EFFECT OF ORGANIC AMENDMENTS ON RHIZOCTONIA AND ACCOMPANYING MICROFLORA IN SOIL

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EFFECT OF ORGANIC AMENDMENTS ON RHIZOCTONIA AND ACCOMPANYING MICROFLORA IN SOIL

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The effect of oat straw, green bean plants, and cellulose on the infection index and saprophytic activity of *Rhizoctonia*, and the effect of chitin, N-supplemented oat straw, and green cotton plants on the infection index, saprophytic activity, and soil microflora was tested. A modified method for counting *Rhizoctonia*-antagonistic bacteria, actinomycetes, and fungi was developed. A relationship between efficiency of the amendments in decreasing infection index and in suppressing the saprophytic activity of *Rhizoctonia* was observed at the end of the experimental period. Decrease in infection index and saprophytic activity was accompanied by increase in counts of general and antagonistic soil microorganisms. This increase was especially pronounced in chitin-amended soil. The possible mechanism of the biological control of *Rhizoctonia* by chitin is discussed.

Introduction

A great deal of information is available regarding the control of *Rhizoctonia* in soil by organic amendments. Yet, the mechanism of this suppression is not fully understood. It is agreed, however, that organic amendments affect *Rhizoctonia* or other soil-borne fungi indirectly, through their influence on the soil microflora (1, 4–6, 11–14, 17). Thus, it has been shown by Papavizas and Davey (13) that general counts of soil microorganisms as well as counts of actinomycetes antagonistic to *Rhizoctonia solani* significantly increased during the suppression of *R. solani* disease of beans by organic amendments.

In the present investigation, the effect of organic amendments on the pathogenic and saprophytic activities of *Rhizoctonia* was tested during three incubation periods in a naturally infested soil. In an attempt to correlate the suppression of *Rhizoctonia* with specific changes in the composition of the soil microflora, these tests were accompanied by counts of soil bacteria, fungi, and actinomycetes that were antagonistic and non-antagonistic to *Rhizoctonia*.

Materials and Methods

The soil used was a slightly alkaline (pH 7.6) sandy loam, naturally infested with *Rhizoctonia* and treated as described in a previous publication (18). For the evaluation of *Rhizoctonia* infectivity, the infection index method was employed, using bean seedlings (*Phaseolus vulgaris* L., variety Brittle Wax) as the test plants. Disease severity was estimated by indexing the visible damage caused to the plants 3 weeks after seed was planted. A 6-degree rating scale was employed: from 0 (no damage) to 5 (plant's stem completely girdled). The relative abundance of *Rhizoctonia* in the soil was estimated by measuring its saprophytic activity, using the bean segment colonization method (18). Green internodal bean stem segments were mixed with the tested soil and incubated for 4 days at 27 °C. The recovered segments were washed, surface-sterilized by being soaked in sodium hypochlorite 1% for 1 minute, washed

with sterile water, and plated on tap water agar containing 250 p.p.m. chloramphenicol. The results are expressed as percentage of segments colonized by *Rhizoctonia*. The detailed procedures as well as the experimental conditions were given in a previous publication (18).

Counts of the soil microflora (bacteria, actinomycetes, and fungi) were made by the soil dilution plate technique. Microorganisms showing antibiotic activity towards *Rhizoctonia* were counted by a modification of Herr's technique (8), in which three agar layers of 5 ml each were used: a base layer of tap water agar; a middle layer of soil extract or tap water agar for bacteria and actinomycetes and Martin's rose bengal agar containing 250 p.p.m. chloramphenicol (instead of streptomycin) for fungi. This layer was mixed with 0.5 ml of the soil dilution (10^{-6} for bacteria and actinomycetes, 10^{-4} for fungal counts), and was poured onto the base layer. After incubation of 3 days at 27 °C, general counts of bacteria, actinomycetes, and fungi were made. To enable the counting of colonies with antibiotic activity towards *Rhizoctonia*, a third layer was prepared with melted Czapek's agar mixed with washed and homogenized mycelium of *Rhizoctonia* grown on potato-dextrose broth for 7 days, at 27 °C. This mixture was poured into sterile petri dishes in 5-ml aliquots and allowed to solidify. The solidified layer was placed on the middle layer containing the microbial colonies by means of a sterile cellophane sheet. By this technique, the spreading of soil microorganisms, especially bacteria, was prevented. Organisms which showed antagonistic activity towards *Rhizoctonia* were recognized by clear inhibition zones around their colonies and were counted after an incubation period of an additional 3 days at 27 °C.

Experiments with amended soil were carried out in 15 × 15 × 16 cm plastic containers, each containing 1000 g of naturally infested soil. Amendments used were 0.2% (w/w) of air-dried soil, ground chitin (N.B.Co., unbleached), 0.2% (w/w) cellulose (Whatman filter paper No. 1, cut into 4 × 4 mm pieces), and 1% (w/w) oat straw, oat straw supplemented with 200 p.p.m. ammonium sulfate, green bean plants, and green cotton plants, respectively. Green amendments were cut into pieces 0.5 cm long. Soil moisture was adjusted to 50% of moisture-holding capacity.

Infection index was determined at zero time and at 3 and 7 weeks after soil amendment, by seeding and evaluating the degree of infection of seedlings after 3 weeks.

The soil microflora and the saprophytic activity of *Rhizoctonia* were estimated 6 and 7 days after each seeding, respectively. Counts of soil microorganisms were made on six replicates of four treatments only, namely chitin, oat straw + ammonium sulfate, cotton, and control (non-amended soil). Incubation temperature in the greenhouse ranged between 22 and 30 °C. Statistical analysis was carried out by the Duncan multiple range test at 5% level.

Results

The Effect of Organic Amendments on Infection of Bean Seedlings by Rhizoctonia

The effect of six amendments incorporated for three different periods on the *Rhizoctonia* infection index of bean seedlings is shown in Table I. In general, the effectiveness of all amendments in reducing infection index improved with

time. Only chitin significantly reduced the infection index during the first experimental period. With the exception of cellulose, all amendments significantly reduced infection index during the second experimental period, chitin again being the most effective. During the third incubation period, significant differences between control and all other treatments were observed, chitin and oat straw being most effective. Nitrogen-supplemented oat straw did not significantly differ from oat straw alone during the first and second experimental periods. However, it was less effective than oat straw during the third experimental period.

TABLE I

Infection index of bean seedlings sown in a naturally infested soil* as affected by various amendments and time

Treatment	Days†		
	0	21	49
Non-amended soil	2.44 *a*‡	2.36 *a*	2.46 *a*
Cellulose	2.13 *a*	1.96 *ab*	2.01 *b*
Cotton	2.50 *a*	1.78 *b*	1.54 *c*
Bean	2.17 *a*	1.70 *b*	1.45 *c*
Oat straw + N	2.64 *a*	1.20 *c*	1.10 *d*
Oat straw	2.49 *a*	1.13 *c*	0.64 *e*
Chitin	0.70 *b*	0.56 *d*	0.50 *e*

*Seeds sown at the beginning of each period and infection indices evaluated after 3 weeks.
†Elapsed time from soil amendment to seeding.
‡Numbers followed by the same letter are not significantly different at the 5% level of probability.

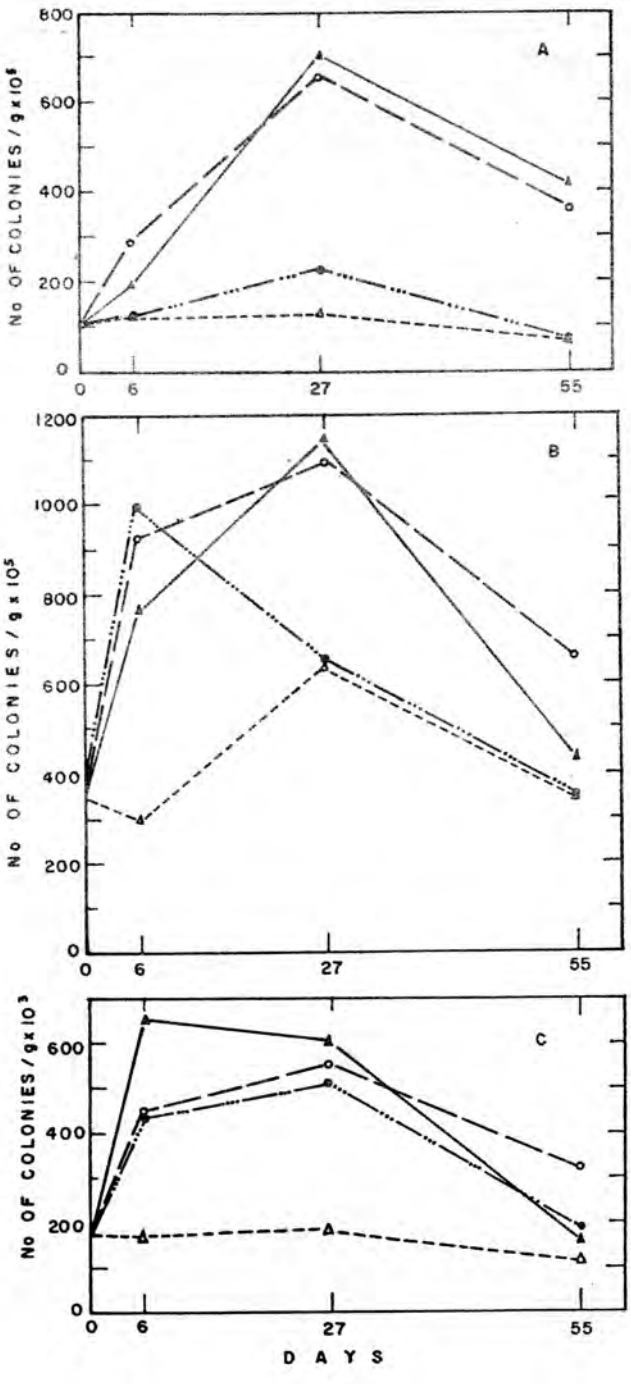
TABLE II

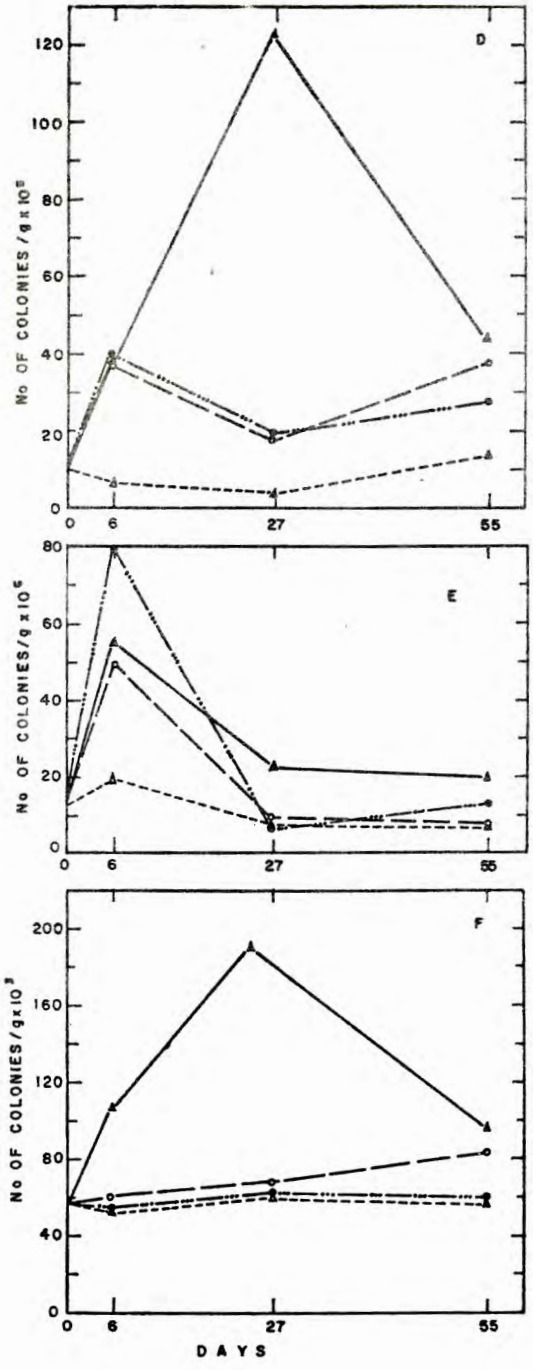
Percentage of bean segments colonized by *Rhizoctonia* as affected by various amendments and time

Treatment	Days*		
	7	28	56
	% colonized		
Non-amended soil	55 *cd*†	57 *a*	50 *a*
Cellulose	52 *d*	30 *c*	37 *b*
Bean	87 *a*	65 *a*	34 *b*
Cotton	64 *bcd*	48 *b*	25 *c*
Oat straw + N	78 *abc*	19 *d*	22 *c*
Oat straw	84 *ab*	16 *d*	13 *d*
Chitin	19 *e*	7 *e*	10 *d*

*Time elapsing from amendment incorporation in soil.
†Numbers followed by the same letter are not significantly different at the 5% level of probability.

FIG. 1. Effect of chitin, nitrogen-supplemented oat straw and cotton amendments on the soil microflora (number of colonies per gram of soil) actinomycetes (A), bacteria (B), and fungi (C); and on the microorganisms antagonistic to *Rhizoctonia*: actinomycetes (D), bacteria (E), and fungi (F). Time in days represents the period elapsing from the incorporation of the amendments into the soil. △ Control, non-amended soil. ▲ Chitin. ○ N-supplemented oat straw. ● Cotton.


A
B
C
No OF COLONIES / g x 10⁵
No OF COLONIES / g x 10⁵
No OF COLONIES / g x 10³
0
200
400
600
800
1000
1200
0
6
27
55
DAYS


D
No OF COLONIES / g x 10⁵
120
100
80
60
40
20
0
0
6
27
55
E
No OF COLONIES / g x 10⁶
80
60
40
20
0
0
6
27
55
F
No OF COLONIES / g x 10³
200
160
120
80
40
0
0
6
27
55
DAYS

The Effect of Organic Amendments on the Relative Abundance of Rhizoctonia in the Soil

Colonization of bean stem segments by *Rhizoctonia* as affected by various amendments was examined 7, 28, and 56 days after soil amendment. The results are shown in Table II. Only chitin decreased *Rhizoctonia* colonization after 7 days, whereas other amendments did not decrease and some even increased the level of colonization by *Rhizoctonia*. During the second incubation period (28 days) *Rhizoctonia* colonization decreased with all the amendments. However, with beans this effect was not statistically significant. In the third period (56 days), colonization with all amendments was significantly lower than that of the control. Their efficiency in decreasing colonization of bean segments by *Rhizoctonia* was very similar to that observed with the infection index method during the same incubation period, chitin and oat straw again being most effective.

The Effect of Some Amendments on the Soil Microflora

Counts of the microorganisms and of organisms antagonistic to *Rhizoctonia* are shown in Fig. 1 (A–F). Fungal counts in the three amendments tested (Fig. 1C) were higher than the control after 6 and 27 days of incubation. Some decrease was observed after 55 days, but the counts remained higher than in the unamended soil. A similar trend was obtained for bacteria (Fig. 1B). However, cotton-amended soil gave higher bacterial counts than the control only during the first period (6 days). The greatest differences (five- to-six-fold) between amended and non-amended soil were observed with actinomycetes in soil amended with chitin and with nitrogen-supplemented oat straw after 27 and 55 days (Fig. 1A).

Numbers of fungi and actinomycetes antagonistic to *Rhizoctonia* were higher than the control mainly in chitin-amended soil, especially after 27 days. In all treatments, a pronounced increase in the number of antagonistic bacteria was observed only during the first period (Fig. 1E).

Discussion

Correlation between the efficiency in decreasing infection index and suppressing of saprophytic activity of *Rhizoctonia* was observed with six soil amendments tested, mainly at the third experimental period, 7 weeks after incorporation. However, correlation between the efficiency of chitin and to some extent of N-supplemented oat straw in controlling the disease and counts of *Rhizoctonia*-antagonistic soil microorganisms was observed throughout the three experimental periods. On the other hand, in cotton amendment, which was less effective in decreasing the infection index, only counts of fungi were relatively high.

It is assumed that organic amendments affect *Rhizoctonia* indirectly by altering the soil microflora (4, 6, 13). In addition, the influence of the amendments on host resistance (14) or on its rhizosphere population (13) could play an important role in the infection index test; this could have resulted in discrepancies from the results obtained with the segment-colonization method and counts of the soil microflora. Also involved in such discrepancies are the different incubations times required for each of the three comparative tests,

as the soil microflora and *Rhizoctonia* population may have changed considerably during the incubation period required for the infection index test.

Among the factors involved in the depressing effect of organic amendments on *Rhizoctonia*, C:N ratio was found to affect their efficiency significantly, best results being obtained with amendments having C:N ratio of 40 to 200, depending on the incubation period (5). These findings are not consistent with chitin efficiency, which has a C:N ratio of approximately 6.4. Chitin is known for its effectiveness in controlling certain plant diseases caused by species of *Fusarium* (11, 12) and it serves as a specific enrichment factor for bacteria and actinomycetes (15) and for phycomycetes (9). It has been suggested that the suppression of chitin containing fungi such as *Fusarium* is the result of the enrichment of mycolytic and (or) toxin-producing microorganisms, especially actinomycetes (12). It has also been suggested (10) that lysis of *Fusarium* and *Glomerella* by chitinase-producing streptomycetes is a secondary process induced by toxic metabolites from these antagonistic microorganisms. According to Potgieter and Alexander (16) cell walls of *R. solani* are not lysed by chitinase. Therefore, the increase in counts of actinomycetes antagonistic to *Rhizoctonia* in the chitin-amended soil suggests the possible production of *Rhizoctonia*-inhibiting compounds in the amended soil as demonstrated by Smith and Ashworth (17) for soil amended with rice hulls and oak sawdust. The effect of chitin as an enrichment factor for phycomycetes (9) may be explained by their relative resistance to antibiotics (2).

Cellulose in the form of pieces of filter paper was somewhat effective in suppressing *Rhizoctonia*. This observation is in agreement with the results obtained by Davey and Papavizas (5) but not with those obtained by Daniels (3). A possible reason for this discrepancy may be the physical form of the cellulose used, a factor which may affect the nature of the developing soil microflora (7).

It was demonstrated recently (18) that a decline in the percentage of colonization by *Rhizoctonia* of plant segments incubated in a naturally infested soil occurred, especially with segments of cereal straw. The relationship between this phenomenon and the efficiency of oat straw as a *Rhizoctonia*-suppressing amendment should be tested. The study of the decolonization process and its mechanism may serve in the future as a useful tool for predicting the efficiency of specific amendments in the biological control of soil-borne plant pathogens.

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