BIOLOGICAL CONTROL OF GINSENG ROOT ROTS WITH SOIL AMENDMENTS

Hoo Sup Chung and Choong Hoe Kim
Department of Agricultural Biology, College of Agriculture, Seoul National University
Suweon, KOREA

Abstract

The phenomenon of "soil sickness" is one of the most important limiting factors for ginseng *(Panax ginseng)* production in Korea. The principal cause is known to be due to the root rots caused by *Cylindrocarpon destructans* and *Fusarium solani*. Attempts were made to control the root rots with non-polluting cultural methods or soil amendments.

Among the nine soil amendments tested, crab shell, cow bone and pig feces were selected for further testing. Each of the three amendments increased the populations of various actinomycetes in the range of 10–25 times over that of non-amended soil, whereas the population of *C. destructans* was reduced to about 50–70% as compared with the control. Five isolates of *Streptomyces* with clear zones on chitin-agar medium were selected and then tested for their antagonistic effects on *C. destructans*. When any one of the five isolates of *Streptomyces* and *C. destructans* was grown together in a modified peptone broth, growth of the latter was highly inhibited. When three levels of crab shell, cow bone, or pig feces were used to amend potted soil infested with *C. destructans*, the root rot ratings of ginseng seedlings were reduced to less than one half in all the treatments as compared to the control. In another similar experiment, crab shell and cow bone amendments resulted in almost complete control of the seedling root rots in soil infested with *C. destructans* or *F. solani*.

In conclusion, biological control with soil amendments of ginseng root rots caused by *C. destructans* and *F. solani* was successful. Further basic studies should be pursued using soil amendments for better control. In addition, field experiments are needed to complement the soil amendment control measures in an integrated pest control program.

Introduction

Korean ginseng (*Panax ginseng* C.A. Meyer) is a medicinal root crop belonging to the Araliaceae family. Ginseng has long been recognized as a medicinal crop to cure all derived from the Greek words *Pan*: all + Axos: healing for more than 2,000 years in the Orient. Since so many pharmacological activities of chemical components unique to ginseng have been recognized(12), its demand increased year after year. However, many problems remain to be solved before increasing yield and expansion of ginseng cultivation can be made. One of the most important limiting factors is "soil sickness" that results in not only poor quality and low yield but ginseng grown once can not be cultivated in the same field again for more than 10 years.

It is believed that the cause of the phenomenon of soil sickness is principally due to soil-borne pathogens causing a severely limiting (6,7,8,9,10, 15,17,24,27,34), although other possibilities such as deficiency of soil nutrients and certain growth inhibitors (21) can not be excluded. Among the soil-borne diseases of ginseng, various root rots caused by *Cylindrocarpon destructans* (Zinss.) Schölten (7, 27) and *Fusarium solani* (Mart.) Appel & Wr. (9) are known to be the principal ones. The losses in yield in 1977 due to various root rots at harvest were 43–59% at Yangji and 20–49% at Kimpo (9). In 1965, a bacterial root rot associated probably with *C. destructans* and/or *F. solani* caused losses of 48% of the crop at Keumsan and as much as 80% of the crop at Buyeo(23).

Since ginseng requires fertile soil, rich in humus and with continuous shade for six years to grow until harvest, there are no practical control measures for the root rots. Fungicidal application to the soil may not be feasible to control the root rots even when the fungicides are available and effective, because of residual problems and other reasons. Furthermore, varieties resistant to the pathogens are not known. None are expected very soon as neither varietal differentiation of Korean ginseng nor response to the pathogens has been established so far. Therefore, because alternative control measures of ginseng roots were unavailable, biological control with non-polluting soil amendments were tried to improve the quantity and the quality of ginseng production in Korea.

Biological control of soil-borne pathogens with soil amendments is not a new approach. About a half century ago in 1926 Sanford(40) suggested a method to control potato scab by applying green amendments to the soil. The control resulted from the inhibiting action of antagonistic soil saprophytes on the growth of *Streptomyces scabies.* Since then, plant pathologists and soil microbiologists realized that it might be possible to suppress other root pathogens by manipulating the soil environment by appropriate crop rotation and soil management practices.

The subject of biological control of soil-borne plant pathogens has been reviewed extensively (2,3,29,37). Nothing has done more to summarize the existing knowledge on the subject than Baker and Cook's book (4) published in 1976. Although there are numerous examples in which biological control is being accomplished in field soil, nothing is known about the control of ginseng root rot using this principle yet.

There are many examples of successful biological control of *F. solani* f. sp. *phaseoli,* the cause of common bean root rot, which may be closely related to successfully cotrolling the ginseng root rot pathogens. Amendments of chitin (19,31, 32,33), crab shell (14,33), spent coffee (1), or higher carbon to nitrogen (C:N) ratios induced by various organic materials (5,36,41) all reduced the severity of bean root rot disease. Mauer and Baker (28) found that chitin and lignin added to soil together significantly reduced the disease development of bean root rot, whereas chitin alone had no effect.

Recently biological control of certain vascular-invading *Fusarium* spp. that cause wilts of vegetables have also been accomplished with soil amendments. Inoue *et al.* (16) and Mitchell *et al.* (30) reported that chitin amendment was effective in reducing *Fusarium* yellows of raddish caused by *F. oxysporum* f. sp. *conglutinans.* Similarly, *Fusarium* spp. that cause wilts of tomato (14) and cucumber (19) also have been reduced by placing various organic amendments into infested soil.

This paper presents evidence that soil amendment of crab shell, cow bone and pig feces act as biological control agents of *C. destructans,* and *F. solani* that cause root rots in ginseng. Brief results of some of this work have been reported (8,9,10).

Materials and Methods

Soil

The soil used was a sandy loam with rich humus and a pH of 5.6 that was taken from a moderately well drained field plot at Yangji,

Kyonggi-Do in which ginseng had been cultivated for five years. The soil screened through a 20-mesh sieve was used throughout the experiments. The soil was infested with *C. destructans* or *F. solani* grown on wheat bran for seven days, then mixed into the soil at the rate of 50g/kg of dry soil. Soil moisture was maintained at about 30% (w/w) of soil capacity throughout the experiments. Unless otherwise indicated, all the experiments were conducted at 24–28°C.

Isolates

Among 8 isolates of *C. destructans* (Zinss.) Schölten obtained from rusty colored root rot lesions collected at Kwachon, Yangji, Kimpo, Pungki and Buyeo, one isolate was selected with the most virulence. This isolate was used in the first year's experiment. In the second year's experiment an additional isolate, also associated with the root rot of ginseng, *F. solani* (Mart.) Appel & Wr., was from the laboratory of the Department of Plant Pathology at the Chungbuk National University. Both isolates were used separately to infest soil for experiments conducted in the second year.

Soil amendments

The crab shell, pig feces, cow bone, chitin, and clam shell were added to the screened soil as soil amendments. Crab shell, pig feces, clam shell, cow bone and spent coffee were airdried and milled to pass a 100 mesh screen. The following micronutrients were also tested : calcium sulfate ($CaSO_4 \cdot 2H_2O$), ferric ammonium sulfate ($FeNH_4(SO_4)_2H_2O$), and calcium superphosphate. Changes of population of *C. destructans* and actinomycetes in the screened soil were followed by plating soil samples prior to following the pot tests.

Soil dilution counts and media

Counts of microfloral changes were made by the soil dilution plate technique. Population changes of *C. destructans* were measured on PCNB medium(35) and the actinomycetes on a chitin agar medium as described by Lingapa and Lockwood(25).

The chitin agar medium contained 2g of colloidal chitin and 20g of agar/liter. Colloidal chitin was prepared as follows : crude unbleached chitin(Nutritional Biochemicals Co., N.Y., U.S.A.) was washed alternatively for 24 hours each time with 1N NaOH for one time and 1N HCl for 3 times, and then with 95% ethanol for 3 times. Fifteen grams of cleaned chitin were moistened with acetone and dissolved in 100 ml of cold, concentrated HCl, then filtered through glass wool pads. The colloidal chitin was washed with distilled water several times, and then remaining acid was neutralized with 1N NaOH.

Results

Population changes of *C. destructans* and actinomycetes in amended soil

Soil amendments and infestation of the screened soil were made simultaneously. The treated soil samples thus treated were kept in Erlenmeyer flasks and incubated for 10 days prior to evaluation. Each treatment was replicated 3 times.

Populations of actinomycetes with both crab shell and pig feces were increased to more than 25 times over those of nonamended soil, whereas populations of *C. destructans* declined to about one half those of the control. Cow bone resulted in about 10 times increase in numbers of colonies of actinomycetes over those of the control and reduced the populations of *C. destructans* to 70% as compared with the control(Table 1).

With clam shell, spent coffee and ferric ammonium sulphate, populations of *C. destructans* were reduced about 10% over those of control while those of actinomycetes varied. On the contrary, chitin and calcium sulfate resulted in slightly increasing the populations of *C. destructans* while those of actinomycetes were reduced over those of the control.

Antagonistic effects of *Streptomyces* to *C. destructans*

Table 1. Number of colonies of *Cylindrocarpon destructans* and actinomycetes when fungal infestation and soil amendments were made simultaneously to screened ginseng-cultivated soil

Soil infested and mixed with amendments	g/50 gr dry soil	*C. destructans*	*Actinomycetes*
		x10²/g of soil	x10³/g of soil
Organic			
Pig feces	5.0	52	598
Crab shell	0.5	54	553
Cow bone	0.5	70	257
Spent coffee	0.5	84	32
Clam shell	0.5	94	23
Chitin	0.5	113	7
Inorganic			
Calcium perphosphate	1.0	119	32
Calcium sulfate	0.5	105	15
Ferric ammonium sulfate	0.01	94	11
None	0	100	22

It was noteworthy that most of the colonies of actinomycetes formed clear zones on the chitin agar medium regardless of the treatment in previous experiments. Among these actinomycetes that formed wide clear zones, 5 colonies of *Streptomyces* were selected for further study of their antagonistic effects to *C. destructans* (Fig. 1). Three colonies from the non-amended soil and one each from the crab shell and cow bone amended soils were selected.

One ml spore suspensions of each isolate of *Streptomyces* and those of *C. destructans* were used as inoculum for 100 ml aliquots in Erlenmeyer flasks of a modified peptone broth containing 5 g of peptone and 5 mg of killed dry fungal mass of *C. destructans*/1000 ml of water. The inoculated flasks were incubated for 20 days at 28°C in the dark. The cultural mass in the flasks were separated from the liquid filtering, and centrifugation. Then volumes of mass growth was measured in a 2 ml-capacity hypodermic syringe. No *Streptomyces* inoculum was added to the control flasks. Each treatment was replicated 5 times.

All the five isolates of *Streptomyces* inhibited the growth of *C. destructans* in a range from 23 to 77% as compared to the control at the modified peptone broth (Table 2.). The mass growth of each isolate of *Streptomyces* was negligible. Inhibition of the mass growth of *C. destructans* resulted in as much as a 77% reduction with the ST-3 and ST-4 isolates of *Streptomyces* obtained from non-amended ginseng cultivated soil. The final pH of the broth was measured and was found to have been raised from pH 8 to pH 9 after 20 days growth of *C. destructans*.

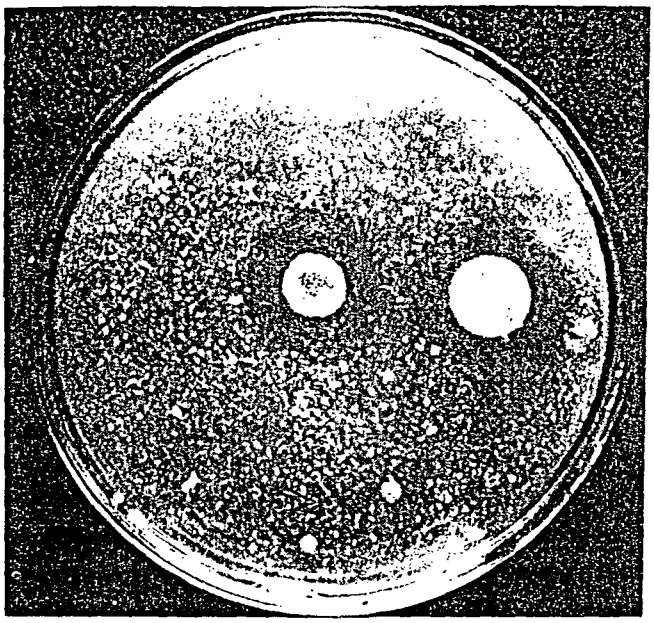

Fig. 1. Clear zones surrounding *Streptomyces* obtained from ginseng-cultivated soil on a chitin agar medium.

Root rot control with soil amendments

Among the nine soil amendments added to the ginseng-cultivated soil, crab shell, cow bone and pig feces were selected for further study because these seemed to be promising as control measures.

Table 2. Effects of antagonistic *Streptomyces* obtained from ginseng-cultivated soil on growth of *Cylindrocarpon destructans* when both organisms were grown simultaneously in a modified peptone-fungal mass broth

Isolate of *Streptomyces*	Soil source	Volume of *C. destructans*(ml)	Volume as % of control
ST-1	crab shell	0.7	54
ST-2	cow bone	0.9	70
ST-3	non-amended	0.3	23
ST-4	non-amended	0.3	23
ST-5	non-amended	1.0	77
Control	none added	1.3	100

The ginseng-cultivated soil was infested with propagules of *C. destructans* and the soil incubated for 2 weeks prior to adding the soil amendments. Each amendment was thoroughly mixed with 1 kg of infested soil and then used to fill plastic pots (20 × 17 × 12 cm). Seedling roots of ginseng were then buried in the pots of treated soil at 28°C in the laboratory.

After 4 weeks incubation, the roots were harvested and severity of root rot was rated on a scale of 0 to 5. A zero rating reflected no rotting, 5 indicated complete rotting of the root, and 1 - 4 were light to severe rotting (Fig. 2). A root rot index was determined by averaging the sum of each of the rottings obtained in three replications. In another experiment, a similar test was carried out using one isolate of *F. solani* obtained from a ginseng root rot lesion.

All the amendments tested, significantly reduced ginseng root rot caused by *C. destructans* to less than 50 % over that of the control regardless of rates of application used in the first experi-

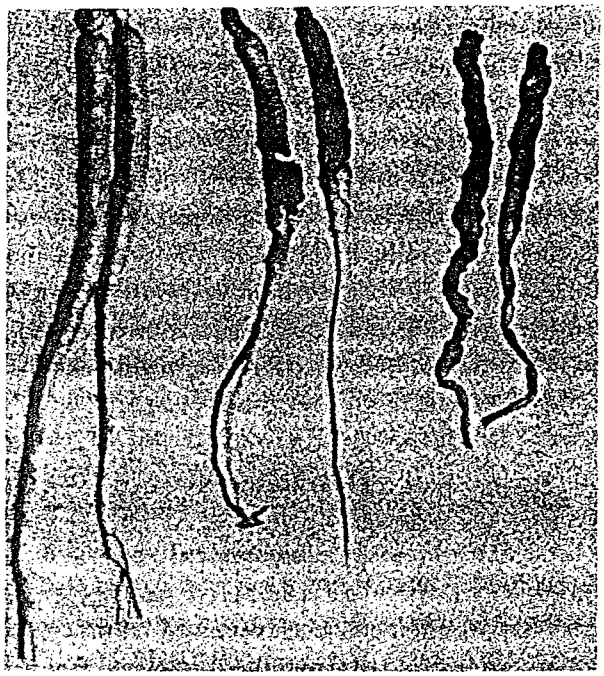

Fig. 2. Root rot ratings of seedling roots caused by *C. destructans* or *F. solani*.
0: no rotting, 5: complete rotting
3: moderate rotting

Table 3. Effects of soil amendments on the severity of ginseng root rots

Amendments to fungal infested soil	g/kg soil	Disease index[a,b]		
		C. destructans		*F. solani*
		Experiment I	Experiment II	Experiment II
Crab shell	3.0	0.9	0.3	1.1
	1.5	1.4	0.3	0.0
	0.5	1.1	0.0	0.2
Cow bone	3.0	1.5	0.3	1.1
	1.5	1.9	0.0	0.3
	0.5	1.5	0.7	0.0
Pig feces	10.0	1.8	...	...
	5.0	1.3	...	...
	1.0	0.8	...	...
None	0.0	3.3	5.0	5.0

[a] Average of three replications, 0: no rotting, 5: complete rotting, and 1-4: light to severe rotting
[b] ...: not tested

ment (Table 3). Although disease indices varied with the amendments and the rates of application, crab shell amendment reduced the disease index of ginseng roots from about 30 to 40 % below that of the control, and cow bone to about 50 % of the control. Pig feces resulted in slightly more severe root rot as the amount of this amendment was increased, but the disease indices were still significantly below that of the control.

In a second experiment with the soil infested with *C. destructans* or *F. solani*, root rots were almost completely controlled as reflected by their disease indices in the range from 0 % to about 20 %. These were of the control in crab shell and cow bone amendments regardless of the rate of application used. In every treatment when the ratings of the ginseng root rots were being made, the growth of the actinomycetes was apparent by the very special odors they imported to the soil. The changes in soil pH were negligible during these experiments.

Discussion

The concept of biological control of soil-borne plant pathogens is based on the principle that a pathogen can be suppressed by saprophytic microorganisms altering the biological equilibrium in the soil by their growth. If the antagonistic flora could be stimulated to grow selectively with certain soil amendments, the populations of pathogenic fungi might decline. Ginseng is a perennial root crop that is faced with severe losses due to various root rots, and so this principle is, therefore, a worthwhile approach because there are as yet no appropriate control measures known today.

There are numerous examples of soil borne diseases that could be controlled substantially by soil amentments that enrich populations of actinomycetes on the soil to inhibit certain pathogens(16, 19, 32, 43). In the present study, with crab shell, cow bone and pig feces, a negative correlation was observed between rising populations of actinomycetes and suppressing populations of the root rot fungus *C. destructans*. However, no such correlation was observed using chitin as a soil amendment.

The clear zones surrounding most actinomycetes colonies on a chitin agar medium may indicate a role of chitinase activity that is likely to be related to degradation of fungal cell-wall constituents as suggested by others (19, 20, 26, 31, 38). It has also been suggested that a marked inhibition of *C. destructans* by five isolates of *Streptomyces* in this study,growing in a modified peptone-fungal mass broth may be the result of mycolytic activity and/or toxin production by the latter. It is well known that actinomycetes produce exocellular chitinase and antibiotics(44), and that the major cell-wall component of *Fusarium* seems to be chitin (11, 38). However, the experimental data presented here are too limited to indicate possible mechanisms of the control of ginseng root rot. The positive correlation between the reduction in the root rot index with decreasing populations of *C. destructans* and the stimulation of growth of actinomycetes antagonistic to the causal fungus may suggest some small part of the mechanism that is involved. It is probable that the nature of *Fusarium* suppressive soil (42) might be one approach to elucidate the mechanisms involved. Further work on the mechanisms of root rot control in ginseng with the soil amendments are in progress in our laboratory.

Practical control of ginseng root rot has been accomplished by the soil amendments to ginseng-cultivated soil with crab shell, cow bone and pig feces. Although pig feces was effective, it may not be useful in that higher amounts of nitrogen may predispose the root rots caused by *C. destructans* and *F. solani* as was pointed out in a previous study(9). This requires further testing.

It is interesting to note that bone meal has been used as a fertilizer in Korea(17) and in the United States of America(45) for ginseng cultivation for a number of years. Bone meal normally contains 20-30 % phosphorous with relatively lower amounts of nitrogen and has been recommended as a good basal fertilizer for ginseng(18). This also coincides with the recommendation that cow bone is a good soil amendment for the control of fungal root rots in ginseng in this study. Similarly crab

shell contains 12—20% chitin resulted in a marked reduction of ginseng root rots as obtained with other fusarial diseases (14, 33). It may not be feasible, however, because large amounts of crab shell are not available for the control of ginseng root rots.

Inorganic amendments may also be used to control certain soil-borne diseases(13, 39). In this connection, Lee and Chung(22) have shown that the activities of cellulolytic and pectolytic enzymes produced by *C. destructans* are much less when certain inorganic nutrients are present. Thus, the mechanisms for the control of *C. destructans* and *F. solani* causing root rot by cow bone amendment must be the subject for further investigation.

Acknowledgement

This investigation was supported by the research grant of the Monopoly Research Institute, Office of Monopoly, Seoul, Korea. Thanks are due graduate students, Mr. Y. W. Lee and Mr. Y. L. Chung for their various help. We are indebted to Professor Oscar H. Calvert, University of Missouri, Columbia, Mo., for his suggestions and help in preparation of the manuscript.

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