Pathogenicity and Host Range of Fusarium oxysporum from Sweet Basil and Evaluation of Disease Control Methods

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ABSTRACT

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Fusarium wilt of basil (Ocimum basilicum) was first detected in a commercial greenhouse in South Carolina in April 1992. Four isolates of Fusarium oxysporum from sweet or bush (cv. Minimum) basil were pathogenic on these hosts in greenhouse tests. Height and leaf area of inoculated sweet basil plants were reduced by 30 and 40%, respectively, compared with noninoculated plants. F. oxysporum reduced fresh weights of sweet and lemon basil (cv. Citriodorum) but had no effect on six other herbs in the family Lamiaceae. Neither mancozeb, iprodione, benomyl, nor Streptomyces griseoviridis prevented Fusarium wilt of sweet basil when applied as a drench 3 days prior to inoculation.

Sweet or common basil (Ocimum basilicum L.) is a cultivated herb grown commercially in the Mediterranean region of southern Europe and North Africa and in the United States. It is used fresh and dried, for flavorings and fragrances and in traditional medicines (18). Three cultivars with distinctive characteristics are recognized as subgroups within O. basilicum grown in the United States: lemon basil cv. Citriodorum, bush basil cv. Minimum, and purple basil cv. Purpurascens (1). In the United States, production of herbs for the fresh market has increased rapidly during the past 5 yr. Over 910 t of miscellaneous fresh herbs (anise, basil, chives, cilantro, dill, mint, and thyme) were produced in 1992 and 1993 (15).

Fusarium oxysporum Schlechtend.:Fr. causes vascular wilt diseases on many different crops, and over 120 formae speciales and races have been identified (2). Fusarium wilt of basil was first detected in southern Russia in 1956 (20) and in the Abkhazia region of Georgia in 1957 (14). It was subsequently found in Italy in 1975 (8) and France in 1982 (16). This disease was first observed on sweet basil in the United States in Massachusetts in 1990 (21). Since then it has been reported in California (3), New Mexico, Nevada, New York, Colorado, and Connecticut (6).

Typical symptoms of Fusarium wilt on basil include defoliation without chloro-

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sis of leaves, browning of vascular tissue, dark longitudinal streaks on stems and petioles, and severe wilting (3,14,21). The pathogen can be seedborne (6,14). Isolates of *F. oxysporum* originating from basil seed and diseased plants collected throughout the United States belong to the same vegetative compatibility group (6).

This report describes isolation of F. oxysporum from basil in South Carolina. The objectives of this study were to confirm pathogenicity, quantify potential yield losses from Fusarium wilt, examine the susceptibility of other herbs in the family Lamiaceae to F. oxysporum from basil, and test several chemical and biofungicides for disease control. A preliminary report has been published (12).

MATERIALS AND METHODS

Fusarium isolates. Several sweet basil plants with characteristic symptoms of Fusarium wilt were obtained from a commercial greenhouse in Charleston County, South Carolina, in April 1992. Stem segments were surface-disinfested in 0.5% NaOCl for 30 sec and placed on 0.25-strength potato-dextrose agar (QPDA). One isolate of F. oxysporum, designated F3, was obtained from these plants. Subsequently, bush basil plants grown at the Coastal Research and Education Center, Charleston, that displayed similar symptoms were obtained, and isolation from stem and root segments yielded three additional isolates of F. oxysporum, designated F1, F2, and F4. All four isolates were grown on PDA slants and carnation-leaf agar and were examined microscopically to verify identification as F. oxysporum (17).

Two seed lots, one of bush basil originating from Italy and one of sweet basil of unknown origin, were assayed for contamination by F. oxysporum. Then 300 seeds from each lot were placed on Komada's agar (13), 25 seeds per 9-cm-diameter petri plate, and incubated at 22-24 C and a 12-hr photoperiod. Colonies of Fusarium originating from the seed were compared with known isolates of F. oxysporum from basil grown on Komada's agar under the same conditions.

Pathogenicity tests. The four isolates of F. oxysporum were grown on QPDA at ambient temperature (22-24 C) and a 12-hr photoperiod for 1 wk. Three 0.5cm-diameter agar plugs were placed in 50 ml of potato-dextrose broth (PDB) in 250-ml flasks and shaken at 150 rpm at ambient temperature for 4 days. Cultures were decanted through sterile cheesecloth, and mycelial mats were washed with 5-10 ml of sterile distilled water. The culture filtrate was centrifuged in a clinical centrifuge at 5,000 rpm for 10 min, the supernatant was decanted, and the pelleted microconidia were resuspended in 10 ml of sterile distilled water. Spore concentration was determined by counting in a hemacytometer and adjusted to 10⁷ microconidia per milliliter. Dilutions were plated onto QPDA to verify viable spore concentrations.

Sweet and bush basil were seeded in vermiculite:peat (60:40, Fafard No. 2 commercial potting mix, Piedmont Nursery Supply, Spartanburg, SC) in 72cell plastic trays. When 4 wk old, seedlings were transplanted individually into 10-cm-diameter plastic pots 2 days before inoculation. Roots were wounded by stabbing with a sterile spatula four times before 10 ml of the spore suspension was pipetted into the pot near the stem base and roots of the plant. In addition to the four F. oxysporum isolates, two sets of control plants, wounded and not wounded, were inoculated with sterile distilled water. Six plants per treatment were used in a randomized complete block design. The experiment was performed twice in a greenhouse.

Plants were harvested 11 and 16 days after inoculation in the first and second trials, respectively. Height and leaf area of sweet basil plants were measured with a LI-COR LI-3100 leaf area meter. The second lowest pair of leaves on the plants was collected, and petioles were surfacedisinfested in 0.25% NaOCl for 30 sec,

cut in half, and placed onto QPDA. Two 1-cm sections of two randomly selected lateral stems of bush basil plants also were surface-disinfested and plated. Plates were incubated at 22–24 C under a 12-hr photoperiod for 3 days. The number of tissue pieces yielding the pathogen was recorded for each plant.

Host range tests. Seven herbs in the family Lamiaceae were tested for susceptibility to F. oxysporum. In addition to sweet basil as a control, catmint (Nepeta cataria L.), lemon basil, and sage (Salvia officinalis L.) were tested in three trials and lemon balm (Melissa officinalis L.), oregano (Origanum vulgare L.), rosemary (Rosmarinus officinalis L.), and thyme (Thymus vulgaris L.) were tested in two trials. All herbs except rosemary, which was raised from cuttings, were grown from seed in commercial potting mix. After 4 wk, plants (except oregano and thyme plants) were transplanted singly into 10-cm-diameter plastic pots. Because individual oregano and thyme plants are considerably smaller than those of the other herbs, each pot contained five to 10 plants of these two herbs.

F. oxysporum isolates F3 and F4 were selected for use in this experiment based on consistent infection and reisolation in the pathogenicity experiment; they also represented the original hosts, sweet and bush basil, respectively. These two isolates were grown in PDB and herbs were inoculated as described previously, except that a 10⁶ microconidia per milliliter suspension was used. Control plants were wounded and inoculated with sterile distilled water. Three weeks after inoculation, plants were checked for symptoms of Fusarium wilt. Herbs were cut at the soil surface, and two 1-cm sections

from the lower portion of the stem were surface-disinfested in 0.25% NaOCl and placed onto QPDA. Plates were incubated at ambient temperature (22-26 C) under a 12-hr photoperiod for 3-5 days. The number of stem sections yielding F. oxysporum colonies was recorded. Colonies and the original isolates were transferred to Komada's agar to verify identification as F. oxysporum. The experiment was designed as a randomized complete block with 10 replicate pots per treatment. The experiment was performed three times in a greenhouse; fresh weights of all herbs were recorded for two trials.

Control of Fusarium wilt on sweet basil. Four-week-old sweet basil plants were grown as described above. The experimental design was a randomized complete block with 10 replicate plants assigned to blocks according to height. Suspensions of mancozeb (Manzate 200DF), iprodione (Rovral 4F), and benomyl (Benlate 50WP) were prepared at 0.1 and 0.01 the concentration applied to rosemary to control web blight (9). and 100 ml of each fungicide concentration was added to treated pots. Mycostop (Streptomyces griseoviridis strain K61, 108 cfu/g of product, AgBio Development, Westminster, CO) was suspended in water and diluted to 0.1 and 0.01% a.i., and 100 ml was added to treatment pots. Two sets of control plants were treated with 100 ml of sterile distilled water. S. griseoviridis was reapplied 14 days later in accordance with the manufacturer's instructions. Three days after treatments were applied, plants were inoculated with F. oxysporum isolate F3 as described previously except that roots of basil plants were not wounded. One set of plants previously

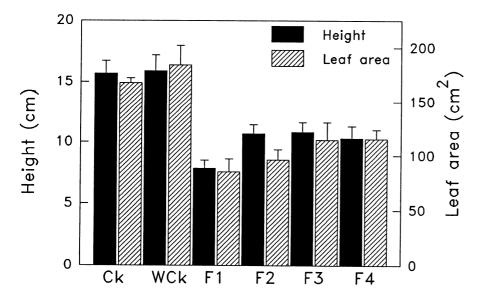


Fig. 1. Height and leaf area of sweet basil inoculated with four isolates (F1, F2, F3, F4) of Fusarium oxysporum obtained from sweet or bush basil compared with a nonwounded, noninoculated check (Ck) and a wounded, noninoculated check (WCk). Error bars show one standard error. Height and leaf area are significantly lower for inoculated plants than for noninoculated plants (Waller-Duncan k-ratio t test, k = 500).

treated with sterile distilled water was also inoculated with F. oxysporum, while the other set received sterile distilled water again.

Approximately 3 wk after inoculation, height of plants was measured. Basil plants were rated for symptoms of Fusarium wilt on a scale of 1-5, where 1 = no visible symptoms, 2 = upperleaves small and curled, 3 = plant stunted with small, curled leaves, 4 = plant stunted with dark streaks on stem, and 5 =plant severely wilted or dead. Herbs were cut at the soil surface and two stem sections were plated as described for host range tests. Colonies of F. oxysporum plus the original isolates were transferred to Komada's agar to verify identification as F. oxysporum. Areas of leaves longer than 1.5 cm, fresh weights of leaves, and dry weight (100 C for 24 hr) of leaves were recorded.

In the first trial, some fungicides were phytotoxic at the 0.01 concentration tested. Therefore, 0.001 and 0.0001 concentrations were tested in the second trial. In the third trial, only benomyl was tested, at the same concentrations as in the second trial. The first trial was conducted in a greenhouse at 14–34 C, the second trial was conducted in a growth chamber at 21 C and 14-hr photoperiod, and the third trial was conducted in a growth cabinet (Labline Instruments, Melrose Park, IL) at 26–28 C day and 20–22 C night (12-hr cycle) and a 12-hr photoperiod.

Data analysis. Plant height, leaf area, fresh and dry weight, and percent recovery of the pathogen were analyzed with PROC GLM of SAS, Version 6.04 (SAS Institute, Cary, NC). Before analysis, data were checked for homogeneity of variance and normality. The squareroot transformation was used with some fresh weights and percent recovery of F. oxysporum from herbs. Percentage recovery also was weighted for analysis by the variance, which was calculated from the predicted probability of each plant being infected (19). Disease ratings were weighted by the percentage of the plant affected (1 = 0%, 2 = 10%, 3 =50%, 4 = 70%, and 5 = 100%) and analyzed with PROC CATMOD of SAS. Treatment means were compared with the Waller-Duncan k-ratio t test or preplanned orthogonal, single-degree-offreedom contrasts.

RESULTS

Pathogenicity tests. All four isolates of F. oxysporum, originally obtained from sweet or bush basil, were pathogenic on these hosts. In both repetitions of the experiment, F. oxysporum significantly (P = 0.0001) reduced height (25 and 37%, respectively) and leaf area (39 and 42%, respectively) of sweet basil (Fig. 1) compared with noninoculated control plants. Nonwounded and wounded control treatments did not differ. In the first

pathogenicity trial, F. oxysporum was recovered from at least one tissue piece of all inoculated sweet and bush basil plants except one sweet basil inoculated with isolate F4. In the second trial, F. oxysporum was recovered from all inoculated sweet basil plants except one plant inoculated with isolate F1 and and one with isolate F2. Recovery of isolates F1 and F2 on bush basil also was slightly lower, five of six and two of six plants, respectively. All control plants in both trials remained healthy and F. oxysporum was not recovered from any noninoculated plant except from two of 12 control bush basil plants in the second trial. Because the bush basil seed lot was subsequently found to contain 12% contaminated seeds, the infected control plants might have originated from contaminated seeds. F. oxysporum was not recovered from any sweet basil seeds, so this seed lot was used in all subsequent experiments.

Host range tests. When inoculated with F. oxysporum isolate F3 or F4, only sweet basil displayed symptoms typical of Fusarium wilt. No symptoms were visible on the other inoculated herbs or on noninoculated plants. Only sweet and lemon basil showed significant differences in fresh weight between inoculated and noninoculated plants (Table 1). Both isolates of F. oxysporum reduced fresh weights of sweet and lemon basil by 43 and 21%, respectively, compared with noninoculated plants (P = 0.0001), and the response was similar for both isolates.

F. oxysporum was recovered significantly more often from inoculated than from noninoculated plants of sweet basil, lemon basil, and thyme (Table 2). F. oxysporum was recovered from 50.5% (±10.8% SE) of inoculated lemon basil and 55.0% (±12.2%) of inoculated thyme plants. Occasionally, a F. oxysporum isolate indistinguishable from isolate F3 was recovered from noninoculated control plants.

Control of Fusarium wilt. In the first trial, all fungicides (mancozeb, iprodione, and benomyl) were phytotoxic at the 0.1 concentration tested (data not shown). All inoculated plants treated with the 0.01 concentration or Mycostop were shorter and had lower leaf fresh and dry weights than noninoculated plants (kratio = 500) (Table 3). In addition, plants treated with mancozeb and iprodione were shorter and those treated with 0.1% Mycostop and mancozeb had smaller leaves (lower leaf fresh and dry weights) than inoculated control plants. Plants treated with 1.88 ml/L of iprodione also showed some leaf necrosis (data not shown)

In the second trial with 0.001 and 0.0001 concentrations of the same three fungicides, only benomyl at 180 mg/L reduced symptoms and increased plant height (k-ratio = 500) relative to the inoculated control plants (Table 4).

Benomyl also increased leaf area and fresh and dry weights (k-ratio = 100). Disease ratings and growth of all other plants inoculated and treated with a fungicide or a biofungicide were not significantly different from those of the inoculated control.

In the third trial, only benomyl was

tested again, at the same concentrations used in the second trial. Disease ratings and fresh and dry weights of leaves were significantly (k-ratio = 100) lower for all inoculated treatments than for the noninoculated control, and there was no significant difference between benomyltreated plants and inoculated control

Table 1. Fresh weights of herbs inoculated with Fusarium oxysporum isolates F3 and F4 from basil

Treatment	Sweet basil	Lemon basil	Catmint	Oregano	Rosemary	Sage	Thyme	Lemon balm
Noninoculated	30.2	23.6	19.9	7.6	6.6	10.7	8.7	16.8
Isolate F3	17.7	19.9	20.0	8.1	6.8	10.4	8.9	17.1
Isolate F4	17.2	17.4	19.8	7.3	7.2	11.4	9.7	15.9
Contrast								
Noninoc. vs. inoc.	0.0001^{z}	0.0001	0.97	0.91	0.50	0.76	0.41	0.86
F3 vs. F4	0.80	0.054	0.90	0.35	0.57	0.23	0.36	0.45

^yHerb roots were wounded by stabbing, then were inoculated with 10 ml of a 10⁶ microconidia per milliliter suspension of *F. oxysporum*.

Probability of a greater F value.

Table 2. Percentage recovery of Fusarium oxysporum from eight herbs inoculated with isolate F3 or F4 from basil or not inoculated^w

Treatment	Sweet basil	Lemon basil	Oregano	Rosemary	Sage	Thyme	Catmint	Lemon balm
Noninoculated	6 ^x	10	5	0	13	0	0	0
Isolate F3	92	44	0	25	13	45	3	Ō
Isolate F4	93	57	16	25	10	65	0	Ö
Contrast								
Noninoc. vs. inoc.	0.0002^{y}	0.0073	0.97	0.20	0.46	0.01	ND²	ND
F3 vs. F4	0.88	0.78	0.43	0.66	0.70	0.45	ND	ND

^{*}Herb roots were wounded by stabbing, then were inoculated with 10 ml of a 10⁶ microconidia per milliliter suspension of *F. oxysporum*. Stem segments were surface-disinfested and plated on 0.25-strength potato-dextrose agar.

² Not determined.

Table 3. Plant growth and recovery of Fusarium oxysporum from inoculated sweet basil plants treated with three fungicides or a biofungicide (trial 1)

Treatment	Plant height (cm)	Fresh weight of leaves (g)	Dry weight of leaves (g)	Recovery of F. oxysporum'
Noninoculated control	37.2 a	29.7 a	9.3 a	0
Inoculated control	31.0 bc	15.6 b	4.1 b	10
Mycostop, 0.1 g/L	32.9 b	13.1 bc	3.5 bc	10
Mycostop, 1 g/L	29.1 bcd	9.7 c	2.6 cd	6
Benomyl, 1.8 g/L	26.7 cd	13.8 b	3.0 bcd	ĺ
Iprodione, 1.88 ml/L	26.2 d	12.5 bc	2.6 cd	4
Mancozeb, 1.2 g/L	25.3 d	9.8 с	2.6 cd	10
Min. sig. dif. ^y	4.3	3.9	1.3	^z

wPlants were drenched with 100 ml of the treatment 3 days before inoculation with 10 ml of a 10⁷ microconidia per milliliter suspension of F. oxysporum isolate F3. Plants treated with 10-fold higher concentrations of the three fungicides were severely stunted (data not shown).

² Treatment totals were not analyzed.

^{*}Percentages are means of three trials (30 per treatment) for sweet and lemon basil, sage, and catmint and of two trials (20 per treatment) for oregano, thyme, rosemary, and lemon balm.

^y Probability of a greater F value.

^x Number of plants (10 per treatment) from which *F. oxysporum* was recovered on 0.25-strength potato-dextrose agar.

^y Minimum significant difference, Waller-Duncan k-ratio t test, k = 500. Means in a column followed by the same letter are not significantly different.

plants (Table 5). Inoculated plants were not significantly shorter than noninoculated plants.

DISCUSSION

Isolates of *F. oxysporum* that cause wilt of basil are specifically pathogenic on basil and have been referred to as *F. oxysporum* f. sp. basilicum (6). Host specificity of *F. o. basilicum* has been reported. *F. oxysporum* isolated from basil in France was not pathogenic on Dianthus (16), and *F. oxysporum* isolated from basil in European Georgia was specific to basil (14). Three different cultivated varieties of basil (sweet, bush, and lemon) were all susceptible to *F. oxysporum*. Plant height, leaf area, and fresh and dry weights of leaves were reduced in sweet basil. Because of this

yield reduction, the persistence of the pathogen in soil (14), and seed transmission (6,14), Fusarium wilt has the potential to limit basil production in the United States.

F. oxysporum was less virulent on lemon basil than on sweet basil, since the former showed no vascular discoloration, dark streaks on stems, or wilting. Although fresh weight of whole plants was reduced by F. oxysporum, the reduction was not as dramatic as with sweet basil. Plant height and leaf area of lemon basil, although not measured, appeared not to be affected. Lemon basil, and perhaps other cultivars or types of basil, may be alternatives for producers who find it difficult to grow sweet basil because of losses to Fusarium wilt.

Although not pathogenic on herbs

Table 4. Disease rating and growth of sweet basil plants treated with three fungicides or a biofungicide and inoculated with Fusarium oxysporum (trial 2)

Treatment*	Disease severity ^x (%)	Plant height (cm)	Leaf area (cm²)	Fresh weight of leaves (g)	Dry weight of leaves (g)
Noninoculated control	1.1*	49.1 a	495.0 a	15.8 a	2.4 a
Inoculated control	53.3	33.1 b	268.4 bcd	8.4 bc	1.2 bc
Mycostop, 0.1 g/L	67.8	26.7 b	201.4 cd	6.5 c	0.8 c
Mycostop, 1 g/L	50.0	35.8 b	206.9 cd	7.2 c	0.9 с
Benomyl, 18 mg/L	58.9	35.2 b	239.7 bcd	7.7 c	1.1 c
Benomyl, 180 mg/L	1.1*	50.1 a	414.6 ab	13.7 a	2.0 ab
Iprodione, 0.019 ml/L	34.4	37.6 ab	237.5 bcd	7.8 c	1.1 c
Iprodione, 0.19 ml/L	35.6	38.0 ab	354.0 abc	9.8 bc	1.5 abc
Mancozeb, 12 mg/L	68.9	29.0 b	159.9 d	5.2 c	0.7 с
Mancozeb, 120 mg/L	60.0	32.0 b	224.6 bcd	6.8 c	1.0 c
Min. sig. dif. ^y	ND^z	12.60	192.67	5.70	0.91

wPlants were drenched with 100 ml of the treatment 3 days before inoculation with 10 ml of a 10⁷ microconidia per milliliter suspension of *F. oxysporum* isolate F3.

Table 5. Disease rating, plant growth, and recovery of *Fusarium oxysporum* from sweet basil plants treated with benomyl before inoculation (trial 3)

Treatment ^t	Disease severity ^u (%)	Plant height (cm)	Fresh weight of leaves (g)	Dry weight of leaves (g)	Recovery of F. oxysporum
Noninoculated control	2.2*	47.2	23.2 a	10.5 a	0
Inoculated control	57.8	40.8	8.7 b	4.9 b	7
Benomyl, 180 mg/L	47.8	44.7	12.0 b	7.6 ab	9
Benomyl, 18 mg/L	44.4	43.3	14.4 b	7.7 ab	5
Min. sig. dif ^w	ND^x	NSy	8.7	3.8	z

¹ Plants were drenched with 100 ml of benomyl 3 days before inoculation with 10 ml of a 10⁷ microconidia per milliliter suspension of *F. oxysporum* isolate F3.

other than basil in the family Lamiaceae, F. oxysporum can infect and colonize stem vascular tissue of several herbs. F. oxysporum was recovered from up to 25 and 65% of inoculated rosemary and thyme plants, respectively. Nonsymptomatic infected rosemary cuttings could be an avenue for dispersal of the pathogen, since rosemary is often propagated by cuttings. In addition, colonization of nonhost herbs may be a mechanism by which F. oxysporum persists in the absence of basil. Incorporating residues of nonhost crops infected with F. oxysporum into soil may increase inoculum density of the pathogen. Other formae speciales of F. oxysporum, such as apii and melonis, have been recovered from roots of nonhost crops grown in rotation with celery (5) and muskmelon (7), respectively. On the other hand, F. oxysporum was rarely recovered from inoculated catmint and never recovered from lemon balm. These herbs could be used in a rotation sequence in mixed herb production and planted into soil infested with F. oxysporum.

Control of Fusarium wilts with fungicides is difficult. Three fungicides (mancozeb, iprodione, and benomyl) and a commercial biofungicide (Mycostop), none of which is registered for use on basil, did not protect sweet basil from infection by *F. oxysporum* when applied as a drench 3 days before inoculation. Moreover, several of the products reduced plant height or leaf weight at concentrations that did not prevent infection. These three fungicides have controlled web blight of rosemary when applied to foliage, apparently without phytotoxicity (9).

Other control measures, such as seed and soil treatments, tolerant or resistant cultivars, and specific fertilization regimes should be explored. For example, raising soil pH to 6.0-7.5 and fertilizing with nitrate nitrogen has successfully controlled Fusarium wilts of tomato, chrysanthemum, and other crops (11). In European Georgia, diseasefree basil could be produced after fumigating infested soil with 750-1,000 kg/ha of methyl bromide (4). Trichoderma viride Pers.:Fr. applied to the rhizosphere reduced wilt incidence and increased basil growth (10). At present, prevention of Fusarium wilt of basil by exclusion of the pathogen combined with eradication after detection is the most promising control strategy. Basil producers can grow catmint and lemon balm in Fusarium-infested soil without increasing the inoculum density.

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^x Disease was rated on a scale of 1-5, where 1 = no symptoms and 5 = plant severely wilted or dead, and ratings were weighted by the percentage of the plant affected (1 = 0%, 2 = 10%, 3 = 50%, 4 = 70%, and 5 = 100%). Disease severities followed by an asterisk are significantly different from the inoculated control (P = 0.0001).

^y Minimum significant difference, Waller-Duncan k-ratio t test, k = 500. Means in a column followed by the same letter are not significantly different.

^z Not done.

^u Disease was rated on a scale of $\hat{1}$ -5, where 1 = no symptoms and 5 = plant severely wilted or dead, and ratings were weighted by the percentage of the plant affected (1 = 0%, 2 = 10%, 3 = 50%, 4 = 70%, and 5 = 100%). Disease severities followed by an asterisk are significantly different from the inoculated control (P = 0.0005).

Number of plants (10 per treatment) from which F. oxysporum was recovered on 0.25-strength potato-dextrose agar.

[&]quot;Minimum significant difference, Waller-Duncan k-ratio t test, k = 100. Means in a column followed by the same letter are not significantly different.

^{*} Not done.

y No significant differences among treatments.

² Treatment totals were not analyzed.

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