Etiology and Control of Root Diseases of Spinach

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ABSTRACT

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Seedling diseases in field tests were more severe in late summer or early fall plantings when soil temperature maxima at 1 cm depths were 25-36 C. Seedling injury also was severe in a winter crop when soil temperature minima were −2.8 C for 2 days in succession 12 days after planting. The fungi most frequently isolated from seedlings were *Fusarium oxysporum, F. solani, Pythium* spp., *Rhizoctonia solani,* and *F. roseum,* in that order. In pathogenicity tests in environmental chambers, *R. solani* caused the most severe damage, but *Pythium irregulare, F. oxysporum,* and *F. roseum* also caused significant root injury. Populations of *F. oxysporum* and *Pythium* spp. were reduced and seedling diseases and injury by root-knot, stubby-root, and ring nematodes decreased by soil fumigation with DD-MENCS and methyl bromide-chloropicrin (2:1, v/v). Soil fumigation sometimes decreased the incidence and severity of yellows and decline and increased yields, but results were variable. Symptoms of decline were associated with nutritional imbalance, wet soil, prolonged cloudiness, abrupt temperature changes, nematodes, and soil-borne fungi, but were not typical of Fusarium wilt.

Additional key words: Spinacia oleracea, Meloidogyne incognita, Trichodorus christiei, Criconemoides ornatus, ethoprop, benomyl, chloroneb.

Spinach decline and wilt (1, 4, 5, 8, 17), caused by *Fusarium oxysporum* Schlecht. f. sp. *spinaciae* (Sherb.) Snyd. & Hans. and *F. solani* (Mart.) Appel & Wr. have severely reduced yields and quality in some areas, especially during periods of high temperatures. Pre-emergence damping-off caused by *Pythium ultimum* Trow and *Rhizoctonia solani* Kühn also greatly limited stands in Virginia (3) and New York (14) before fungicides for seed treatment were utilized.

Several companies in the foods industry have recently shown interest in growing spinach for processing in the Georgia Coastal Plain. Since there was no history of successful commercial production in Georgia, this research was undertaken to determine whether Fusarium wilt occurs in Georgia, and if root diseases would limit the establishment of commercial spinach production.

Tests for controlling root diseases and nematodes with fungicides, fumigants, and a nematicide were run to study the role of soil fungi and nematodes in spinach production. The pathogenicity of several soil-borne fungi to spinach was studied under various temperature cycles in growth chambers.

Preliminary reports of this research have been published (9, 15, 16).

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MATERIALS AND METHODS

Root diseases.—Spinach cultivars were grown in experimental field plots in Tifton and Dothan loamy sand (approximately 85% sand, 10% silt, and 5% clay) at the Coastal Plain Station, Tifton, Georgia. In addition, spinach was observed in a grower's field near Autreyville, Georgia, from 1973 to 1976. Spinach was planted at various times from September through January and harvested from January through April.

Seeds were planted 1-3 cm apart and 1-2 cm deep on raised beds with Planet Junior® (Planet Junior Corp., Boise, Idaho) seeders. Rows were spaced 21-40 cm apart. A randomized complete block design with four or five replications was used for each experiment.

Dolomitic limestone was applied periodically to maintain pH from 5.7-6.5. Before planting, fertilizer was broadcast and tilled into the surface 15-20 cm of soil at the following rates (kg/ha): N, 54 to 112; P, 108 to 112; and K, 108 to 112. Plants were periodically side-dressed with N as $NaNO_3$ or $CaNO_3$, depending on the amount of rainfall and appearance of growth.

In some tests the herbicides chloropropham or cycloate were applied before planting. Cycloate was incorporated 5-cm deep and the soil was immediately irrigated. Chloropropham was sprayed on the surface immediately after planting. An insecticide (methomyl) and a fungicide

(chlorothalonil) were applied to help prevent foliage injury.

Soil temperatures were monitored continuously with a Tempscribe® (Bacharach Industrial Instrument Co., Pittsburgh, Pa.) recorder in the 1972 winter crop and with a YSI Model 47 Tele-Thermometer and YSI Model 80 A recorder (Yellow Springs Instrument Co., Yellow Springs, Ohio) from 1973 until 1975.

Stand counts and growth ratings were recorded intermittently. Growth ratings were based on color and overall vigor using a scale of 1 to 7, where 1 = yellow, spindly plants and 7 = green, vigorous plants. Foliage was harvested by hand or with a sickle-bar mower, weighed, and evaluated for color.

Fifteen cultivars were planted and grown (without using pesticides) in a preliminary evaluation trial 5 December 1972. Cultivars that were used are listed in Table 1.

Plants were examined for root injury 2 weeks after planting and at 1- to 2-week intervals until the end of January. In other tests, seedlings were removed 12-40 days after planting and examined for root and hypocotyl discoloration and decay. Tissue sections 1-2 cm long were removed from hypocotyls and adjacent primary and secondary roots, washed 0.5-2 hours in running tap water (10-20 C), aseptically blotted dry on sterile filter paper, and transferred to water agar. Fungi that grew from the tissue sections were transferred to PDA and identified. Cultures of *Pythium* spp. were grown on hempseed agar and identified to species.

Soil assays.—Ten soil cores 2.5 cm in diameter and 15 cm in depth were collected from each plot, combined, mixed, and assayed for fungi and nematodes.

Soils were assayed for fungi by plating soil on selective media as follows: for *Pythium* spp., modified gallic-acid (6); for *Fusarium* spp., PCNB-peptone as modified by Papavizas (12); and for *Rhizoctonia solani,* the basal medium as for *Pythium* spp., but without gallic-acid and with 600 mg neomycin, 120 mg tannic acid, and 0.1 ml pyroxychlor [Dowco 269 (95%), Dow Chemical Co., Midland, Michigan]. Populations of *Trichoderma* spp., *Penicillium* spp. + *Paecilomyces* spp., and *Neocosmospora vasinfecta* Smith were also estimated on modified PCNB-peptone agar. In 1975, soil samples were taken from each plot 5 and 120 days after planting. The latter samples were taken 5-10 cm from the row and in the root zone. In 1974, soil removed from roots by vigorous shaking was combined with soil directly under the plants, blended, passed through a sieve (1- to 2-mm openings), and assayed for fungi.

In 1975, rhizosphere samples were taken from 10 plants in the center of each plot at harvest, then roots were examined for decay and discoloration. Tap roots were surface disinfested by soaking them for 2-5 minutes in 1% NaOCl, rinsed in tap water, and sectioned transversely with a sterile scapel. Vascular tissue from just below the crown of each plant was transferred to water agar, and cultures of fungi were transferred to PDA for identification.

Soil was assayed for nematodes with the centrifuge-sugar flotation method (10). Samples were collected at planting and 3-4 months later. At harvest roots were examined for root-knot nematode damage and indexed for percentage of galled roots on a 1-5 scale, in which: 1 = 0 galls, and 5 = 75-100% of all roots galled.

Chemical control.—In 1973, the land was fallow before spinach was planted, but in 1974 southern peas [*Vigna unguiculata* (L.) Walp.] were grown before spinach to increase populations of soilborne pathogenic fungi and nematodes.

Soil pesticides used and dosages per hectare were: 20% methylisothiocyanate and 80% chlorinated C_3 hydrocarbons, 376.9 kg (DD-MENCS) (Vorlex®); 67% methyl bromide and 33% chloropicrin, 336 kg (MBC) (Dowfume MC-33®); sodium methyl dithiocarbamate (Metham) (Vapam®) 234 liters; ethoprop, 8.96 kg; chloroneb, 14.56 kg; benomyl, 11.2 kg; and chloroneb + benomyl. Metham, DD-MENCS, and MBC were injected 25-30 cm deep with chisels spaced 30 cm apart, 23 days before planting. Plots treated with Metham and MBC were immediately covered with plastic [0.152-mm (6-mil)], and plots treated with DD-MENCS were either covered or noncovered. Noncovered fumigated plots were sealed with a packing board attached to the injection equipment. Fumigated plots were irrigated immediately, and the plastic cover was removed 11 days later. Ethoprop, chloroneb, benomyl, and chloroneb + benomyl were applied 6 days before planting by spraying on the soil just ahead of a powerdriven rototiller that incorporated them 15-cm deep. Plots were irrigated with 5 cm of water 2-5 hours later.

Pathogenicity.—Twenty-five different cultures of fungi were grown on an autoclaved cornmeal-sand mixture (3:100, w/w) (CMS) for 8 to 13 days. Pasteurized field soil (1 hour at 82 C dry heat) or nontreated field soil was infested (1:100, v/v) with CMS inoculum, and 75 mg N, 75 mg K, and 3 g of finely ground $CaCO_3$ were added to each liter of soil. After thorough mixing, the infested soil was placed in 11.5 × 11.5 × 5.3 cm plastic trays. In one experiment, 10 seeds of cultivars Chesapeake, Early Hybrid 7, and Marathon were planted 1 cm deep in each tray. Three replicates of each treatment were placed in an environmental chamber at a 10 C night and a 22 C day (±1 C), with 12 hours of light (20,000 lux) per diurnal cycle. Seedlings were harvested after 16 days and examined for root discoloration.

A second experiment was conducted to determine the effect of temperature on pathogenicity. The same methods as in the first experiment were used, but only Chesapeake was tested. Three replicates of each treatment were grown in separate growth chambers at diurnal temperature ranges of: (i) 19 ± 2 C night, 36 ± 4 C day (25,000 lux); (ii) 9 ± 3 C night, 26 ± 3 C day (20,000 lux); and (iii) 11 ± 2 C night and 26 ± 2 C day (6,000 lux). The last chamber was designed for low temperatures, and the air temperature was lowered to 0 to −3 C for 15 hours when the plants were 8-10 days old and 1-2 cm tall; then the chamber was re-set to the original temperature range. When the plants were 12-17 days old, the roots were evaluated for discoloration and isolations were made from hypocotyls and primary roots of 10 plants in each treatment in the two chambers with the lowest temperature regimes.

RESULTS

Seedling diseases in field tests.—The most severe root and hypocotyl injury was noted in a September planting,

when the daily soil temperature maxima 1 cm deep were 25-36 C during the 2 weeks following planting. Severe seedling injury also was observed in a December planting when the soil temperature minima 1 cm deep were −2.8 C for 2 days in succession, 12 days after planting. The least root injury and the best plant stands were in the mid-fall plantings when the soil temperatures at the 1 cm depth ranged from 6 to 30 C.

Isolation of fungi and cultivar evaluation.—Fungi most frequently isolated from seedlings were *Fusarium oxysporum, F. solani, Pythium* spp. (primarily *P. irregulare* Buis.), *Rhizoctonia solani,* and *F. roseum* (Lk.) Fr., in that order. *Pythium* spp. were isolated more commonly from seedlings in the late fall and winter and *F. solani* in the early fall. *Fusarium oxysporum* was frequently isolated from seedlings throughout the growing season, but *F. roseum* was rarely isolated. *Rhizoctonia solani* was not isolated from seedlings in some fields, but frequently was isolated from seedlings in others, irrespective of the season.

There were no significant differences in root damage among the 15 cultivars, but there were differences in numbers of fungi isolated from roots (Table 1). The fungi most frequently isolated from 1,419 seedlings, and the percentage of total plants yielding each fungus were: *R. solani*–24%, *Pythium* spp.–19%, *F. oxysporum*–7%, and *F. solani*–3%. Seedlings yielding one or more of the preceding fungi increased from 41% at 2 weeks to 76% at 8 weeks after planting. The percentage of seedlings with >10% root and hypocotyl discoloration also increased from 19% in 2-week-old seedlings to 64% in 7- to 8-week-old seedlings.

Significantly more cultures of *Pythium* spp. were isolated from Alf-Christianson Hybrid No. 7 than from Asgrow Early Hybrid Seven or from Alf. Christianson Chesapeake (Table 1). Also, Asgrow Hybrid Marathon yielded significantly more cultures of *F. oxysporum* than Charter Hybrid 50. The number of seedlings yielding one or more fungi was significantly less in Charter Hybrid 51 than in Alf. Christianson Hybrid 62, Asgrow Hybrid Marathon, and Ferry Morse Hybrid 612. There were no other significant differences among cultivars in numbers of fungi isolated.

Charter Hybrid 52 produced significantly higher stand counts than the other cultivars (Table 1). Stand counts were not correlated with the total number of fungi isolated from seedlings, but there was a highly significant negative correlation between the number of plants yielding cultures of *Pythium* spp. and the percentage of seedlings with <10% root and hypocotyl discoloration ($r = -.31^{**}$). The frequency of isolation of *F. oxysporum* from seedlings was correlated with poor growth ratings ($r = -.33^{**}$).

Root-galls were rarely observed on randomly selected plants. No root-knot, *Meloidogyne incognita* (Kofoid & White) Chitwood, larvae were found in soil samples, but stubby-root, *Trichodorus christiei* Allen, and ring, *Criconemoides ornatus* Raski, nematodes were present in low numbers.

Yellows and decline.—A deterioration of older plants was observed for several years in commercial fields in Thomas County. Foliage symptoms were similar to decline described in other areas (1, 4), except that fewer plants had discolored vascular systems, and there was very little decay of external tap and feeder roots. Symptoms of the decline were more severe on the oldest leaves. Plants frequently were stunted and older leaves were light green to yellow. After leaves yellowed, the blades and petioles frequently died and the crown rotted. In some plants, a brown rot produced a hollow crater in the center of the crown that extended into the hypocotyl.

Isolations made from vascular tissues in roots and crowns of 62 plants in various stages of decline from four fields yielded *Fusarium oxysporum* and *F. solani* from 27

TABLE 1. Fungi isolated from seedlings, stand counts, and growth ratings of 15 spinach cultivars planted 5 December 1972 in Dothan loamy sand at the Georgia Coastal Plain Station

		Percentage of seedlings yielding:				
Seed company	Cultivars	*Pythium* spp.	*Fusarium oxysporum*	Total[w] potential pathogens	Stand[x] at 10 weeks	Growth[y] rating at 18 weeks
Alf. Christianson	Hybrid No. 7	36 a[z]	4 ab	57 bc	66 cd	3.6 abcd
	Hybrid No. 7R	32 ab	4 ab	50 bc	33 fg	2.4 d
	Hybrid No. 62	26 ab	10 ab	69 a	42 efg	3.4 bcd
	Hybrid Avon	26 ab	6 ab	56 bc	49 def	2.6 cd
	Chesapeake	12 b	4 ab	40 bc	93 b	4.8 a
Asgrow	Hybrid Marathon	16 ab	16 a	62 ab	24 g	2.8 cd
	Early Hybrid Seven	12 b	8 ab	52 bc	25 g	2.8 cd
	Hybrid Seven R	14 ab	10 ab	48 bc	31 fg	3.2 bcd
Charter	Hybrid 50	18 ab	2 b	48 bc	90 b	4.2 ab
	Hybrid 51	16 ab	4 ab	35 c	78 bc	3.4 bcd
	Hybrid 52	22 ab	12 ab	54 bc	131 a	3.8 abc
Ferry Morse	Hybrid No. 7	20 ab	8 ab	54 bc	62 cde	3.8 abc
	Hybrid 612	20 ab	6 ab	62 ab	36 fg	2.8 cd
	Avon	24 ab	10 ab	48 bc	68 cd	3.6 abcd
	Chesapeake	20 ab	8 ab	50 bc	72 cd	3.8 abc

[w] *Pythium* spp., *Rhizoctonia solani, Fusarium oxysporum, F. solani,* and *F. roseum.*
[x] Plants per 8 m of row.
[y] Rating scale: 1 = very few plants, mostly yellow and stunted, <15 cm tall; 7 = green, abundant foliage, greater than 30 cm tall.
[z] Numbers followed by the same letter are not significantly different according to Duncan's multiple range test; $P = 0.05$.

and 14% of the plants, respectively, and *Pythium* spp., *F. roseum,* and *R. solani,* in that order, were isolated less frequently.

In grower fields with the most severe symptoms of decline, populations of *F. oxysporum* ranged from 960 to 2,040 propagules/g (p/g) of oven-dry soil in soil under declining plants compared with 660 to 950 p/g in soil from adjacent bare ground. Populations of *F. solani* ranged from 240 to 900 p/g under declining plants and from 180 to 660 p/g in bare soil.

In February, soil was collected from under declining plants grown in nontreated soil in one field, and under green plants growing in methyl bromide-treated soil in an adjacent field. Populations of fungi in the two soils were: *Pythium* spp., 91 and 4 p/g; *F. oxysporum,* 800 and <130 p/g; and *F. solani,* 5,750 and 1,070 p/g, respectively.

The pathogenicity to spinach of individual isolates of fungi from soil was not studied.

Pathogenicity.—The most virulent pathogen on spinach seedlings in both experiments in environmental chambers was *Rhizoctonia solani. Pythium irregulare* and *P. aphanidermatum* (Edson) Fitzpatrick also significantly increased root discoloration. *Fusarium roseum* reduced stands in Early Hybrid 7 but not in other cultivars. In the second experiment, stands were reduced by two of 10 isolates of *F. oxysporum* but not by three isolates of *F. solani* or an isolate of *F. roseum*. Brown, decayed root tips frequently were observed in plants in soil infested with *F. oxysporum*. Each of the pathogenic fungi was reisolated from seedlings grown in soil with which it had been infested.

The roots of plants grown at the highest temperature

TABLE 2. Root and hypocotyl discoloration and isolation of fungi from 19- to 20-day-old spinach seedlings, and root-gall indices of 63- and 146-day-old plants, grown in field plots treated with soil pesticides

Soil treatment	RDI[x]	Percentage of seedlings yielding cultures of: *Rhizoctonia solani*	Total potential[y] pathogens	RGI[w] 63	RGI[w] 146
DD-MENCS + film	1.22 a[z]	0 a	2.5 a	1.02 ab	1.00 a
DD-MENCS	1.25 a	5 ab	27.5 bc	1.00 a	1.04 a
MBC + film	1.12 a	0 a	12.5 ab	1.01 ab	1.04 a
Metham + film	1.73 abc	8 abc	22.5 bc	1.31 abc	1.48 bcd
Ethoprop	2.12 bc	35 c	62.5 d	1.20 ab	1.38 ab
Chloroneb	1.67 abc	30 bc	52.5 cd	2.00 cde	2.11 cd
Benomyl	1.50 ab	8 abc	32.5 bcd	2.08 de	2.01 bcd
Chloroneb + benomyl	1.71 abc	0 a	30.0 bcd	1.76 bcde	1.75 bcd
Control	1.71 abc	25 bc	40.0 bcd	2.41 e	2.34 d
Control + film	2.18 c	28 bc	42.5 cd	1.51 abcd	1.41 abc

[w]Root-gall index: 1 = no galls, 5 = 75-100% of the roots galled.
[x]Root disease index: 1 = 2%, 2 = 2-10%, 3 = 11-50%, 4 = >50% of roots and hypocotyls discolored, and 5 = dead or dying seedlings.
[y]*Rhizoctonia solani, Pythium* spp. (primarily *P. irregulare*), *Fusarium oxysporum, F. solani,* and *F. roseum.*
[z]Numbers followed by the same letter are not significantly different according to Duncan's multiple range test; $P = 0.05$.

TABLE 3. Effect of soil treatments on populations of soilborne fungi 5 and 111 days after planting spinach 17 October 1974[w]

Soil treatment and rate, A.I./ha[y]	Fungal propagules per gram of oven-dry soil: *Pythium* spp.[x] After days 5	*Pythium* spp.[x] After days 111	*Fusarium solani* After days 5	*Fusarium solani* After days 111	*Fusarium oxysporum* After days 5	*Fusarium oxysporum* After days 111
	Propagules/g of oven-dry soil					
DD-MENCS + film, 376.9 kg	0 a[z]	2	151 a	43 a	0 a	64 a
DD-MENCS, 376.9 kg	0 a	0	757 ab	1,479 b	368 b	515 b
MBC + film, 336 kg	2 a	1	1,116 b	2,139 b	109 ab	172 a
Metham + film, 234 liters	27 b	2	1,943 b	4,594 b	1,748 c	2,085 b
Ethoprop, 8.96 kg	64 c	8	1,878 b	1,420 b	2,642 c	2,002 b
Chloroneb, 14.56 kg	61 c	8	1,769 b	1,998 b	2,206 c	2,706 b
Benomyl, 11.2 kg	76 c	6	2,178 b	1,484 b	2,699 c	2,104 b
Chloroneb + benomyl	64 c	15	2,317 b	2,190 b	1,923 c	2,816 b
Control	70 c	6	2,513 b	2,706 b	2,864 c	2,538 b
Control + film	49 bc	9	1,570 b	1,398 b	2,178 c	2,988 b

[w]Fumigants were applied 23 days, and the other treatments 6 days, before planting.
[x]Primarily *Pythium irregulare.*
[y]DD-MENCS = 20% methylisothiocyanate and 80% chlorinated C_3 hydrocarbons (Vorlex®), MBC = 67% methylbromide and 33% chloropicrin (Dowfume MC-33®), Metham = sodium methyldithiocarbamate (Vapam®), ethoprop = Mocap® 10G, chloroneb = Demosan® 65 WP, and benomyl = Benlate® 50 WP. A.I. = active ingredient.
[z]Numbers followed by the same letters are not significantly different according to Duncan's multiple range test; $P = 0.05$. No letters indicate no significant differences.

(19 ± 2 C night, 36 ± 4 C day) were the most severely damaged. Freezing injury did not reduce stands, but root discoloration was increased compared with the plants grown in the chambers at 9 ± 3 C night and 26 ± 3 C day.

Soil pesticides.—Discoloration of seedling roots and hypocotyls was significantly reduced by DD-MENCS + film and MBC + film but not by DD-MENCS without film (Table 2). Other soil treatments did not reduce seedling diseases. The fungi most frequently isolated from seedlings were *R. solani, F. oxysporum,* and *P. irregulare,* in that order. Significantly fewer cultures of *R. solani* were isolated from plants grown in soil treated with DD-MENCS + film, MBC + film, and chloroneb + benomyl than the controls. Isolation of other fungi did not differ among treatments. However, DD-MENCS + film and MBC + film significantly reduced the total number of potentially pathogenic fungi (*Fusarium* spp., *Pythium* spp., and *R. solani*) isolated from seedlings.

Populations of *Pythium* spp. and *F. oxysporum* were significantly reduced 5 days after planting by soil fumigation with DD-MENCS and MBC as shown in Table 3. In addition, DD-MENCS also reduced populations of *F. solani* and total *Fusarium* spp. Both soil fumigants covered with film significantly suppressed populations of *F. oxysporum* and total *Fusarium* spp. 111 days after planting, but only DD-MENCS + film had a lingering effect on the inoculum density of *F. solani*. Populations of *Penicillium* spp. were significantly reduced by DD-MENCS with film and MBC at both samplings, and by DD-MENCS without film at the last sampling. There were no significant differences in populations of *Trichoderma* spp. at the first sampling, but populations were decreased by SMDC and DD-MENCS at the last sampling, compared to controls. The inoculum density of *R. solani* was not determined at the first sampling, but there were no significant differences among treatments at the last sampling. Benomyl, chloroneb, benomyl + chloroneb, and ethoprop did not influence populations of the soil fungi that were assayed.

There were no differences in the number of roots yielding cultures of various fungi 5 months after planting. None of the roots sampled showed external discoloration and only 2% had tan-to-brown streaking in the vascular tissues. No vascular discoloration was observed in plants grown in fumigated soil, and only 5% or less of the roots yielded cultures of *F. oxysporum,* compared with 15 and 32% in the controls, with and without film, respectively. A *Cephalosporium* sp. was isolated from vascular tissues of 35 to 60% of the plants in all treatments, but other fungi were isolated only occasionally.

Populations of fungi in rhizosphere soil 5 months after planting were similar in all treatments and were not related to stand counts, growth ratings, or yield. However, populations of *F. solani* and total *Fusarium* spp. were still significantly suppressed in MBC + film as compared to the controls (40 vs. 3,460 and 2,770 vs. 10,440 p/g, respectively). Seedling root and hypocotyl discoloration was correlated with populations of *F. solani* in the rhizosphere (r = .44**).

The nematodes most frequently found in soil assays were root-knot larvae, stubby-root, and ring, in that order, but nematode populations were low in all field tests. In soils treated with benomyl, populations of root-knot larvae were significantly greater than the control at 120 days (35 vs. 2/150 ml of soil). No parasitic nematodes were recovered after 120 days in soil treated with DD-MENCS or MBC. Root-knot nematodes caused very little injury in the control + film treatment. Without film, DD-MENCS and ethoprop reduced galling (Table 2), but other soil pesticides did not.

There were no differences in stands or growth ratings among treatments, but seedling root and hypocotyl injury, stands, and growth ratings were related to populations of soilborne fungi (Table 4), though the coefficients of variability among populations of fungi sampled at different times ranged from 30 to 100%. There was also a highly significant negative correlation between the root-gall index at 63 days and growth ratings at 62 days after planting. Isolation of *R. solani* from seedlings was correlated with root discoloration (r = .63**) and stand counts of 20-day-old seedlings (r = −.46**). The isolation of *Pythium* spp. was negatively correlated with stand counts 35 and 117 days after planting (r = −.50** and −.55**, respectively).

Foliage was harvested 7 January and again 12 March 1975. There were no significant differences in yield among treatments for either harvest or the total of the two harvests. Yields ranged from 12.8 to 16.1 metric tons per hectare(ha) in the first cutting and from 10.8 to 14.5 tons/ha in the second cutting. All of the first harvest was green and acceptable for processing, but 20-40% of the leaves in the second harvest were yellow and nonacceptable. Total yields of green, usable leaves were 26.5, 24.0, 23.9, and 23.8 tons/ha in the MBC + film,

TABLE 4. Correlation coefficients of populations of fungi in soil, and the root-gall index, with root and hypocotyl discoloration of seedlings, stand counts, and growth ratings in the 1974-75 winter crop of spinach grown under various soil treatments[a]

Variable	Days after planting	Root and hypocotyl discoloration, 19-20 days	Stand, days		Growth rating, 62 days
			11	117	
Pythium spp.	5	.54**[a]	−.40**	−.35*	−.45**
Fusarium oxysporum	5	.50**	−.48**	−.35*	−.49**
Fusarium solani	5	NS	−.40**	−.31*	−.51**
Total *Fusarium* spp.	5	.33*	−.44**	−.36*	−.55**
Root-gall index	63	NS	NS	NS	−.48**
Pythium spp.	120	.49**	NS	NS	−.34*
F. oxysporum	120	.52**	−.48**	−.39*	NS
F. solani	120	NS	−.48**	−.45**	−.43**
Total *Fusarium* spp.	120	.38*	−.48**	−.46**	NS

[a]* = Significant, $P = 0.05$; ** = highly significant, $P = 0.01$: and NS = not significant.

benomyl + chloroneb, chloroneb, and DD-MENCS + film treatments, respectively; and 21.3 and 21.1 in the controls (with and without film).

In one October test, soil was treated with the herbicide chloropropham, and plants in all plots were stunted, regardless of soil treatment. However, when the plots were tilled and replanted in January, plants in plots treated with DD-MENCS were a darker green throughout the experiment than were plants in other treatments. Also, when irrigation was occasionally delayed early in the crop, plants in the ethoprop and check plots wilted while plants in the DD-MENCS plots appeared normal. When the herbicide cycloate was used, no stunting was observed.

There was a significant negative correlation of yield with the root disease index in seedlings ($r = -.32^*$), but not with populations of fungi in the soil or the isolation of fungi from seedlings. Yield was negatively correlated with total populations of stylet-bearing nematodes 63 days after planting ($r = -.33^*$) but not with the root-gall index.

DISCUSSION

Our data showing that high soil temperatures increased the severity of root rot and damping-off in young spinach plants in both field and growth chamber experiments agree with the results of Leach (11) who reported that spinach emerged best at constant temperatures of 20 and 25 C in pasteurized soil, but did not emerge at 35 C; in soil infested with *P. ultimum,* severe damping-off occurred at 8 C and above, but not at 4 C; and with *R. solani* damping-off and root rot was severe only at 16 C and above.

We found that root rot also was severe at low temperatures, especially when young seedlings were subjected to freezing. Freezing injury was tested only once, and more research is needed to determine the interactions of specific soilborne pathogens and cold injury. In Kansas, severe injury was not observed with temperatures as low as −21 C during 2 successive years (13). In Virginia, however, freezing injury was reported to cause symptoms similar to Fusarium wilt (4).

Root-knot nematodes did not cause severe injury to plants in our tests, but when present, were controlled with DD-MENCS, MBC, and ethoprop. Nevertheless, the negative correlation between root-gall indices and growth ratings indicate that root-knot nematodes, even in low numbers, adversely affect plant growth. Good (7) reported that yield of winter spinach in Georgia was greatly reduced in untreated soil heavily infested with *M. incognita* as compared with the yield in soil treated with ethylene dibromide. In our tests, yield was negatively correlated with total populations of stylet-bearing nematodes, suggesting that polyspecific populations of *M. incognita, T. christiei,* and *C. ornatus* reduce yields. Thus, it is possible that significant yield reduction could result from nematode injury in some grower fields if initial populations of nematodes were greater than in our tests.

We showed that soil fumigation provided full season control of soilborne pathogenic fungi and nematodes, but yields were not always increased. In a commercial field near Autreyville, Georgia, approximately 1 ha was fumigated with DD-MENCS in 1974. Spinach grown in that area yielded 22 metric tons/ha and the plants were green and succulent at harvest. In contrast, spinach grown on nearby, nontreated areas yielded only 9 metric tons/ha and exhibited various severities of decline at harvest. Similar results were noted in 1975 on the same farm. In plots of Chesapeake at the Station decline was less severe and fumigation gave less stimulation of growth.

Spinach is very sensitive to nitrogen deficiency; smooth-leafed cultivars are more sensitive than savoy-type cultivars (2). We top-dressed spinach in soil-pesticide tests with nitrogen every week, and in some instances applied as much as 300 kg/ha during the season. Also, the plants were never under stress for soil moisture during the last few weeks before harvest. These factors could have delayed or decreased the symptoms of decline. Because we rarely observed symptoms of severe vascular discoloration or decay of the lower tap root that are associated with decline in other states (1, 4), we have concluded that the decline and yellows syndrome that we have observed in south Georgia is not caused by Fusarium wilt, but results from the interactions of several factors. The onset of decline was invariably associated with cultural problems, such as nutrient imbalance, or periods of excess soil moisture; or with climatic factors, such as abrupt temperature changes or prolonged periods of cloudy, wet weather. Therefore, we have concluded that decline results from the deleterious interaction among cultural and climatic factors, and populations of soil-borne fungi and nematodes.

Many soil-borne fungi and nematodes present in Coastal Plain soils are potential pathogens and could cause problems in commercial spinach production; however, chemicals are available to provide adequate control of the deleterious soilborne pathogens.

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