Root Diseases, Populations of Soil Fungi, and Yield Decline in Continuous Double-Crop Corn

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ABSTRACT

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Grain yield in the spring crop of continuous double-cropped irrigated corn (*Zea mays*) declined from 11.3 to 7.2 t/ha during 1978–1983. From 1985 to 1987, tillage, soil, and fertility treatments were applied to determine the causes of yield decline. Soil fumigation with DD-MENCS in February reduced root disease, eliminated symptoms of decline, increased yield, and reduced populations of basidiomycetes, *Phoma* spp., *Fusarium* spp., and total fungi in soil. Fertility and tillage practices, a winter crop of rye (*Secale cereale*), and soil treatments with fenamiphos and metalaxyl did not prevent decline. *Phoma terrestris, Pythium arrhenomanes*, and *Pythium* spp. were isolated most frequently from lesions on roots of 11- to 15-wk-old corn. In greenhouse experiments with soil from the field, heat and benomyl treatments reduced chlorosis and increased plant weight in corn. In pathogenicity experiments with corn, *Pythium arrhenomanes, P. aphanidermatum*, and *P. irregulare* were moderately virulent and *Phoma terrestris* and *P. americana* were slightly virulent.

Much of the southeastern United States has an average frost-free growing season of 240 or more days, and there is the potential for growing two crops of corn (*Zea mays* L.) per year in a double-crop system for grain or ensilage (27). Corn was grown as a continuous double-crop from 1978 through 1984, except that grain sorghum (*Sorghum bicolor* (L.) Moench) was substituted for the second crop of corn in 1982. Yield of grain in the first crop each year declined from approximately 11.3 to 7.2 t/ha during that time, and frequently plants were stunted or chlorotic. Tillage and fertility treatments had little effect on the decline in yield. Experiments with nematicides gave variable results and did not control decline consistently. Several fungi were isolated and identified from lesions on roots during 1978–1984, including a sterile white basidiomycete that was first identified in Georgia in that field (22). Root disease severity was reduced in soil turned with a moldboard plow compared with disking, chiseling, or in-row subsoiling, but differences were usually not significant.

In June 1984, *Pythium arrhenomanes* Drechs., *Pythium* spp., and *Phoma terrestris* Hans. (= *Pyrenochaeta terrestris* (Hans.) Gorenz, Walker, & Larson) were isolated more frequently than other fungi from lesions on corn roots 20–60 cm deep in soil. Seedling decline in sweet corn has been documented in the Georgia coastal plain (25). Root diseases and nematodes may reduce growth in corn (20,24), but yield decline in monocrop field corn has not been reported.

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This paper reports a series of investigations undertaken to identify the cause of yield decline. Specifically, the 1984 field experimental area where the problem occurred was subdivided with treatments to examine a broad spectrum of possible problems—fertility, tillage, nematodes, and soilborne pathogenic fungi—while continuing double-crop corn management. In this phase of the research, root growth was examined, soil compaction was documented, nematode populations were assayed, and plant and soil nutrient status was tested. This paper reports the relationship of root diseases and populations of soil fungi to yield decline in corn.

MATERIALS AND METHODS

The experiment was conducted in one quadrant of the circle of a 6-ha center-pivot overhead sprinkler irrigation system on Bonifay sand (loamy, siliceous, thermic, grossarenic Plinthic Paleudult). Corn was grown as a double-crop (11 crops of field corn, two second crops of sweet corn, and one second crop of grain sorghum) during 1978–1984, with most fertilizers and pesticides applied through the irrigation water (chemigation). Herbicides and N, K, and S were applied through the irrigation water in this test during 1985–1987. Corn cultivars Pioneer 3320 and X304C (tropical corn) were planted 12 March and 18 July 1985, respectively, and Pioneer 3058 (ensilage corn) was planted 24 March 1986 and 26 March 1987. The first and second crops were harvested in July and November, respectively.

Experimental design and treatments. A split-split-plot design with randomized complete blocks was used. In 1985, whole plots were tillage treatments (no-till or in-row subsoiling 30–45 cm deep) and subplots were fertility levels (low [14] and high [15]). Sub-subplots in the first crop were rye (*Secale cereale* L.) as winter cover (planted 3 December 1984); fumigation with DD-MENCS (20% methyl isothiocyanate + 80% chlorinated C_3 hydrocarbons), 618 g a.i./L, 327 L/ha, 14 January 1985; and a winter fallow control. For fumigation, the soil was turned 20–25 cm deep with a moldboard plow 11 December 1984, to bury corn debris, and the fumigant was injected 25 cm deep with eight chisels spaced 25 cm apart on a bed 1.8 m wide.

In a separate test adjacent to the first experiment, and at the outer edge of the center pivot, four more replications of plots with rye, soil fumigation with DD-MENCS, and winter fallow were established in a randomized complete block design. That area was in no-till corn in 1984 but otherwise was treated the same as the corn with the low fertility level in the first test area in 1985. Each plot was 5.4 m wide and 23 m long.

Whole plots and subplots in both tests were reestablished for the second crop. The subplots were the residual effects from the first crop, except that the nematicide fenamiphos was applied to the rye treatment sub-subplots. Granules of fenamiphos (6.72 kg a.i./ha) were broadcast onto the soil surface, and 1.2 cm of water was applied through the irrigation system immediately.

In the second year (1986), whole plots were rye as winter cover (planted in mid-November 1985) or winter fallow (disk-harrowed in mid-November 1985) instead of tillage treatments, and the entire experimental area was disk-harrowed and turned 20–25 cm deep with a moldboard plow in mid-March 1986. Subplots were the same fertility treatments in the same location as in 1986. In sub-subplots, the residual effect of DD-MENCS was maintained in the same plots. Four additional treatments were randomized around those subplots: fumigation with DD-MENCS (327 L/ha injected 22 January), fenamiphos (6.72 kg a.i./ha broadcast 19 March), metalaxyl (2.24 kg a.i./ha broadcast 19 March), and the control. A second crop was not grown that year.

In the third year (1987), whole plots (rye was planted and fallow plots were disk-harrowed 1 December 1986) and subplots were the same as in the second year. Sub-subplots were the same except an additional fumigation with DD-MENCS (327 L/ha 20 January 1987) was substituted in the sub-subplots treated with metalaxyl in 1986. The soil was disk-harrowed in mid-March, when the rye was 20–30 cm high, and was turned 20–25 cm deep with a moldboard plow; the fenamiphos treatment was applied 25 March.

Soil assays for fungi. Soil samples were collected with a probe (2.5 cm diameter, 10 per plot) in the second and fifth rows, 0–15 and 15–30 cm deep 8 March 1985 and 0–15 cm deep 21 August 1985, 14 January and 17 April 1986, and 31 July 1987. Soil was assayed on tannic acid-benomyl agar (TABA) for *Rhizoctonia solani* Kühn and other basidiomycetes with a multiple-pellet soil sampler (4), modified pentachloronitrobenzene (PCNB) agar for *Fusarium* spp. (13), pimaricin-ampicillin-rifampicin-PCNB (PARP) agar for *Pythium* spp. (9), and Ohio Agricultural Experiment Station (OAES) agar (except in 1986) containing streptomycin sulfate, chloramphenicol, and oxbile for numerous saprophytic fungi and total populations of fungi (26).

Isolation of fungi. Each year fungi were cultured and identified from lesions on diseased roots on 6- to 17-wk-old corn plants. Roots were washed and 5- to 10-mm sections from the edge of lesions were either surface-disinfested 15–60 sec in 70% ETOH or washed 0.5–2.0 hr in running tap water (20–25 C); blotted dry on sterile filter paper; and incubated on TABA, PARP, or water agar. Hyphal tips were transferred to potato-dextrose agar (PDA), and fungi were identified.

Root disease severity and stalk rot. Each year 10 plants per plot were dug 5–7 wk after planting (V4–V6 growth stages) (18). A plant was selected at random in the center of the second and fifth rows, then every other plant was collected, for a total of five plants. The root systems were washed and evaluated for discoloration and decay on a 1–5 scale, where 1 (none) = <2%, 2 (slight) = 2–10%, 3 (moderate) = 11–50%, 4 (severe) = >50% discoloration and decay, and 5 = dead plant. In both crops in 1985, root systems on an additional 10 plants per plot were collected in a similar manner and rated for disease severity in the R5 growth stage (just before harvest). The total number of crown and brace roots and the number of roots with lesions and dead apices 0–5, 5–10, and >10 cm below the soil surface were recorded. The number of stalks rotted per 25 stalks per plot was determined empirically at harvest in 1986 and 1987 by squeezing the second internode above the ground by hand.

Growth and yield. The total number of plants at the V3 growth stage and the number of chlorotic, stunted, and dead plants from the V3 to the V7 growth stages were counted weekly or biweekly. Average height was measured every 2–4 wk from the V3 to the R1 growth stages, and the average daily gain in height was calculated for each plot. Corn was harvested for ensilage (yields adjusted to 30% dry matter) and for grain (yields adjusted to 15.5% moisture). Because the cultivars used were developed for ensilage, the grain yields are not included here.

Greenhouse experiments. Soil (150 L) was collected from the field to a depth of 15–20 cm in early February 1985. The soil (containing corn debris) was mixed in 15- to 20-L batches in a concrete mixer. Then 15 L of soil was heated in an oven until the soil temperature reached 68 C for 30 min. Ammonium nitrate was mixed with half the heat-treated soil (161 mg/kg of soil), and the remainder was not fertilized. For each treatment in each soil, 1.4 L of soil was placed into each of four 2-L plastic cans containing drainage holes, three seeds of the corn cultivar Pioneer 3320 were planted in each can, and the seed was covered 3–4 cm deep with an additional 400 ml of soil. Treatments with nonheated soil were mixed as follows: metalaxyl (0.5 mg a.i./kg of soil), metalaxyl + NH_4NO_3, pencycuron (4.3 mg a.i./kg of soil), pencycuron + NH_4NO_3, NH_4NO_3, and nonamended. Cans were placed on a greenhouse bench in a randomized complete block design with four replications. One soil sample from each mixture was collected at planting and assayed on selective media as described for field experiments.

In a second experiment, soil was collected from the same area 14 January 1986 and mixed before treatments were

applied. A split-plot experiment with a randomized complete block design with four replications was used. Whole plots were metalaxyl (0.5 mg a.i./kg of soil), pencycuron (4.3 mg a.i./kg of soil), carbofuran (4.3 mg a.i./kg of soil), fenamiphos (0.5 mg a.i./kg of soil), benomyl (13.4 mg a.i./kg of soil), a heat-treated control (64 C for 30 min), and a control not treated with heat. Subplots were one pot each at rates of NH_4NO_3 of 0, 80, and 160 mg of nitrogen per kilogram of soil. Nitrogen was added in solution 22, 30, and 36 days after planting so that treatments totaled 160, 320, and 480 mg of nitrogen per kilogram of soil, respectively. One soil sample from each treatment was collected after mixing and assayed for populations of *Pythium* spp. and basidiomycetes.

In both experiments, growth characteristics were observed biweekly. Foliage was harvested and weighed, and plants were dug and roots evaluated for disease severity after 5–6 wk (V5–V7 growth stages).

More than 270 isolates of fungi from corn roots, soil, and roots of other crops, including 22 isolates of *P. irregulare* Buisman, five isolates of *P. aphanidermatum*, and one isolate of *P. arrhenomanes*, were tested for pathogenicity to corn roots from 1976 to 1984 (10,18,20,22). Fungi were grown on 3% cornmeal-sand and mixed with soil treated by heat (65–75 C) or with methyl-bromide, 1:100 or 1:500 (v/v). Corn was planted in pots or trays and grown 2–4 wk; the roots were then dug, washed, and rated for root disease severity. In two tests, the oven-dry weight of the roots was recorded.

Data analysis. Data were analyzed by least squares regression with general linear models and stepwise multiple regression (19). Square-root transformations were used with data containing zeros and a range of small numbers, logarithmic transformations with data covering a wide range of values, and arcsin transformations with percentage data covering a wide range of values. Data shown in tables are actual values before transformation.

RESULTS

First crop of 1985. There were no visible differences in plant growth among treatments 4 wk after planting and no significant differences in emergence or plant stand 5 wk after planting. Symptoms of decline were evident by 6 wk, and there were significantly more stunted (<15 cm) yellow plants in nonfumigated than in fumigated plots, and in no-till than in subsoil-plant plots. There were more yellow plants in the low than in the high fertility plots, but there was no difference in the number of stunted plants between fertility treatments (Table 1). There were no dead plants in the fumigated plots and only 2% in nonfumigated plots. Treatments did not influence the number of dead plants significantly.

There was interaction among tillage, fertility, and soil treatments. In nonfumigated soil, the lowest numbers of yellow plants were in plots that were subsoil-planted with high fertility and the highest numbers were in no-till plots with low fertility. In contrast, there were very few yellow plants in any fumigated plot regardless of tillage or fertility treatment. Plant growth (centimeters per day) was similar in all fumigated plots, but high fertility increased growth in nonfumigated plants. There was no interaction among treatments in root disease severity.

Root disease severity was slight to moderate (8% of the plants with >10% discoloration and decay) 7 wk after planting and moderate to severe (67% of the plants with >10% discoloration and decay) 15 wk after planting. Compared with rye and fallow treatments, fumigation significantly reduced the percentage of plants with moderate root rot and the percentage of crown and brace roots with lesions, but tillage and fertility treatments did not affect root disease severity. Mean daily growth and yield of silage were increased greatly by soil fumigation and high fertility but were not affected by tillage (Table 1). There were no significant interactions among treatments. In the adjacent experiment on the edge of the center pivot following no-till corn, the yields were 18.9, 45.6, and 45.1 t/ha in the fallow, rye, and soil fumigation

Table 1. Marginal means for root disease severity, average daily growth, and silage yield in the first crop of corn, 1985

Treatment	Plants/9.1 m of row at 6 wk		Plants with root disease[w] (%)		Crown and brace roots with lesions at 7 wk (%)	Growth[x] (cm/day)	Silage yield[y] (t/ha)
	Stunted (<15 cm)	Yellow	7 wk	15 wk			
Tillage							
No-till	1.4 a[z]	18.1 a	7	65	17	3.9	40.6
Subsoil-plant	0.5 b	7.9 b	9	68	17	4.0	43.4
Fertility							
High	0.8	7.0 b	8	69	17	4.3 a	46.9 a
Low	1.0	18.9 a	7	64	17	3.6 b	37.1 b
Soil treatment							
DD-MENCS	0.1 b	4.5 b	1 b	24 b	2 b	4.7 a	68.0 a
Rye	1.4 a	16.4 a	11 a	91 a	28 a	3.7 b	32.2 b
Fallow (control)	1.3 a	18.2 a	11 a	84 a	23 a	3.5 b	30.6 b

[w] Plants with root systems having more than 10% visible discoloration and decay.
[x] Average daily gain in height 6 through 12 wk after planting.
[y] Adjusted to 70% moisture.
[z] Means of tillage, fertility, or soil treatments followed by different letters are different according to the Waller-Duncan *k*-ratio *t* test, $P = 0.05$. Absence of letters indicates no significant differences.

Table 2. Populations of fungi in soil with different treatments before planting corn 8 March 1985[x]

Treatment	BNR[y]	*Rhizoctonia zeae*	*Pythium* spp.	*Fusarium* spp.	*Trichoderma* spp.	*Penicillium* spp. + *Paecilomyces* spp.	*Phoma* spp.	*Zygorhynchus* spp.	Total fungi
Tillage									
No-till	3.2	3.8	1.9	480	6,870	21,450	1,840	1,050	104,300
Subsoil-plant	1.4	4.9	3.6	600	13,060	35,030	2,360	2,150	123,000
Fertility									
High	1.0	4.8	3.5 a[z]	570	10,490	29,630	2,250	1,630	121,100
Low	3.6	4.0	2.0 b	510	9,440	26,850	1,940	1,570	106,200
Soil treatment									
Rye	2.4 ab	5.7 a	3.1	850 a	15,340 a	49,000 a	4,250 a	3,700 a	168,700 a
DD-MENCS	1.0 b	1.0 b	3.5	320 c	6,370 b	13,530 b	710 c	390 b	62,500 c
Fallow (control)	3.6 a	6.4 a	1.6	460 b	8,180 b	22,180 ab	1,340 b	710 b	109,700 b

[x] Basidiomycetes in colony-forming units per 100 g of oven-dried soil, other fungi in cfu/g of oven-dried soil.
[y] Binucleate *Rhizoctonia*-like fungi CAG-2 and CAG-4.
[z] Numbers within tillage, fertility, or soil treatments followed by the same letter are not different according to the Waller-Duncan *k*-ratio *t* test, $P = 0.05$. Absence of letters indicates no significant differences.

treatments, respectively.

In the first crop, the fungi isolated most commonly from lesions on roots of 7-wk-old plants in nonfumigated plants were *Trichoderma* spp. (43%), *Fusarium oxysporum* Schlecht. (14%), a sterile white basidiomycete (8%), and binucleate *Rhizoctonia*-like fungi (6%). From lesions on 15-wk-old plants, *Phoma terrestris* (18%), *Pythium* spp. (primarily *P. arrhenomanes*) (10%), *Trichoderma* spp. (9%), and *Rhizoctonia solani* AG-4 (8%) were isolated most frequently.

In soil samples collected 8 March 1985, tillage treatments did not affect populations of soil fungi. Populations of *Pythium* spp., *Aspergillus* spp., and *Penicillium* spp. + *Paecilomyces* spp. were greater in the high than in the low fertility treatment, but there were no differences in populations of total fungi. Soil fumigation reduced populations of total fungi, *Rhizoctonia zeae* Voorhees, and binucleate *Rhizoctonia*-like fungi, compared with fallow or rye (Table 2). Compared with fallow, rye increased populations of *Fusarium* spp., *F. solani* (Mart.) Sacc., *Trichoderma* spp., *Phoma* spp., *Zygorhynchus* spp., *Helminthosporium* spp., and total fungi. There were few differences between populations of fungi in soil 0–15 cm deep and those 15–30 cm deep. *F. solani* and the binucleate *Rhizoctonia*-like fungi CAG-2 and CAG-4 were predominantly in the 0–15 cm vs. the 15–30 cm depth, 120 vs. 70 cfu/g and 3.6 vs. 0.3 cfu/100 g of oven-dried soil, respectively. There were few significant tillage × fertility × soil treatment interactions. Populations of the sterile white basidiomycete and *R. solani* AG-4 were very low (av. 0.1 and 0.3 cfu/100 g of oven-dried soil, respectively) and not different among treatments; *R. solani* AG-2 type 2 was not detected.

Corn in the second test in an adjacent area following no-till corn responded to soil treatments in a manner similar to that in the first test. Compared with the control and rye treatments, soil fumigation increased growth (final height 185, 205, and 246 cm in control, rye, and DD-MENCS treatments, respectively) and reduced root disease severity at both 7 and 15 wk after planting,. Populations of soil fungi were similar in rye and fallow treatments, but compared with these treatments, soil fumigation reduced populations of *Fusarium* spp., *Trichoderma* spp., *Penicillium* spp. + *Paecilomyces* spp., *Phoma* spp., *Zygorhynchus* spp., total phycomycetes, and total fungi.

Second crop of 1985. Plant populations were greater following fumigation than following fallow or fenamiphos treatments 3 wk after planting (57,340, 48,080, and 50,450 plants per hectare, respectively). However, plants were taller and greener in plots treated with fenamiphos than in fumigated or fallow plots. Plants in subsoiled plots were taller than those in no-till plots, but there were no differences among fertility treatments.

The percentage of the crown and brace roots with lesions was significantly less in fumigated plots than in other plots, but root disease severity was minimal (Table 3). There were no differences in the percentage of crown and brace roots with lesions among tillage or fertility treatments, but more plants had moderate root rot in the no-till than in the subsoil-plant tillage treatment. In contrast, 15-wk-old plants had moderate to severe symptoms of root diseases in all plots of one replication examined 31 October. Roots were mostly brown to black, with occasional pink discoloration. There were more stubby roots 10–15 cm below the soil surface in no-till than in subsoiled plots. The average daily growth was greater in in-row subsoiled than in no-till plots and in plots treated with fenamiphos than in fumigated plots, but both soil treatments were superior to fallow. Yield of silage was greater with subsoiling than with no-till, with high fertility than with low, and with fenamiphos and fumigation than with fallow (Table 3).

The fungi isolated most frequently from lesions on roots of 6-wk-old plants were *Pythium graminicola* Subramanian (20%) and *F. moniliforme* J. Sheld. (4%); no other fungus was isolated from more than 1% of the lesions. In 15-wk-old plants, the fungi isolated most frequently with PARP agar were *Pythium* spp. (91%, including 62% *P. arrhenomanes* and 15% *P. irregulare*). With TABA, the fungi isolated most frequently were *Fusarium* spp. (53%), *Phoma terrestris*

Table 3. Root disease severity, average daily growth, and silage yield in the second crop of corn, 1985

Treatment	Plants with root disease at 6 wk[v] (%)	Crown and brace roots with lesions at 6 wk (%)	Growth[w] (cm/day)	Silage yield[x] (t/ha)
Tillage				
No-till	6 a	11	2.7 b[y]	19.0 b
Subsoil-plant	2 b	7	4.0 a	35.0 a
Fertility				
High	4	10	3.4	31.6 a
Low	4	8	3.3	31.4 b
Soil treatment[z]				
DD-MENCS	1	2 b	3.4 b	30.3 a
Fenamiphos	5	12 a	3.8 a	29.9 a
Fallow (control)	6	13 a	2.8 c	19.6 b

[v] Plants with root systems having more than 10% visible discoloration and decay.
[w] Average daily gain in height 3 through 10 wk after planting.
[x] Adjusted to 70% moisture.
[y] Means of tillage, fertility, or soil treatments followed by different letters are different according to the Waller-Duncan *k*-ratio *t* test, $P = 0.05$. Absence of letters indicates no significant differences.
[z] The sub-subplots in rye before the first crop were treated with fenamiphos 19 July one day after planting. The DD-MENCS and the control were the residual effects from the first crop.

Table 4. Root disease severity, stalk rot, growth, and silage yield in corn, 1986

Treatment	Plants with root disease at 5 wk[w] (%)	Crown and brace roots with lesions (%)	Stalk rot (%)	Growth[x] (cm/day)	Silage yield[y] (t/ha)
Winter crop					
Rye	6 a[z]	10	9	4.2	48.4
Fallow	3 b	11	5	4.2	49.8
Fertility					
High	4	9	13 a	4.3 a	49.8
Low	4	12	1 b	4.1 b	48.4
Soil treatment					
DD-MENCS, 1986	0 b	4 b	3 b	4.2 ab	58.3 a
DD-MENCS, 1985	3 ab	10 a	1 b	4.2 ab	48.7 b
Fenamiphos	7 a	13 a	9 a	4.1 b	45.1 b
Metalaxyl	4 a	12 a	8 a	4.3 a	45.3 b
Control	7 a	14 a	14 a	4.3 a	48.0 b

[w] Plants with root systems having more than 10% visible discoloration and decay.
[x] Average daily gain in height 3 through 12 wk after planting.
[y] Adjusted to 70% moisture.
[z] Means of tillage, fertility, or soil treatments followed by different letters are different according to the Waller-Duncan *k*-ratio *t* test, $P = 0.05$. Absence of letters indicates no significant differences.

(27%), *Phoma americana* Morgan-Jones & White (24%), and binucleate *Rhizoctonia*-like fungi (7%). Because more than one fungus was isolated from some lesions on TABA, numbers do not total 100%.

Soil samples were collected 14 January 1986 to assay for residual effects of treatments on populations of *R. solani* AG-4, binucleate *Rhizoctonia*-like fungi, *R. zeae*, the sterile white basidiomycete, *Laetisaria arvalis* Burdsall, and *Pythium* spp. *Pythium* spp. were not detected, and populations of basidiomycetes were <10 cfu/100 g of soil. There were no significant differences among treatments.

Growing season of 1986. Only one crop of corn was grown in 1986. Root disease was limited, and few crown and brace roots had lesions 5 wk after planting (Table 4). Symptoms of decline were not observed.

The winter rye treatment differed from fallow only in the percentage of plants with moderate root rot, and the only difference in parameters measured in fertility treatments was that stalk rot and plant growth were greater with high fertility. Soil fumigation with DD-MENCS in 1986 reduced root disease severity, the number of lesions on crown and brace roots, and stalk rot and increased yield of silage compared with controls (Table 4). The residual effect of fumigation with DD-MENCS in 1985 reduced root stalk rot but did not influence other parameters, and soil treatments with fenamiphos and metalaxyl did not influence root disease severity, stalk rot, or yield. Fenamiphos slightly reduced the average daily growth 3–12 wk after planting, but the other soil treatments had no effect on growth. Stalk rot was much greater (45%) in the control plots in the high fertility treatment following rye than in any other treatment. There were no interactions among treatments in other parameters measured.

Fungi isolated most frequently from lesions removed from roots of 6- to 11-wk-old plants were *F. oxysporum* (29%), *P. arrhenomanes* and other *Pythium* spp. (27%), and *Phoma americana* (6%). In soil samples collected 24 days after planting, populations of *R. solani* AG-4, binucleate *Rhizoctonia*-like fungi, and *Pythium* spp. were low and did not differ among treatments. Populations of *F. solani, F. moniliforme*, other *Fusarium* spp., and other saprophytic fungi did not differ among treatments.

Growing season of 1987. There were no differences in plant stand among treatments. Plants did not show symptoms of decline in any treatment. Root disease severity was slight to moderate 9 wk after planting. The winter crop of rye did not affect root disease severity compared with fallow, and there were no differences between the high and low levels of fertility. Soil fumigation in 1985–1987 did not reduce root disease severity in either the fallow or rye treatments, and soil fumigation in 1987 only reduced the percentage of crown and brace roots with lesions in the fallow treatment (Table 5). With the fenamiphos treatment, the percentage of plants with moderate root rot and crown and brace roots with apical decay was increased in the fallow treatment but not in the rye treatment. Growth was greater in the high than in the low fertility level and was increased by soil fumigation in 1987 compared with other treatments (Table 6).

There was <5% lodging, and stalk rot was moderate but did not differ among treatments. Yield of silage was increased 13% by high fertility compared with low fertility and 15% by soil fumigation in 1987 compared with the control, but other treatments had no significant effects (Table 6). Fungi isolated most frequently from lesions on roots of 15-wk-old plants were *Phoma terrestris* (52%), *Pythium graminicola* (35%), and *F. oxysporum* (10%).

During the 3 yr, the principal nematodes in control plots were root knot (*Meloidogyne incognita* (Kofoid & White) Chitwood) and ring (*Criconemella ornata* (Raski) Luc & Raski). Fumigation with DD-MENCS and soil treatment with fenamiphos reduced populations of root-knot nematodes, but only fumigation with DD-MENCS reduced populations of ring nematodes. Tillage and fertility treatments had no significant influence on populations of nematodes. In control plots, numbers of root-knot nematodes ranged from <15/150 cm^3 of soil at planting to 1,400/150 cm^3 of soil at harvest.

Multiple regression analysis showed that 87, 32, 33, and 18% of the variation in silage yield could be explained by populations of soil fungi, root and stalk rot disease severity, and populations of nematodes in the four consecutive crops, respectively ($P = 0.01$). In the first crop, 68% of the variation in yield was positively related to the number of plants with <10% root discoloration and decay per plot and the average number of crown and brace roots per plant 15 wk after planting. In subsequent crops, root parameters were not closely related to yield.

Greenhouse experiments. Root disease severity was slight to moderate, and the only soil treatments that reduced root disease severity were heat in the first experiment and benomyl, pencycuron, and metalaxyl in the second experiment (Table 7). None of the soil treatments reduced the number of mesocotyl lesions, but fenamiphos increased the number. Heat treatment consistently increased plant dry weight and reduced the number of chlorotic leaves. In the second experiment, all chemical treatments increased plant dry weight but only

Table 5. Root disease severity in continuous corn following winter fallow or rye with different soil treatments in 1987

	Plants with root disease at 9 wk[y] (%)		Crown and brace roots terminated (%)	
Soil treatment	Fallow	Rye	Fallow	Rye
DD-MENCS, 1987	1 b[z]	5 b	4 c	6 b
DD-MENCS, 1986	4 b	6 ab	7 c	8 ab
DD-MENCS, 1985	4 b	15 a	9 bc	16 a
Fenamiphos	52 a	8 ab	34 a	13 a
Control	12 b	11 ab	16 b	12 ab

[y] Plants with root systems having more than 10% visible discoloration and decay.
[z] Means within a column followed by different letters are different according to the Waller-Duncan *k*-ratio *t* test, $P = 0.05$.

Table 6. Stalk rot, growth, and silage yield in continuous corn in 1987

Treatment	Stalk rot (%)	Growth (cm/day)	Silage yield (t/ha)
Winter crop			
Rye	23	5.2	54.3
Fallow	27	5.1	52.2
Fertility			
High	25	5.3 a[z]	56.5 a
Low	25	5.0 b	50.2 b
Soil treatment			
DD-MENCS, 1987	18	5.5 a	61.9 a
DD-MENCS, 1986	26	5.2 b	51.8 b
DD-MENCS, 1985	26	4.9 c	48.6 b
Fenamiphos	26	5.0 bc	50.7 b
Control	28	5.2 b	53.8 b

[z] Numbers within a column followed by different letters are different according to the Waller-Duncan *k*-ratio *t* test, $P = 0.05$.

benomyl reduced chlorosis.

Root disease severity increased as nitrogen levels increased, but there were more mesocotyl lesions at low than at high nitrogen levels. Nitrogen increased plant dry weight and reduced chlorosis in the first experiment. In contrast, in the second experiment nitrogen had no significant effect on plant dry weight, and chlorosis increased as nitrogen level increased (Table 7).

In the first experiment, no *Pythium* spp. or *R. solani* was detected in soil in any treatments at planting. Populations of *R. zeae* were low to moderate in the control (13 cfu/100 g of oven-dried soil), but the fungus was not detected in heated soil. Populations of *Penicillium* spp. + *Paecilomyces* spp. were 12,400 cfu/g in the control and ranged from nondetectable to 2,600 cfu/g in other treatments. Populations of total fungi were 40,000 cfu/g in the control and were reduced 80–90% by soil treatments.

In the second experiment, populations of *Pythium* spp., *R. zeae*, and binucleate *Rhizoctonia*-like fungi were 4.2, 20, and 10 cfu/100 g, respectively, in the control at planting. *Pythium* spp. were not detected in the other soil treatments, and populations of *R. zeae* and binucleate *Rhizoctonia*-like fungi were similar to those in the control, except that *R. zeae* was not detected in soil treated with metalaxyl and that populations of the binucleate *Rhizoctonia*-like fungus CAG-2 were high (40 and 73 cfu/100 g, respectively) in soil treated with benomyl and fenamiphos.

Pythium arrhenomanes or *P. graminicola* and binucleate *Rhizoctonia*-like fungi were isolated from one or more root lesions in all soil treatments in the first experiment, and *F. oxysporum* was isolated from lesions in all treatments except heated soil. *R. solani* AG-2 type 2 was isolated from a lesion in the control but not in the other treatments.

In the second experiment, *P. arrhenomanes* was isolated from lesions in the control and the pencycuron and fenamiphos treatments but not from lesions in other treatments. *F. moniliforme* was isolated from lesions in all treatments, and *F. oxysporum* was isolated from lesions in all except the carbofuran and heated treatments. *Phoma* spp. were isolated only from lesions from the carbofuran treatment. Other fungi isolated included *R. zeae, Trichoderma* spp., CAG-2, and *Helminthosporium rostratum* Drechs.

In pathogenicity tests with numerous isolates of several fungi from corn roots, isolates of *P. arrhenomanes* and *P. irregulare* caused moderate root disease and reduced oven-dry weight of roots 48 and 29%, respectively. *Phoma terrestris, P. americana, R. zeae*, the sterile white basidiomycete, and *F. moniliforme* caused slight to moderate root disease but had little or no effect on growth. *Pythium aphanidermatum* caused moderate to severe root disease and also chlorotic streaks or yellowing in lower leaves. *R. solani* AG-2 type 2 caused severe crown and brace root rot but few lesions on fibrous roots. Other fungi either were not pathogenic or caused only slight discoloration of cortical tissues and did not affect plant growth.

DISCUSSION

Our research has shown that decline in double-cropped corn in Georgia is induced by soilborne organisms. Fumigation with DD-MENCS in February eliminated symptoms of decline and increased yield in both spring and fall experiments in 1985. Corn growth and silage yields were similar in fumigated plots each year, but symptoms of decline, poor growth, and low yield were observed only in control plots in the first year. In the second and third years, silage yield in the control was 82 and 87%, respectively, of the yield in fumigated plots.

We do not know why symptoms of decline failed to appear in the control plots in the last 2 yr of the study. The experimental area was turned 20–25 cm deep with a moldboard plow before planting in the second and third years, in contrast to the first year, but symptoms of decline were observed in plots treated with different tillage practices, including plowing in years immediately preceding these experiments.

The decline in double-crop corn is similar to stubble decline of sugarcane in Louisiana (5). Symptoms of decline in sugarcane were associated with infection by *P. arrhenomanes* and other *Pythium* spp., and we isolated *P. arrhenomanes* from lesions on corn roots more frequently than any other fungus. *P. arrhenomanes* and *P. graminicola* are similar morphologically, and both are pathogenic on corn and commonly found in soil (3,7,11,16,17). *P. arrhenomanes*, like *P. graminicola*, is not easily isolated from soil (17), and that may be why populations of *Pythium* spp. were low in many of the soil samples in this research.

In previous research in a greenhouse, chlorosis and necrosis of leaves on sweet corn were increased in field soil infested with *P. arrhenomanes* or the sterile white basidiomycete compared with corn

Table 7. Root disease index, growth parameters, and mesocotyl lesions in corn grown in a greenhouse in field soil with different soil treatments[v]

	Experiment 1				Experiment 2			
Soil treatment	Plants with root disease[w] (%)	Plant dry weight (g)	Chlorotic leaves at 20 days (no.)	Nitrogen in plants[x] (%)	Plants with root disease[w] (%)	Plant dry weight (g)	Chlorotic leaves at 30 days (no.)	Mesocotyl lesions (no.)
Pencycuron	62 a[y]	1.3 b	2.6 b	3.25	3 c	3.4 b	7.9 ab	2.3 abc
Metalaxyl	66 a	1.4 b	2.4 b	3.10	0 c	2.6 c	8.1 ab	2.3 abc
Carbofuran	···	···	···	···	14 bc	2.6 c	8.8 a	1.7 cd
Fenamiphos	···	···	···	···	33 a	2.5 c	8.5 ab	2.8 a
Benomyl	···	···	···	···	0 c	4.4 a	5.8 c	1.8 bcd
Heat[z]	0 b	2.2 a	0.1 c	2.89	19 ab	3.5 b	7.3 b	1.3 d
Control	79 a	1.1 b	3.6 a	2.30	22 ab	2.2 d	9.0 a	1.8 bcd
Nitrogen levels (mg/kg)								
0	42 b	1.3 b	2.8 a	2.36	···	···	···	···
67	···	···	···	···	3 b	3.0 a	6.8 c	2.5 a
135	···	···	···	···	14 a	3.0 a	8.1 b	1.9 b
168	68 a	1.7 a	1.6 b	3.92	···	···	···	···
202	···	···	···	···	21 a	3.2 a	8.9 a	1.6 b

[v]Soil was collected from the field where decline occurred in February 1985 and on 14 January 1986 in experiments 1 and 2, respectively.
[w]Plants with root systems having more than 10% visible discoloration and decay.
[x]Combination of all replications; data not analyzed.
[y]Numbers within columns of soil treatments or nitrogen levels followed by the same letter are not different according to Duncan's multiple range test, $P = 0.05$. Absence of letters indicates data were not analyzed.
[z]At 68 and 64 C for 30 min in an oven in experiments 1 and 2, respectively.

grown in heat-treated or noninfested soil (25). With field corn, *P. aphanidermatum* caused severe chlorosis and the sterile white basidiomycete, *F. moniliforme*, and *F. oxysporum* caused mild chlorosis, chlorotic streaking, or necrosis that could be misconstrued as mineral deficiency (10). In contrast, *Phoma terrestris* and *P. americana* (12) did not induce discoloration of foliage. *P. terrestris* is widely distributed in soil but may not be a limiting factor in growth of corn (1,6). *Helminthosporium sativum* Pam. King & Bakke (= *Bipolaris sorokinianum* (Sacc. or Sorok.) Shoem.) was isolated by D. R. Sumner from corn roots on the same farm where the research was done in 1980. The fungus produced prehelminthosporol that induced stem collapse and general necrosis in corn seedlings (2) but was rarely isolated from lesions on corn roots in this investigation.

In decline of sweet corn, symptoms were frequently reproduced in soils treated with carbofuran alone or in combination with butylate and butylate + atrazine (25). In this research, carbofuran was applied to soil five times from 1978 to 1983 for insect control but was not used later. Butylate was applied four times from 1980 to 1984, and atrazine was applied seven times from 1980 to 1985. Other soil pesticides applied frequently from 1979 to 1984 were chlorpyrifos and metolachlor (seven times each) and fenamiphos (six times). Herbicides increased root disease severity induced by *R. solani* AG-2 type 2, but symptoms of decline were not observed (23). Crown and brace root rot was observed infrequently in this research, and *R. solani* AG-2 type 2 was isolated rarely.

Changing from a double-crop to a mono-crop system of producing corn may have contributed to the disappearance of decline. We have not observed decline in any mono-crops of corn or multi-cropping systems with corn and other crops. It is possible that double-cropping corn maintains high inoculum levels of several soilborne fungi that are pathogenic to corn, because there is little time between crops for roots to decay. Winter rye in rotation with double-crop corn did not reduce decline, except in the small test with no-till corn in 1985, but winter rye or other winter crops rotated with mono-crop corn may allow populations of pathogens to decline while supporting growth of saprophytic microorganisms. The role of saprophytic soil microorganisms in biological control of root pathogens on corn in Georgia is not known, but fungi that are known biocontrol agents are common in the soil of the coastal plain (21). There would be more time between crops for natural control to occur in soil in mono-crop corn than in double-crop corn, and numerous microorganisms are antagonistic to *P. arrhenomanes* (8).

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