized for their potential as antagonists of pathogens such as *Phytophthora* de Bary, *Pythium* Pringsh., *Rhizoctonia* DC., and *Sclerotium* Tode (Marchi & Utkhede, 1994).

Pseudomonas putida population was negatively correlated ($r = -0.83$, $P < 1\%$) to incidence of disease in soybean (Fig. 1J). No statically significant ($P < 5\%$) correlation was shown between disease incidence and population of other microorganisms (*Bacillus megaterium*, *Enterobacter aerogenes*, *Trichoderma koningii*, *Penicillium citreonigrum*, and *P. herquei*). The lowest levels of disease incidence (~ 5-15%) were reached with populations of *P. putida* around log ~ 5.5-6.1 CFU g^{-1}. In soils amended with kudzu, velvetbean, and pine bark the population of *P. putida* was around, log ~ 5.4 CFU g^{-1}, log ~ 5.6 CFU g^{-1}, and log ~ 5.5 CFU g^{-1}, respectively. Earlier study reported that *P. putida* reduced vascular wilt in cotton caused by *F. oxyporum* f.sp. *vasinfectum* W.C. Snyder & H.N. Hansen (Chen *et al*., 1995). *Pseudomonas fluorescens* Migula and *P. putida* have been implicated (Sivasithamparam *et al*., 1979) in suppression of *Gaeumannomyces graminis* var. *tritici* J. Walker in wheat (*Triticum aestivum* L.) take-all disease.

The mechanisms involved in pathogen control may vary, depending on bacterial species. Parasitism, antibiosis, siderophore production, plant growth, induced resistance, and competition (nutrients and sites of colonization) might be involved in suppression of pathogen populations and in disease incidence or severity (Kloepper & Schroth, 1981). Marchi & Utkhede (1994) showed that *E. aerogenes* acted by antibiosis against *Phytophthora cactorum* (Lebert & Cohn) J. Schröt. on apple trees (*Malus domestica* Borkh.). *Bacillus megaterium* is an efficacious competitor and extracellular enzyme producer that inhibit soil-borne plant pathogens, such as *Rhizoctonia solani* J.G. Kühn (Bertagnolli *et al.*, 1996). Competition or antibiosis might be biological control mechanisms used by *P. putida* against several soil-borne pathogens (Scher & Baker, 1982; Weller & Cook, 1983).

Kudzu amended soil stimulated increases in populations (Non-amended soil, ~ log 1.3 CFU g^{-1}; amended soil, ~ log 2.5 CFU g^{-1}) of *P. herquei* (Fig. 1G). This fungus is known to produce antifungal metabolites which might be associated with disease suppression (Domsch *et al*., 1980).

The incorporation of crop residues into soil is a strategy to control soil-borne plant pathogens by promoting selectively enhanced activity of antagonists or to immobilize essential elements for successful infection by a plant pathogen (Pankurst & Lynch, 1995). Bourbos & Skoudridakis (1987) reported that some antagonists, such as *P. herquei* and *T. viride* Pers. ex Gray, were found in greater numbers in the rhizosphere of resistant tomatoes (*Lycopersicon esculentum* Mill.) to vascular diseases caused by *F. oxysporum* f.sp. *lycopersici* W.C. Snyder & H.N. Hansen and *Verticillium albo-atrum* Reinke & Berthold. *P. herquei* produces an alkaloid (herquline) with antimicrobial activities (Omura *et al*., 1979). This information indicates the potential of *P. herquei* as a biological control agent against *S. rolfsii*.

Methods for increasing populations of beneficial microorganisms by soil amendments have the advantage that no improved methodology is needed to facilitate microbial growth and survival. Indigenous microorganisms, in general, have higher competence for survival in their own habitat than introduced antagonists. Other cultural methods, such as mulching and crop rotation, also induce microbial increases in soil (Rodríguez-Kábana *et al*., 1992).

Adding organic amendments to soil such as velvetbean (C:N ~ 32) and kudzu (C:N ~ 17) limits disease intensity, possibly due to release of toxic compounds (ammonium derived compounds and others) or to increases in levels of resident antagonistic soil microorganisms (Punja, 1985). Mian & Rodríguez-Kábana (1982) reported that the relative efficacy of the amendment on suppressing pathogens depends on their nitrogen content and C:N ratio. These authors, also reported that materials with narrow C:N ratio (high N content) were more effective than those with broad C:N ratios. Materials with C:N ratios of 15-20 are optimal to stimulate soil microflora when applied at around 1-2% rate (Mian & Rodríguez-Kábana, 1982). Punja & Grogan (1982) published that nitrogen derived compounds were toxic to *S. rolfsii*, inhibiting sclerotial germination. Nogueira *et al*. (1996) reported that an aliphatic alcohol (1-triacontanol) and an ester (triacontyl tetracosnate) extracted from leaves and stems of *Mucuna aterrima* Merr. had nematicidal effects on *Meloidogyne incognita* (Kofoid & White) Chitwood. These compounds could be released during velvetbean decomposition in soil and then inhibit *S. rolfsii*.

Probably a group of factors like soil pH, secondary metabolites from decomposition of amendments, microorganisms (*B. megaterium*, *E. aerogenes*, *P. putida*, *T. koningii*, *P. citreonigrum*, and *P. herquei*), small animals, type of pathogen inoculum (Blum & Rodriguez-Kábana, 2004) is involved in the control of *S. rolfsii* by amendments to soil. Single factors might have a major role, but are not alone responsible for the suppression of soil-borne diseases.

ACKNOWLEDGEMENTS

The first author gratefully acknowledges the fellowship provided by CNPq.

REFERENCES

BEUTE, M.K. & RODRÍGUEZ-KÁBANA, R. Effect of volatile compounds from remoistened plant tissues on growth and germination of sclerotia of *Sclerotium rolfsii*. Phytopathology 69:802-805. 1979.

BERTAGNOLLI, B.L., DALSOGLIO, F.K. & SINCLAIR, J.B. Extracellular enzyme profiles of the fungal pathogen *Rhizoctonia solani* isolate 2B-12 and of two antagonists, *Bacillus megaterium* strain B153-2-2 and *Trichoderma harzianum* isolate Th008. I. Possible correlations with inhibition of growth and biocontrol. Physiologycal Molecular Plant Pathology 48:145-160. 1996.

BLUM, L.E.B. & RODRÍGUEZ-KÁBANA, R. Effect of organic amendments on sclerotial germination, mycelial growth, and *Sclerotium rolfsii*-induced diseases. Fitopatologia Brasileira 29:66-74. 2004.

BOURBOS, V.A. & SKOUDRIDAKIS, M.T. Das Verhalten einiger pilzlicher Antagonisten in der Rhizosphäre resistenter und anfälliger Gewächshaustomaten. Journal of Phytopathology, 120:193-198. 1987.

CHEN, C., BAUSKE, E.M., MUSSON, G., RODRÍGUEZ-KÁBANA, R. & KLOEPPER, J.W. Biological control of fusarium wilt on cotton by use of endophytic bacteria. Biological Control 5:83-91. 1995.

DOMSCH, K.H., GAMS, W. & ANDERSON, T.H. Compendium of soil fungi. Vol. 1. Academic Press, London, England. 859 p. 1980.

HARDY, G.E.ST.J. & SIVASITHAPARAM, K. Effects of sterile and non-sterile leachates extracted from composted eucalyptus bark and pine-bark container media on *Phytophthora* spp. Soil Biology & Biochemistry 23:25-30. 1991.

KLOEPPER, J.W., RODRÍGUEZ-KÁBANA, R., MCINROY, J.A. & YOUNG, R.W. Rhizosphere bacteria antagonistic to soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne incognita*) nematodes: identification by fatty acid analysis and frequency of biological control activity. Plant & Soil 139:75-84. 1992.

KLOEPPER, J.W. & SCHROTH, M.N. Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and displacement of root microflora. Phytopathology 71:1020-1024. 1981.

KOKALIS-BURELLE, N. & RODRÍGUEZ-KÁBANA, R. Effects of pine bark extracts and pine bark powder on fungal pathogens, soil enzyme activity, and microbial populations. Biological Control 4:269-276. 1994.

MARCHI, A. & UTKHEDE, R.S. Effect of *Enterobacter aerogenes* on the rhizosphere microflora of apple trees. Journal Phytopathology 141:127-132. 1994.

MIAN, I. H. & RODRÍGUEZ-KÁBANA, R. Survey of the nematicidal properties of some organic materials available in Alabama as amendments to soil for control of *Meloidogyne arenaria*. Nematropica 12:235-246. 1982.

NOGUEIRA, M.A., OLIVEIRA, J.S. & FERRAZ, S. Nematicidal hydrocarbons from *Mucuna aterrima*. Phytochemistry 42:997-998. 1996.

OMURA, S., HIRANO, A., IWAI, Y & MASUMA, R. Herquline, a new alkaloid produced by *Penicillium herquei*. Fermentation, isolation and properties. Journal of Antibiotics 32:786-790. 1979.

PANKHURST, C.E. & LYNCH, J.M. The role of soil microbiology in sustainable intensive agriculture. Advances in Plant Pathology 11:229-247. 1995.

PUNJA, Z.K. The biology, ecology and control of *Sclerotium rolfsii*. Annual Review of Phytopathology 23:97-127. 1985.

PUNJA, Z.K. *Sclerotium* (*Athelia*) *rolfsii*, a pathogen of many plant species. Advances in plant pathology. 6:523-535. 1988.

PUNJA, Z.K. & GROGAN, R.G. Effects of inorganic salts, carbonate-bicarbonate anions, ammonia, and the modifying influence of pH on sclerotial germination of *Sclerotium rolfsii*. Phytopathology 72:635-639. 1982.

RODRÍGUEZ-KÁBANA, R., PINOCHET, J., ROBERTSON, D.G. & WELLS, L. Crop rotation studies with velvetbean (*Mucuna deeringiana*) for the management of *Meloidogyne* spp. Journal of Nematology (Supplement) 24(4s):662-668. 1992.

SHER, F.M. & BAKER, R. 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressive to Fusarium wilt pathogens. Phytopathology 72:1567-1573. 1982.

SIVASITHAMPARAM, K., PARKER, C.A. & EDWARDS, C.S. Bacterial antagonists to the take-all fungus and fluorescent pseudomonads in the rhizosphere of wheat. Soil Biology & Biochemistry. 11:161-165. 1979.

STEAD, D.E. Identification of bacteria by computer-assisted fatty acid profiling. Acta Horticulturae. 225:39-46. 1988.

WELLER, D.M. & COOK, R.J. Suppression of take-all of wheat by seed-treatment with fluorescent pseudomonads. Phytopathology 73:463-469. 1983.