Development of Suppressiveness to Diseases Caused by *Rhizoctonia solani* in Soils Amended with Composted and Noncomposted Manure

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ABSTRACT

Voland, R. P., and Epstein, A. H. 1994. Development of suppressiveness to diseases caused by *Rhizoctonia solani* in soils amended with composted and noncomposted manure. Plant Dis. 78:461-466.

Fresh and composted dairy manure were compared along with other amendments in the greenhouse and in field microplots for their effects on induction of suppression to plant diseases caused by *Rhizoctonia solani*. Damping-off of radish induced by *R. solani* was least severe for seedlings planted in the greenhouse in soil media amended with urea and straw, more severe with manure or compost, and most severe after urea treatment. Radish damping-off was less severe at all inoculum density levels in the urea with straw treatment than in the other amendment treatments. At very high infestation levels (20 and 30 cfu/g *R. solani*), disease severity did not differ among the least effective amendments. The population of *R. solani* did not differ among treatments, despite differences in radish damping-off severity. Manure and compost were more effective than urea alone in inducing suppression of damping-off of radish at low inoculum levels. Neither amendment was effective at high inoculum levels. Bean hypocotyl lesions were least common for seedlings planted in field microplots amended with manure, and more severe in those treated with compost. Composting of animal manure did not significantly enhance the effectiveness of manure for inducing suppression in soil of plant diseases caused by *R. solani*.

Additional keywords: biological control, sustainable agriculture

Concerns about groundwater pollution by nitrates and other agricultural chemicals, and renewed attention to the costs of agricultural inputs (22) have revived interest in efficient management strategies for animal manure. Some farmers have considered composting manure before field application. Numerous reports indicate that manure has significant effects on the soil biota (8,10,11) and on the development of some plant diseases (8). Manure is an important factor in the management of Phytophthora root rot of avocado in Australia (17). Kadir (15) observed that soils from fields with different fertilization practices also differed in levels of suppressiveness to plant disease caused by *Rhizoctonia solani* Kühn. Two soils from fields with a history of manure application had higher levels of disease suppression than did soils from fields not treated with manure. Compost controlled some plant diseases when used as a component of plant growth media or as a soil amendment for field crops (14).

Few reports on the effects of compost application on plant disease involved composted agricultural wastes (3,18). Even though manure is commonly applied to crop fields, Kadir's report on the influence of manure on plant disease is the first in the United States since the 1940s (7). None of the previous research compared the effects of manure and compost on plant disease. The objective of this study was to compare suppression of *R. solani* and *R. solani*-incited damping-off in the presence of organic amendments, including composted and fresh manure.

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Iowa State University Experiment Station Journal No. J-14316, Project 2405.

Accepted for publication 28 January 1994.

MATERIALS AND METHODS

Inoculum. Isolate RHA-3 of *R. solani* (AG-4) was obtained from C. A. Martinson, Iowa State University, and maintained on potato-dextrose agar. Inoculum was produced on green bean pods (31) in glass petri dishes and dried in a forced-air oven at 27–28 C for 48 hr. Dried mats were ground in a Waring blender and stored at ambient conditions in an air-conditioned laboratory. Inoculum for field use was apportioned in the laboratory and transported in capped culture tubes.

Amendments. Soil amendments were selected for a hierarchical arrangement of treatments to facilitate designed comparisons (16); they included fertilizer-grade urea (urea), dairy manure including straw bedding (manure), and composted dairy manure (compost). Urea was selected as a synthetic source of nitrogen containing little organic matter. Manure was selected as an animal-based, organic source of nitrogen, and was obtained from the Iowa State University Dairy Farm for both experiments and for the production of compost. Compost was selected as a stabilized source of animal-based, organic nitrogen (14), and was collected from the same pile for the microplot and greenhouse experiments. The compost pile was formed in November 1985. The manure and straw mixture was mixed with a manure spreader and allowed to accumulate after several passes of the spreader in a mound about 10 m long, 3 m wide, and 2 m high. During the first 2 wk, the internal temperature reached 49 C. The pile was turned for the first time at 2 wk. The compost contained 0.49% N (wet matter basis, April 1987) and 0.54% N (wet matter basis, October 1988), as determined by the Minnesota Valley Testing Laboratories, Inc., Nevada, Iowa.

Microplot experiment. The treatment design for the outdoor microplot experiments was a factorial with four soil amendment levels and two infestation levels. The eight treatments were arranged in six complete blocks. A microplot consisting of 15 or 25 bean seeds was the experimental unit. Each microplot was infested and planted three times.

Microplots consisted of circular areas 60 cm in diameter separated from the surrounding soil by an aluminum barrier extending 25 cm below and 5 cm above ground level. The soil was neither fumigated nor treated with fungicide. The area between microplots was maintained in a mixed turfgrass sod.

The microplots were located about 5 km southwest of Ames, Iowa. The site was sampled for soil fertility, and analyses were conducted at the Iowa State University Soil Testing Laboratory before the first application of amendments. The soil contained 3.5% organic matter, 60.5 ppm phosphorus (very high [designations from the ISU Soil Testing Laboratory]), 110.0 ppm potassium (high), 13.8 ppm zinc (high), and 6.0 ppm sulfur (high). The soil had a pH of 7.65 and a buffer pH of 7.30.

Soil amendments consisted of a nonamended control, 12 g of urea per plot (424 kg/ha, 199 kg N/ha), 1.50 kg of manure per plot (53 Mg/ha wet matter [WM], 93 kg N/ha), and 1.69 kg of compost per plot (60 Mg/ha WM, 93 kg N/ha). Amendments were applied and incorporated on 5 June 1987.

Microplot soil was infested with ground bean pod cultures of *R. solani* (not sifted). Soil was infested at each planting with 7.5 g per microplot (27 g/m^2) on 8 June, 7.5 g (27 g/m^2) on 7 July, and 6.0 g (21 g/m^2) on 28 July. On 8

June 1987, Asgrow cultivar Eagle bush bean (15 seeds per microplot), selected for its susceptibility to two isolates of *R. solani* AG-4, (30) was planted by hand. Seeds were covered with soil after application of inoculum. For subsequent plantings, a wooden template was used to make 25 equally spaced depressions in the soil of each microplot. Each hole held one seed and inoculum. A bean planting cycle refers to one complete sequence of planting, germination, and harvesting. The plant residue remaining from each previous bean planting cycle was used as mulch over the freshly planted seeds. Plots received at least 5 cm of rain or irrigation during the first week after planting. Emergence of bean seedlings in microplots was expressed as the percentage of seeds planted that emerged as symptomless, apparently healthy seedlings. Numbers of bean seedlings with lesions typical of damage by *R. solani* were recorded for the second bean planting cycle. The yield of plant tops above the cotyledonary node was measured for plants in the third bean planting cycle at 6 wk after planting.

Greenhouse experiment. The treatment design for the greenhouse experiments also was a factorial with four soil amendment levels and four infestation levels. The experimental unit was a planting tray with 100 radish seeds. The planting trays were arranged with two replications on each of two greenhouse benches (four replications total). Each planting tray was infested at the first of four planting dates and infested again at the first of three more planting dates.

Soil amendments consisted of urea, manure, compost, and urea + straw (fertilizer-grade urea mixed with straw bedding that was visibly free of manure and urine). The urea amendment was considered the control because nonleguminous crops normally receive nitrogen fertilizer. Soil amendment levels were chosen to provide a nitrogen loading rate (75 ppm) typically used for Iowa crops. The loading rates were 161 ppm urea, 1.60% (w/w of soil medium) manure, 1.39% (w/w) compost, and 161 ppm urea + 2.88 g/kg straw bedding. Suppressiveness to *R. solani* has been associated with large carbon/nitrogen ratios (23). Therefore, the quantity of straw bedding in the urea + straw treatment was chosen to provide the same rate of organic matter application (1% w/w) as in the compost and manure treatments (12). The manure, compost, and urea + straw amendments contained the same carbon/nitrogen ratio, but the organic matter was in different stages of decomposition to isolate the influence of organic matter type on disease suppression.

Inoculum was sifted to obtain inoculum propagules uniform in size (250 μm $\leq d \leq$ 710 μm). Viability of inoculum was assayed less than 1 wk before infestation by counting the number of inoculum pieces from weighed samples that produced hyphae in water agar.

Two forms of replication were used: two soil media × two time blocks. Two soil media were prepared in a soil shredder/mixer as follows: A contained sand, soil, and peat (1:3:1, v/v/v); and B contained sand and soil (1:1, v/v). Neither soil medium was treated with heat, steam, fungicide, or fumigant. Planting trays on one greenhouse bench were considered a time block and were processed on the same day for each procedure. The two time blocks were processed on consecutive days during each phase of the experiment. Each time block contained 16 trays with soil medium A and 16 trays with soil medium B.

The manure with bedding was ground in a Wiley mill to pass a 5-mm mesh sieve. Moisture from the manure caused the pieces of straw bedding contained in the manure amendment to tear and partially disintegrate during the grinding process. Dry straw bedding also was ground in this mill for the urea + straw treatment. The compost was processed in a soil shredder to pass a 13-mm-mesh sieve. The manure, compost, and straw bedding were individually mixed with soil media in a cement mixer, and 2.7 kg were placed in each planting tray (28 × 19 × 2.5 cm). Urea was applied directly to individual planting trays as a 73 mM solution in tap water. All soil media not receiving urea solution were watered with an equivalent volume of tap water. The amended soil media were incubated in a moist condition in the greenhouse for 1 wk before infestation to avoid rehydration effects (26).

Early Scarlet Globe radish seeds (100 per tray) were planted 23 × 13 mm apart and covered 1 cm deep. Six days after planting, the seedlings were rated for damping-off severity according to lesion diameter (d), expressed as a fraction of the hypocotyl circumference (c) (healthy, $d < c/4$, $c/4 \leq d < c$, girdled), and discarded. Damping-off severity for each experimental unit (100 seeds in each tray) was calculated as $D = 100 - [(4H + 3S + 2L + G)/4]$, where D = disease severity index, H = number of symptomless seedlings, S = number of seedlings with small lesions ($d < c/4$), L = number of seedlings with large lesions ($c/4 \leq d < c$), and G = number of girdled seedlings.

The population of *R. solani* was assayed on the seventh day after planting by using 50 dry-autoclaved cultivar

Table 1. Bean seedling emergence in outdoor microplots in the presence of *Rhizoctonia solani* and three soil amendments during 1987

	Bean emergence (%)	
	Not infested	Infested
Soil amendment		
Not amended	52.1[a]	40.0
Urea (424 kg/ha)	46.8	39.9
Compost (59.7 Mg/ha)	43.6	49.6
Manure (53.1 Mg/ha)	59.9	57.8
Planting date		
June 8	46.1[b]	42.8
July 7	47.8	35.0
July 28	57.8	62.7

[a] Each value is the mean of three planting dates for six complete blocks.
[b] Each value is the mean of four amendment levels replicated in six complete blocks.

Table 2. Analysis of variance for emergence of bean seedlings in the presence of *Rhizoctonia solani* and three soil amendments

Source of variation	*df*	Mean square	*F* value	Prob. > *F*
Blocks	5	361.38	1.75	0.1492
Infestation	1	513.78	2.49	0.1238
Soil amendment	3	1,720.72	8.33	0.0003
(Not amended vs. others)[a]	(1)	341.33	1.65	0.2071
(Urea vs. animal-based N)[b]	(1)	2,115.63	10.24	0.0029
(Compost vs. manure)	(1)	2,705.21	13.10	0.0009
Infestation × amendment	3	536.07	2.60	0.0680
[I × (Not amended vs. others)[a]]	(1)	825.94	4.00	0.0534
[I × (Urea vs. animal-based N)[b]]	(1)	478.03	2.31	0.1372
[I × (Compost vs. manure)]	(1)	304.22	1.47	0.2330
Error A	35	206.57		
Time	2	4,909.42	19.91	0.0001
(T1: linear effect of time)	(1)	5,995.57	30.08	0.0001
[T2: Lack of fit (T1)]	(1)	3,823.27	13.01	0.0008
Infestation × time	2	938.11	3.80	0.0264
(I × T1)	(1)	400.17	2.01	0.1643
(I × T2)	(1)	1,476.06	5.02	0.0306
Amendment × time	6	324.02	1.31	0.2605
Infestation × amendment × time	6	260.70	1.06	0.3952
Error (time)	79	246.57		
Error (T1)	40	199.34		
Error (T2)	40	293.79		

[a] Not amended vs. mean of urea, compost, and manure.
[b] Urea vs. mean of compost and manure.

Redpack beet seeds in a flat, nylon-mesh sack as bait. The beet seeds were recovered from the soil medium after 24 hr, surface-sterilized, and implanted in melted agar (Difco) which had been cooled to 50 C and acidified with one drop of 50% (v/v) lactic acid per plate (25 ml of agar). After 3 days, beet seed cultures were examined microscopically for the presence of *R. solani* mycelium.

The procedure of planting, incubating, counting, discarding, and baiting constituted one planting cycle, each of which required 7 days to complete. The group of planting cycles following an infestation is defined as a planting series.

Soil medium was infested with *R. solani* inoculum at 0, 10, 20, or 30 cfu/g at the beginning of the first planting cycle. The sacks of beet seeds were buried in the soil medium of all trays during planting cycles 1–3, but only the beet seeds from the 0 and 20 cfu/g treatments of the second time block were saved for observation. The first planting series consisted of four planting cycles.

The soil media were reinfested prior to the fifth planting cycle. The same weights of inoculum were used as at the beginning of the first planting series, but inoculum viability in water agar had decreased from 8.5×10^3 cfu/g for the first infestation to 4.7×10^3 cfu/g for the second infestation. The 30 cfu/g treatments were not repeated. The beet seed baiting bioassay was used to detect the presence of *R. solani* after all three planting cycles in this second planting series. The sacks of beet seeds were buried in the soil medium of all trays, but only the beet seeds from the 0 and 20 cfu/g treatments of the second time block were saved for observation.

Isolations to confirm identification and pathogenicity were taken from 28 beet seeds that were determined to have been colonized by *R. solani* during the final baiting. Identifications were based on Parmeter and Whitney (24). Pathogenicity determinations were based on ability to cause visible seedling necrosis by forming an infection cushion on 3-day-old radish seedlings.

Statistical analyses. All statistical analyses were performed using SAS (27) procedures for analysis of variance (ANOVA and GLM). Treatment effects were determined using designed comparisons expressed as orthogonal contrasts (16) in keeping with the hierarchical arrangement of amendments. Compost and manure were considered animal-based sources of nitrogen, and urea, a synthetic source of nitrogen. Probability values presented in the text are for these contrasts or factors in the analysis of variance. The disease and Rhizoctonia population ratings were considered repeated measures. Univariate analyses of variance were performed for these repeated measures. Beet seed data from noninfested treatments were excluded from statistical analysis because the variances in these treatments were much smaller than in the infested treatments. The two planting series in the greenhouse experiment were analyzed separately.

RESULTS

Microplots. More beans emerged from manure-amended soil than from soil amended with urea (Table 1) or compost ($P < 0.01$) (Tables 1 and 2). Differences in emergence resulting from infestation did not vary significantly between urea and the mean of compost and manure ($P = 0.14$) (Table 2), nor between compost and manure ($P = 0.23$) (Table 2). Damping-off was less severe in all microplots after the first two planting cycles (Table 1). Emergence increased with time ($P < 0.01$) (Tables 1 and 2), and infestation no longer reduced emergence ($P = 0.03$) (Tables 1 and 2) during the third planting cycle.

Bean plants from manure-amended soil had fewer visible lesions ($P < 0.01$) (Tables 3 and 4) and produced more fresh matter ($P < 0.01$) (Tables 3 and 5) than plants from compost-amended soil. Manure increased the yield of fresh matter over the yield in the nonamended soil without increasing visible disease (Table 3).

Greenhouse experiment. Disease was less severe ($P < 0.01$) on radish in the urea + straw treatment than in urea, manure, and compost treatments during the first three planting cycles (Fig. 1A–C). The respective means over the first four planting cycles for each amendment reflect this trend (Fig. 1E). Disease severity declined faster ($P < 0.01$) in the urea + straw treatment than in other treatments, so that disease severity for the urea + straw treatment during each of planting cycles 1–3 (Fig. 1A–C) was similar to disease severity for the three other treatments during planting cycles 2–4 (Fig. 1B–D). Disease severity was greater ($P < 0.01$) for the urea treatment than for the manure and com-

Table 3. Diseased bean seedlings from outdoor microplots in the presence of *Rhizoctonia solani* and three soil amendments

Soil amendment	Diseased plants (%)[a]	Yield of plant tops[b] (g fresh matter/ microplot)
Not amended	9.0[c]	135.2[d]
Urea	15.3	218.1
Compost	16.7	223.2
Manure	8.0	284.4

[a] Planted on 7 July 1987; diseased seedlings counted at 10 days.
[b] Planted on 28 July 1987; fresh matter measured at 57 days.
[c] Each value is the mean percent diseased plants arising from 300 seeds distributed among six infested and six noninfested microplots (0.3 m^2 each).
[d] Each value is the mean fresh matter above the cotyledonary node arising from 300 seeds distributed among six infested and six noninfested microplots (0.3 m^2 each).

Table 4. Analysis of variance for number of diseased bean seedlings in the presence of *Rhizoctonia solani* and three soil amendments[a]

Source of variation	*df*	Mean square	*F* value	Prob. > *F*
Blocks	5	124.53	2.65	0.0392
Infestation	1	75.00	1.59	0.2157
Soil amendment	3	230.56	4.90	0.0060
(Not amended vs. others)[b]	(1)	169.00	3.59	0.0664
(Urea vs. animal-based N)[c]	(1)	72.00	1.53	0.2243
(Compost vs. manure)	(1)	450.67	9.58	0.0039
Infestation × amendment	3	69.67	1.48	0.2368
Error	35	47.05		

[a] Second planting 1987: planted on July 7; diseased seedlings counted at 10 days.
[b] Not amended vs. mean of urea, compost, and manure.
[c] Urea vs. mean of compost and manure.

Table 5. Analysis of variance for yield of plant tops from beans grown in the presence of *Rhizoctonia solani* and three amendments[a]

Source of variation	*df*	Mean square	*F* value	Prob. > *F*
Blocks	5	11,282	3.96	0.0060
Infestation	1	7,179	2.52	0.1214
Soil amendment	3	45,078	15.80	0.0001
(Not amended vs. others)[b]	(1)	22,448	7.87	0.0082
(Urea vs. animal-based N)[c]	(1)	10,224	3.58	0.0668
(Compost vs. manure)	(1)	102,560	35.96	0.0001
Infestation × amendment	3	1,374	0.48	0.6983
Error	35	2,852		

[a] Third planting 1987: planted on July 28, fresh matter measured at 57 days.
[b] Not amended vs. mean of urea, compost, and manure.
[c] Urea vs. mean of compost and manure.

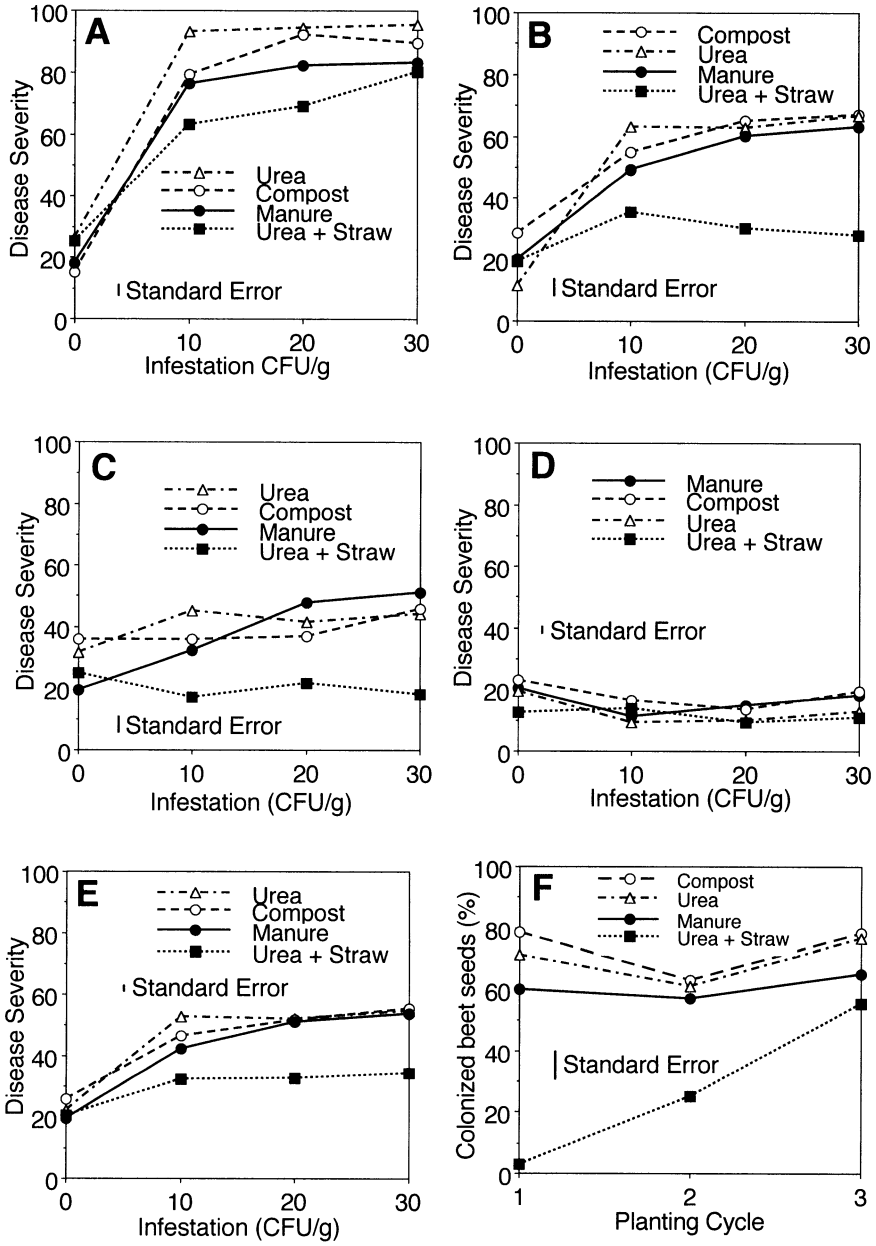

Fig. 1. Incidence of Rhizoctonia disease of radish **(A–E)** and population of *Rhizoctonia solani* **(F)** resulting from the first infestation in soil receiving four amendments. A–D represent planting cycles 1–4, respectively (four experimental units, 400 radish seeds total at each point). E represents the average of planting cycles 1–4 (16 experimental units, 1,600 seeds total at each point). F presents the population density of *R. solani* after each of planting cycles 1–3 (two experimental units, 100 seeds total at each point).

post treatments at all infestation levels during planting cycle 1 (Fig. 1A). It remained greater than in the manure and compost treatments at the 10-cfu/g infestation level during planting cycles 2 and 3 (Fig. 1B and C). This trend for the urea, manure, and compost treatments at the 10-cfu/g infestation level was reflected in the respective means over planting cycles 1–4 (Fig. 1E). The manure and compost treatments did not differ in disease severity during planting cycle 1 ($P = 0.23$) (Fig. 1A), planting cycle 2 ($P = 0.16$) (Fig. 1B), planting cycle 3 ($P = 0.83$) (Fig. 1C), planting cycle 4 ($P = 0.34$) (Fig. 1D), and in the average of planting cycles 1–4 ($P = 0.34$) (Fig. 1E).

The population of *R. solani* measured by colonized beet seeds did not differ ($P = 0.25$) among amendments (Fig. 1F). The population increased over time ($P = 0.06$), but the relative rankings among amendment treatments did not change significantly ($P = 0.23$) over time.

Radish disease after the second infestation was less severe for the urea + straw treatment than for the other amendments during planting cycle 5 ($P < 0.01$) (Fig. 2A) but not during planting cycle 6 ($P = 0.76$) (Fig. 2B). Disease severity during planting cycle 7 did not differ significantly among the four amendments ($P = 0.76$) (Fig. 2C). Disease severity was greater ($P = 0.03$) for the compost treatment than for manure in planting cycle 5 (Fig. 2A). *R. solani* was isolated from symptomatic radish seedlings harvested during cycle 5. The population of *R. solani* increased more ($P = 0.05$) over time in the urea + straw treatment than in the other amendments (Fig. 2D). Twenty-five isolates from the final set of beet seed baits were confirmed to be *R. solani,* were pathogenic, and had similar colony morphology.

DISCUSSION

Greater suppressiveness to damping-off caused by Rhizoctonia in soil amended with manure or compost than with nitrogen sources lacking organic matter is consistent with reports for a wide range of pathogens (1-4,7,15,21, 28,29). Manure application was associated with increased disease incidence in two reports (5,20). Böning (5) speculated, however, that increased disease incidence might be associated with an unfavorable soil pH or an imbalance in relative nutrient levels following manure application. Moubasher and Abdel-Hafez (20) applied amendments at high levels, and Chung et al (6) observed that excessive cellulose application negated suppression of damping-off caused by *R. solani* in a suppressive potting medium. Lower application rates were used in our experiments. The response by seedlings to urea + straw amendment is consistent with a decrease in disease caused by small additions of cellulose (6) and oat straw (9).

All soil media became suppressive, as indicated by the smaller infestation effect during the second planting series of the greenhouse experiment and the third planting cycle of the microplot experiment. All soil media became suppressive eventually, but they differed in the rates at which suppressiveness developed. Kadir (15) also observed this phenomenon. However, Henis et al (13) reported that not all soils became suppressive.

Suppression of Rhizoctonia disease on radish was overcome in this experiment by high levels of infestation (20–30 cfu/g). An inoculum density of 21.5 cfu/g was required to overcome one suppressive soil (32).

Suppression of disease caused by *Rhizoctonia* spp. without pathogen suppression is consistent with observations reported by Rouse and Baker (25). They allowed soil to recover after rehydration and observed that populations of *R. solani* stabilized and continued to survive after 8–9 days. Other workers have used air-dry soil in various studies involving *R. solani,* but ecological relationships in rehydrating soil are unstable (26). Martinson (19) observed much more frequent isolation of species of *Penicillium, Aspergillus, Trichoderma,* and fungi in the Mucorales from freshly rehydrated soil than from soil kept moist for several weeks prior to use. Intense competition from the combined soil biota in the rehy-

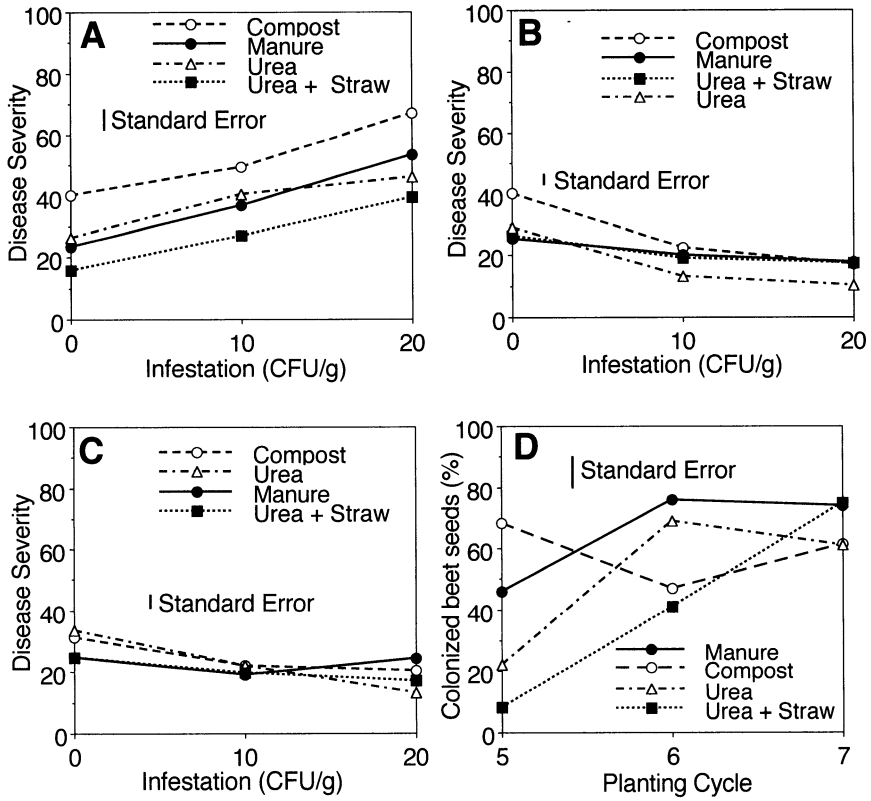

Fig. 2. Incidence of Rhizoctonia disease of radish **(A–C)** and population of *Rhizoctonia solani* **(D)** resulting from the second infestation in soil receiving four amendments. A–C represent planting cycles 5–7, respectively (four experimental units, 400 radish seeds total at each point). D represents the population density of *R. solani* after each of planting cycles 5–7 (two experimental units, 100 seeds total at each point).

drating soil used by previous workers may have impeded establishment of *R. solani*.

This research demonstrated that disease suppression can be induced in soil by organic amendments applied at conventional rates; however, one must keep in mind that these experiments included one source of manure and one source of compost. Further research is necessary to determine mechanisms that control induction of plant disease suppression by manure and compost. For example, large amounts of cellulose added to some composts may interfere with disease suppression (6).

Based on these observations, the quality of organic matter was as important as the quantity for inducing suppression to *R. solani* in soil, even though nitrogen levels were constant. Although organic amendments hastened the development of disease suppression in soil media compared to nitrogen sources deficient in organic matter, pathogen suppression was not consistently associated with suppression of disease. It appears that composting may not necessarily improve the value of manure as an amendment for inducing disease suppression in soil.

ACKNOWLEDGMENTS

Research was supported in part by the Iowa Agricultural Experiment Station, Ames, and by the Center for Rural Affairs, Hartington, Nebraska.

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