Comparison of Ammonium Sulfate and Calcium Nitrate Fertilization Effects on Verticillium Wilt of Eggplant

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ABSTRACT

Elmer, W. H., and Ferrandino, F. J. 1994. Comparison of ammonium sulfate and calcium nitrate fertilization effects on Verticillium wilt of eggplant. Plant Dis. 78:811-816.

Eggplants fertilized with either $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ were grown over three seasons in soil with high, low, or no inoculum of *Verticillium dahliae*. Early in the season before the onset of symptoms, eggplants treated with $(NH_4)_2SO_4$ had more nonstructual carbohydrates in the roots and leaves than plants treated with $Ca(NO_3)_2$, and the rhizosphere was more acidic. Densities of total rhizobacteria and fluorescent pseudomonads were not affected by nitrogen fertilizers. After anthesis when symptoms appeared, the fertilizer treatments did not affect the percentage of diseased foliage or the amount of root and stem colonization by *V. dahliae*. However, fertilization with $(NH_4)_2SO_4$ was associated with increased leaf and root levels of N, P, and Mn, compared with fertilization with $Ca(NO_3)_2$. In soils with high inoculum density, there was no difference between the nitrogen fertilizers in effect on eggplant growth or yield. In soils with low inoculum densities, however, plants fertilized with $(NH_4)_2SO_4$ were significantly larger, had more large leaves and fewer small leaves, and had 33–44% more marketable yield than plants fertilized with $Ca(NO_3)_2$. Fertilization with $(NH_4)_2SO_4$ in soil with low inoculum densities may be useful in the management of Verticillium wilt of eggplant.

Additional keywords: mineral nutrition, soilborne pathogens

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a major yield-limiting disease of eggplant (*Solanum melongena* L.) in the northeastern United States. Yield losses of 50% are not uncommon in certain years (9). In addition to wilt, symptoms include stunting, chlorosis, and defoliation. No cultivars are resistant (9), and disease management includes fumigation and/or 2- to 5-yr rotations. However, the wide host range of *V. dahliae* (33) makes rotation beyond the 2-yr schedule difficult to arrange with nonsusceptible crops. Shortened 2-yr rotations rarely result in sufficient reduction in inoculum densities to prevent Verticillium wilt of eggplant from reappearing. Other practices that could complement shortened rotation practices and avoid fumigation are urgently needed.

Verticillium wilt diseases are reportedly less severe when nitrogen is supplied as an ammoniacal source than as a nitrate source (19,20,30,31,37). However, much of the literature reports data from seedling studies in the greenhouse and not from field trials (7,24,31,36). The use of highly susceptible cultivars (22) and/or the rapid conversion of NH_4-N to NO_3-N (20,22) in the field may have prevented this practice from being widely accepted. It would be useful to know whether or not suppression of Verticillium wilt with NH_4-N in the field is practical.

In greenhouse and laboratory studies, NH_4-N fertilizers have been reported to have a direct deleterious effect on the pathogen (7,25,42). However, it is well known that NH_4^+ absorption reduces uptake in cations such as K^+ and Ca^{+2}, whereas NO_3^- tends to reduce absorption of PO_4^{-3} and SO_4^{-2} (17,29). Compared to NO_3-N, NH_4-N encourages flowering (12,17), stimulates carbohydrate production (4,29), and affects the rhizosphere differently (20,22,37). Ammonium nutrition reduces the rhizosphere pH, which may also influence the antagonistic activity of rhizobacteria (38). Rhizosphere acidification also increases the availability and uptake of trace elements (22), which are associated with Verticillium wilt suppression (2,6). The effect of NH_4-N fertilizers on the host response in the field has been neglected. Our objectives were to compare the effects of $(NH_4)_2SO_4$ with those of $Ca(NO_3)_2$ on yield and Verticillium wilt of eggplant and to understand how each nitrogen fertilizer affects plant growth, flowering, tissue composition, and colonization by *V. dahliae* and rhizosphere pH and soil densities of bacteria in the rhizosphere.

MATERIALS AND METHODS

Production of plants. Seeds of the high-yielding, moderately tolerant eggplant cultivar Agway Super Hybrid (Agway, Inc., Syracuse, NY) were sown into 7.5 × 7.5 cm cells containing potting mix (Promix BX, Premier Brand, New Rochelle, NY). Seeds were sown in mid- to late March, transplanted to pots for 6–7 wk in the greenhouse, and transplanted into the field on 12 June 1990 and 30 June 1991 and 1992.

Plot establishment. The field experiments were conducted at the Lockwood Farm in Hamden, Connecticut. The soil was a Cheshire fine sandy loam (57% sand, 33% silt, 10% clay) with a pH of 6.1. Soil fertility was determined at the time of planting by taking a composite soil sample containing 16 randomly sampled soil cores to a depth of 15 cm. Soil fertility each year did not vary greatly and averaged, per gram of soil, 25.0 μg of NO_3-N, 12.0 μg of NH_4-N, 70.0 μg of PO_3-P, 160 μg of K, 1,600 μg of Ca, and 152 μg of Mg, as determined according to the procedures of Lunt et al (26). Each year at planting, fenamiphos (Nemacur 2E) was incorporated into the soil at 6.25 L/ha to suppress lesion nematodes (*Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven). Plots received superphosphate and KCl at 56 kg/ha and $CaSO_4$ at 22 kg/ha to provide adequate P, K, Ca, and S and to ensure optimal plant growth.

The three fields used in 1990 varied in cropping history and inoculum densities. One had been cropped to eggplant for two consecutive years and had a 100% incidence of Verticillium wilt the previous year, one had been cropped to tomatoes for 2 yr and had mild symptoms of Verticillium wilt the second year, and one had been cropped to rye for more than 20 yr. Microsclerotia densities in each field were determined (28), and the three fields contained, on an average, 13 ± 4, 0, and 0 microsclerotia per gram of soil, respectively. On the basis of their cropping histories, the three fields were given high, low, and no inoculum treatments of *V. dahliae*, respectively. Transplants were spaced 1.5 m apart in rows spaced 1.8 m apart, eight plants per row. Each soil inoculum treatment (field) consisted of two blocks containing six rows each that were treated with the two fertilizer regimes (three replicate rows per treatment). The plants in the row were treated by hand at planting with $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ at the rate of 112 kg/ha of N and side-dressed again on 12 July at the same rate, for a total of 224 kg/ha of N. Nitrapyrin (N-Serve 24), 2.3 L/ha, was applied to each row in approximately 9.0 L of H_2O immediately after the fertilizers were applied to inhibit nitrifying bacteria.

Accepted for publication 5 May 1994.

In order to reproduce soil with the same relative inoculum densities observed in 1990, infested soil was fumigated under plastic on 19 June 1991 with methyl bromide plus 1% chloropicrin (Brom-O-Gas) at a rate of 243 kg/ha. Twenty-four circular microplots (1 m in diameter) enclosed in flexible plastic sheeting material were dug 30–40 cm deep and spaced 4.5 m apart. Soils were prepared to contain high, low, and no inoculum of *V. dahliae* by filling the plots with naturally infested soil, with a 1:1 (v/v) fumigated soil:infested soil mixture, and with fumigated soil, respectively. Soils in each microplot were then assayed for microsclerotia and found to contain 17 ± 5, 7 ± 3, and 0 microsclerotia per gram of soil for the infested soil, fumigated:infested soil, and fumigated soil, respectively. Four transplants were placed into each microplot and treated with the nitrogen fertilizers as described above. There were four replicates per nitrogen fertilizer/soil inoculum level.

In 1992, only two inoculum densities were used, low and none. A new field containing 9 ± 5 microsclerotia per gram of soil was divided in half and one part was fumigated in April 1992 with 217.6 L/ha of methyl isocyanate and 1,3-dichloropropene (Vorlex). Five plants were transplanted 1.8 m apart in six rows spaced 2.1 m apart in fumigated and nonfumigated soils and treated with the nitrogen fertilizers as described above. There were three replicate rows per treatment.

Yield and sampling. One plant in each row (or microplot) was carefully dug 14 days after transplanting (DAP). This date was before anthesis. A second plant in each replicate row or microplot was sampled after anthesis when at least half of the plants in infested soils had developed symptoms of Verticillium wilt. This date was 38 DAP in 1990, 53 DAP in 1991, and 50 DAP in 1992. All leaves were separated from stems and weighed individually. Leaves were classed into seven known size categories representing 0.15, 0.3, 0.7, 1.2, 1.7, 2.2, and 3.3 dm^2. The total leaf area per plant was estimated as the number of leaves in each size category multiplied by the leaf area of the category. The percentage of leaves with symptoms and the number of flowers per plant were also recorded. At least one asymptomatic leaf from each size class per plant was sampled, washed, rinsed in deionized water, bulked, and freeze-dried for assays described below. Unwashed roots with adhering soil were placed in plastic bags and held at 4 C for 2–8 hr until assayed. Remaining roots were thoroughly washed, rinsed in deionized water, and freeze-dried.

Marketable fruits were weighed individually every 5 days from late July through September. At final harvest, all fruits weighing more than 200 g were removed and weighed. In 1992, all of the plants were harvested at the second sampling, so the yields represent only those fruits on the sampled plants.

Host colonization assays. Stem colonization by *V. dahliae* was determined by isolating the fungus on ethanol-streptomycin sulfate-water agar (ESA) (27) as described elsewhere (8). In 1991, roots from each plant were assayed by placing 2 g of disinfested roots (4% bleach for 4 min, then rinsed) in 100 ml of sterile H_2O and homogenizing them in a Waring blender for 45 sec. Two 10-ml aliquots of the root homgenate were each mixed in a 125-ml flask containing 50 ml of cooled (48–50 C) molten ESA, and five petri plates were poured from each flask (12 ml per plate). Plates were incubated at 20–25 C for 2–3 wk, and colonies with *V. dahliae*-like microsclerotia were counted under a dissecting microscope. There were two samples per plant, and data were averaged and expressed as colony-forming-units of *V. dahliae* per gram of fresh root.

Rhizosphere soil assays. Rhizosphere soil pH was determined in 1991 and 1992 by excising a 15-g sample of feeder roots from each replicate plant with adhering soil, saturating the roots and soil with 0.05 M $CaCl_2$ to form a slurry, then measuring the pH of the slurry. A duplicate 15-g root sample with adhering soil was cut into 1- to 2-cm pieces, and two 5-g root subsamples were agitated for 20–25 min on a wrist-action shaker in 100 ml of sterile distilled water amended with 10 g/L of sodium hexametaphosphate. The soil suspensions were serially diluted in sterile saline for enumeration of total rhizobacteria on Difco nutrient agar and fluorescent pseudomonads on King's medium B agar (5). There were three plates per dilution. Plates were incubated for 3 days at 20–25 C, and plates with colonies between 30 and 300 were counted. Data were enumerated by averaging duplicate plates and subsamples and were expressed as log colony-forming units per gram of rhizosphere soil (oven dry weight equivalent). The actual weight of the rhizosphere soil was quantified by subtracting the washed root weight (roots were caught on 100-μm sieves, rinsed, pressed between absorbent paper towels, and reweighed) and correcting for the percent soil moisture, which was determined independently.

Carbohydrate assays. Two 50-mg samples of ground freeze-dried leaf or root tissue from each plant grown in noninfested soils were digested in α-amylase, and reducing sugars were measured colorimetrically as described elsewhere (39). The average of two determinations from each digested sample was calculated, and values were presented as milligrams of glucose per gram dry weight.

Elemental analyses. Root and leaf tissue samples were prepared for analysis of N and P by H_2SO_4 digestion and analyzed colorimetrically for total N by Nessler reaction and total P by reaction in molybdate (21). Potassium, Ca, Mg, S, Fe, Mn, Cu, and Zn were determined by inductively coupled plasma emission spectroscopy (21). The dried tissue (0.5 g) was digested in HCl and HNO_3 in a CEM MDS 81D microwave (CEM Co., Matthews, NC) and analyzed for B, Ca, Cu, Fe, K, Mg, Mn, S, and Zn on a ARL 3520 ICP-OES spectrophometer (Fison Instruments, Deerborn, MI) according to instructions by the manufacturers. Mineral concentrations were expressed as micromoles per gram of dried tissue.

Statistical procedures. Data from the field experiments in 1990 and 1992 were combined and analyzed as a split-plot design, with inoculum treated as the main plot variable and nitrogen fertilization and time of sampling treated as subplot variables. The 1991 microplot data were analyzed by randomized complete block design, with inoculum, nitrogen fertilization, and the time of sampling all treated as main effects. Pairwise comparisons were made using Tukey's test at $P < 0.05$. Leaf class data were analyzed using the nonparametric Kolmogorov-Smirnov two sample test (3) at $P < 0.05$.

RESULTS

Yield and plant sampling. In the 1990 field experiment, yields were reduced 76 and 40% as a result of Verticillium wilt in the soils with high and low soil inoculum of *V. dahliae*, respectively (Table 1). However, compared with $Ca(NO_3)_2$, fertilization with $(NH_4)_2SO_4$ in 1990 resulted in a significant 44 and 69% higher yield from plants exposed to low and no soil inoculum, respectively. The 1991 microplot yields were also reduced 69 and 44% in the high and low soil inoculum treatments, respectively, and in microplots with low soil inoculum, a significant 33% higher yield was obtained from the $(NH_4)_2SO_4$ treatment than from the $Ca(NO_3)_2$ treatment. In both experiments, there was no fertilizer effect on yield in the soils with high inoculum densities. The yield in 1992 was taken only at the second sampling and therefore did not represent the entire season, but plants fertilized with $(NH_4)_2SO_4$ had a 52% higher fruit weight than those fertilized with $Ca(NO_3)_2$ (*data not presented*).

Regardless of the fertilizer treatment, eggplants grown in soil with high inoculum density were stunted and exhibited severe symptoms of Verticillium wilt. Since the two fertilizer treatments did not differ in their effect on plant growth, these data are not presented. However, when eggplants were grown in soil with low inoculum, plants treated with $(NH_4)_2SO_4$ grew faster and accumulated more dry weight than those treated with $Ca(NO_3)_2$ in 2 of 3 yr, but these growth differences were observed only after the

onset of symptoms (Table 2). There was no effect on the dry weight of plants in the absence of disease.

The first signs of wilt and chlorosis were detected on 30, 30, and 37 DAP for 1990, 1991, and 1992, respectively. Symptoms appeared on plants exposed to low inoculum as readily as on those grown in soil with high inoculum density. Plants in fumigated soil treated with $(NH_4)_2SO_4$ had more flowers than those treated with $Ca(NO_3)_2$, but this difference was not significant on infected plants (Table 3). The amount of symptomatic foliage was never greater than 10% of the total leaf area of the plant, and the fertilizer treatments had no effect on the percentage of leaf symptoms. Between 30 and 85% of the stem nodes from infected plants were colonized by *V. dahliae*, and $(NH_4)_2SO_4$ fertilization reduced colonization compared to $Ca(NO_3)_2$ in only 1 yr (Table 3). In 1991 when the roots were assayed for *V. dahliae*, the fungus was detected only after anthesis when symptoms appeared, and the fertilization treatments had no effect (*data not presented*).

Although total leaf area was not significantly affected by the fertilizers (*data not presented*), plants treated with $(NH_4)_2SO_4$ had more large leaves (1.2–3.3 dm^2) and fewer small leaves (0.15–1.2 dm^2) in the second sampling than those treated with $Ca(NO_3)_2$ in low inoculum soils (Fig. 1). The Kolmogorov-Smirnov two sample test (3) at $P < 0.05$ was used to test for a significant difference in the leaf size class distribution from eggplants in each fertilization treatment. The distribution was significant in the 1990 field trial and in the 1991 microplot study at $P < 0.001$, indicating that plants treated with $(NH_4)_2SO_4$ tended to have more of the total leaf area in large leaves than in small ones.

Effect on rhizosphere. In 1991 and 1992, the mean rhizosphere pH in soils fertilized with $(NH_4)_2SO_4$ was significantly more acidic ($P = 0.001$) than in those treated with $Ca(NO_3)_2$, but only before anthesis (Table 4). The mean densities (log colony-forming units per gram of rhizosphere soil) of total rhizobacteria and fluorescent pseudomonads were not affected by the fertilizers. In the 1991 microplot experiments, the densities of fluorescent pseudomonads in the rhizosphere soil increased ($P = 0.001$) between the first and second sampling, whereas densities of rhizobacteria decreased, but this was not observed in 1992.

Effect on root and leaf carbohydrates. In 1990 and 1992, only tissue from eggplants grown in noninfested soils was analyzed for carbohydrates. Therefore, data were analyzed as a completely randomized block design with N form and time treated as main plot factors. Leaf carbohydrate data from the 1991 microplots were analyzed separately. In the first sampling before anthesis, fertilization with $(NH_4)_2SO_4$ resulted in more total nonstructual carbohydrates in roots and leaves than did fertilization with $Ca(NO_3)_2$ in 2 of 3 yr (Table 5). Averaging over the nitrogen fertilizer, root concentrations were usually higher after anthesis than before, but the levels of leaf carbohydrates did not change over time.

Effect on mineral composition. Leaf and root concentrations of minerals in 1990 and 1992 were not significantly different between the healthy plants grown in noninfested soil and those grown in

Table 1. Effect of nitrogen fertilizers and different inoculum densities of *Verticillium dahliae* on yield of eggplant

Inoculum density[a]	1990 (field)		1991 (microplots)	
N form	kg/plant	no./plant	kg/plant	no./plant
Control				
$(NH_4)_2SO_4$	11.61*[b]	17.4*	3.09	4.7
$Ca(NO_3)_2$	8.04	12.1	3.60	6.0
Low				
$(NH_4)_2SO_4$	7.50*	15.0	2.40*	3.7
$Ca(NO_3)_2$	4.43	11.0	1.61	2.8
High				
$(NH_4)_2SO_4$	2.20	6.6	1.28	3.4
$Ca(NO_3)_2$	2.46	7.5	1.15	3.2

[a] Infested soil was enumerated for microsclerotia of *V. dahliae* using the procedure of Nicot and Rouse (28). The high and low densities were 13 ± 4 and 0 microsclerotia per gram of soil, respectively, in 1990 and 17 ± 5 and 7 ± 3, respectively, in 1991.
[b] The means of three or four replicates, depending on the year. Values followed by an asterisk are significantly different from their respective paired value, using Tukey's test at $P = 0.05$.

Table 2. Effect of nitrogen fertilizers on eggplant dry weights (g) before anthesis and after appearance of wilt symptoms in field or microplot soils infested with low or no inoculum of *Verticillium dahliae*

	Field				Microplots	
	Infested[b]		Control		Infested	Control
Sampling time[a] N form	1990	1992	1990	1992	1991	1991
Before						
$(NH_4)_2SO_4$	5.3[c]	6.2	4.5	9.8	9.3	10.6
$Ca(NO_3)_2$	3.6	6.2	5.3	4.8	10.4	10.8
After						
$(NH_4)_2SO_4$	152.0	175.8*	148.8	140.0	148.7*	208.0
$Ca(NO_3)_2$	145.0	118.8	159.0	131.1	56.0	185.0

[a] Before = before anthesis (14 days after transplanting), after = after anthesis when symptoms appeared in 50% of the plants (37, 53, and 50 days after transplanting in 1990, 1991, and 1992, respectively).
[b] Infested soil was enumerated for microsclerotia of *V. dahliae* using the procedure of Nicot and Rouse (28).
[c] The means of three or four replicates, depending on the year. Values followed by an asterisk are significantly different from their respective paired value, using Tukey's test at $P = 0.05$.

Table 3. Effect of nitrogen fertilizers on flowering, symptoms of wilt, and colonization of stem nodes by *Verticillium dahliae*

	Number of flowers per plant						Percent stem pieces colonized[a]					
	Field				Microplots		Field				Microplots	
	Infested[b]		Control		Infested	Control	Infested		Control		Infested	Control
N form	1990	1992	1990	1992	1991	1991	1990	1992	1990	1992	1991	1991
$(NH_4)_2SO_4$	0.7[c]	8.6	0.7	14.5*	5.8	6.5*	39	43*	0	5	69	0
$Ca(NO_3)_2$	0.6	6.6	0.5	10.6	3.8	3.5	29	84	0	0	56	0

[a] Determined by isolating *V. dahliae* on ethanol-streptomycin agar (27).
[b] Infested soil was enumerated for microsclerotia of *V. dahliae* using the procedure of Nicot and Rouse (28). Densities were 0 and 9 ± 5 microsclerotia per gram of soil, respectively, in 1990 and 1992 and 7 ± 3 in 1991.
[c] The means of three or four replicates, depending on the year. Values followed by an asterisk are significantly different from their respective paired value, using Tukey's test at $P = 0.05$.

soils with low inoculum density except for S, which was greater in diseased leaf tissue ($P = 0.007$, *data not presented*). Root and leaf concentrations of N, P, and Mn were greater in plants fertilized with $(NH_4)_2SO_4$ in 1990 and 1992 (Table 6). The time of sampling or the year in which the study was done did not affect leaf concentrations, but levels of N, P, and Mn were greater in the roots in the second sampling than in the first. The tissue concentrations of K, Ca, S, Mg, Fe, Cu, and Zn did not differ between the nitrogen fertilizers. Leaf tissue in the 1991 microplots followed the same trend, with concentrations of N, P, and Mn being significantly greater in plants treated with $(NH_4)_2SO_4$ than in those fertilized with $Ca(NO_3)_2$ (*data not presented*).

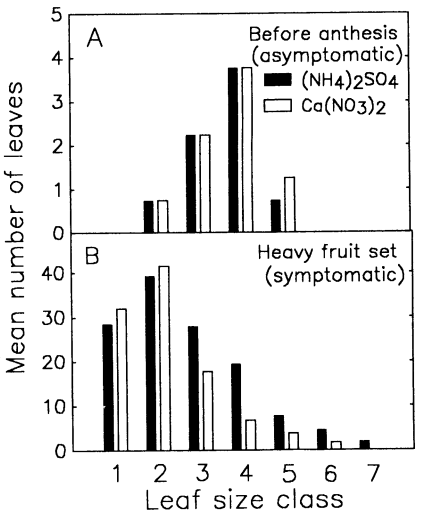

Fig. 1. Effect of $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ on the mean number of eggplant leaves in seven size categories, where 1 = 0.15 dm^2, 2 = 0.3 dm^2, 3 = 0.7 dm^2, 4 = 1.2 dm^2, 5 = 1.7 dm^2, 6 = 2.2 dm^2, and 7 = 3.3 dm^2. Plants were grown in soil infested with *Verticillium dahliae* and measured **(A)** before anthesis when plants were asymptomatic and **(B)** during fruit set after 50% of the plants had symptoms of Verticillium wilt. The Kolmogorov-Smirnov two-sample test (3) indicated a significant difference ($P < 0.001$) between the leaf class distribution from $(NH_4)_2SO_4$-treated plants and plants treated with $Ca(NO_3)_2$ after anthesis but not before.

DISCUSSION

Ammonium sulfate increased yields of eggplants grown in soil with low inoculum densities that averaged <10 microsclerotia per gram of soil, but the beneficial effects of fertilization with $(NH_4)_2SO_4$ disappeared in soils with higher inoculum densities. Likewise, in the absence of disease, the form of nitrogen fertilizer was less important in influencing marketable yields than when disease was present. The ameliorating effect of $(NH_4)_2SO_4$ can apparently be overwhelmed by high inoculum densities. Ashworth et al (1) similarly observed that disease suppressive amendments of KCl to pistachio produced greater yields and less disease when *V. dahliae* inoculum was low (<0.1 microsclerotia per gram of soil) but found the amendments worthless when soil densities of *V. dahliae* were high (>20 microsclerotia per gram of soil). Assuming rotation is strictly enforced and soil inoculum densities are kept low, the inclusion of $(NH_4)_2SO_4$ as a nitrogen fertilizer may enhance disease suppression and increase eggplant yields.

Verticillium wilt reduced plant weights, but fertilization with $(NH_4)_2SO_4$ produced larger plants than fertilization with $Ca(NO_3)_2$. The increased flowering observed in healthy plants as a result of $(NH_4)_2SO_4$ treatment was not significant in diseased plants, but this response may still contribute to disease suppression. Flowering in apple (12), carnation (13), chrysanthemum (40), and aster (16) was enhanced by ammoniacal fertilizers when compared to nitrate sources. More important, physiological interactions between different nitrogen forms and flowering may affect host defense. Huber (19) reported that before flowering, Verticillium wilt-susceptible potato cultivars maintained fungistatic levels of phenols in the vascular tissue, but at flowering, levels dropped below a threshold necessary to suppress the fungus; resistant cultivars maintained high levels (19). It is interesting that nitrate forms of nitrogen reportedly suppress phenol production in plants (10). However, in the present study, compared with $Ca(NO_3)_2$, stem colonization was reduced by $(NH_4)_2SO_4$ in 1 yr only, so no convincing comparisons can be drawn.

The nitrogen fertilizer did not affect the percentage of diseased leaves; however, in soils with low inoculum densities, plants fertilized with $(NH_4)_2SO_4$ had more large leaves and fewer small ones than did plants fertilized with $Ca(NO_3)_2$. We suggest that leaves on $Ca(NO_3)_2$-treated plants did not expand as rapidly as those on $(NH_4)_2SO_4$-treated plants. Selmann and Pegg (34) reported that the number of leaves of *Verticillium*-infected tomato plants did not differ from that of healthy controls, but the leaves were smaller and denser than healthy leaves. The enhanced leaf expansion under $(NH_4)_2SO_4$ fertilization may additionally serve as a yield-promoting mechanism in infected plants. Alternatively, we recognized that large leaves become symptomatic before smaller leaves do and can be shed from the plant, so large leaves may not have been counted on $Ca(NO_3)_2$-treated plants. It is possible that this rapid leaf drop may have also prevented us from measuring significant differences in chlorosis and wilt between the nitrogen forms, since wilted leaves do not remain on the plant for long.

Horsfall (18) classified *V. dahliae* as a "low sugar pathogen," implying it could not easily extract carbon substrates from low sugar tissues except by invading pectin-containing cell walls and, thus, incite disease. In the present study,

Table 4. Effect of nitrogen fertilizers on rhizosphere pH and population density of rhizobacteria of eggplants affected by Verticillium wilt before and after anthesis

	1991 (microplots)						1992 (field)					
	Infested soils[b]			Controls			Infested soils			Controls		
Sampling time[a] N form	pH[c]	Rhizo-bact.[c]	Fluor. pseud.[c]	pH	Rhizo-bact.	Fluor. pseud.	pH	Rhizo-bact.	Fluor. pseud.	pH	Rhizo-bact.	Fluor. pseud.
Before												
$(NH_4)_2SO_4$	4.9[d]	7.68	4.94	5.0	7.31	4.79	4.8	6.81	4.87	5.0	6.98	4.71
$Ca(NO_3)_2$	5.3*	7.78	4.99	5.4*	7.39	4.67	5.1*	6.93	4.84	5.5*	6.75	4.39
After												
$(NH_4)_2SO_4$	5.5	6.79	5.32	5.4	6.34	5.34	5.3	6.83	5.03	5.4	6.91	4.60
$Ca(NO_3)_2$	5.6	6.34	5.16	5.7	6.25	5.40	5.5	6.94	4.63	5.6	6.75	4.90

[a] Before = before anthesis (14 days after transplanting), after = after anthesis when symptoms appeared in 50% of the plants (53 and 50 days after transplanting in 1991 and 1992, respectively).
[b] Infested soil was enumerated for microsclerotia of *V. dahliae* using the procedure of Nicot and Rouse (28). Densities were 7 ± 3 microsclerotia per gram of soil in 1991 and 9 ± 5 in 1992.
[c] pH = Rhizosphere soil pH, Rhizobact. = log cfu of total bacteria per gram of rhizosphere soil, Fluor. pseud. = log cfu of fluorescent pseudomonads per gram of rhizosphere soil.
[d] The means of three or four replicates, depending on the year. Values followed by an asterisk are significantly different from their respective paired value, using Tukey's test at $P = 0.05$.

$(NH_4)_2SO_4$ fertilization increased leaf and root carbohydrates more than $Ca(NO_3)_2$, but only early in the season. Since *V. dahliae* reportedly infects roots early in the season (33), the lower carbohydrates in $Ca(NO_3)_2$-treated plants may have favored more colonization. However, the fungus was never isolated from stems or roots until wilt symptoms appeared later, when carbohydrate concentrations in the roots and leaves did not differ. Therefore, we offer no evidence that would suggest carbohydrate levels influenced disease. We recognize, however, that infection may have occurred below detection by our methods.

We questioned whether the initial increase in root carbohydrates in $(NH_4)_2SO_4$-treated plants would influence root exudation and the rhizobacteria. Our findings, however, indicated no strong effect from $(NH_4)_2SO_4$ compared with $Ca(NO_3)_2$ on densities of rhizobacteria. Smiley (38) suspected that suppression of take-all of wheat was associated with NH_4-N nutrition and interactions with fluorescent *Pseudomonas* spp. We observed the fluorescent pseudomonads to be in relatively low densities and not associated with $(NH_4)_2SO_4$-treated roots any more than with $Ca(NO_3)_2$-treated roots. Although this may not preclude their potential in suppression of Verticillium wilt of eggplant, their densities may need to be elevated to compete sufficiently with other rhizobacteria.

The increased tissue concentrations of N, P, and Mn in $(NH_4)_2SO_4$-treated plants compared with $Ca(NO_3)_2$-treated plants were consistently observed in all years. Increased N concentrations are common under NH_4-N nutrition because of the more rapid assimilation of NH_4^+ ions than of NO_3^- ions (29), and increased P (PO_4^{-3}) is believed to be a result of maintaining ion balance during NH_4-N absorption (17). Of the micronutrients examined, Mn levels in the leaves and roots were consistently higher in $(NH_4)_2SO_4$-treated plants than in plants treated with $Ca(NO_3)_2$. Manganese availability has been implicated with disease suppression, possibly through its role in enzyme activation for synthesis of phenol and lignin products (10,11,23). Conditions that immobilize Mn and limit uptake, such as NO_3-N nutrition (10), may compromise plant defenses and lead to greater disease severity. Higher levels of Mn and other trace metals in plant tissue have been associated with suppressed Verticillium wilt in cotton (2,35), potato (6), and tomato (6) and take-all in wheat (32). The increased availability of these metals may have resulted from the rhizosphere acidification under $(NH_4)_2SO_4$ regimes. This may explain why soil acidification was once recommended as a control for Verticillium wilt of eggplant (14,15). Although Wilhelm (41) pointed out that severe Verticillium wilt could still occur in acid soil, his soils may have had high inoculum densities or were possibly deficient in trace nutrients.

In summary, it should be stressed that yield benefits from $(NH_4)_2SO_4$ fertilizers may only be realized in soils with low inoculum densities. Furthermore, our use of a nitrification inhibitor may be significant to the effectiveness of $(NH_4)_2SO_4$ compared with $Ca(NO_3)_2$. Since nitrapyrin (N-Serve 24) is not currently registered for commercial use on eggplant, other nitrification inhibitors, such as chloride salts (19), may also serve the same purpose.

ACKNOWLEDGMENTS

We thank E. O'Dowd, B. Berger, J. Adinolfi, and S. Devlin for technical assistance and E. Naughton, J. Rich, and M. Amore for help in field preparation.

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Table 5. Total nonstructural leaf and root carbohydrates (mg glucose/g dry weight) of eggplants treated with $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ assayed before and after anthesis

Sampling time[a] / N form	Field 1990 Leaf	Field 1990 Root	Field 1992 Leaf	Field 1992 Root	Microplots 1991 Leaf
Before					
$(NH_4)_2SO_4$	165*[b]	126*	72	135	100*
$Ca(NO_3)_2$	120	80	67	123	74
After					
$(NH_4)_2SO_4$	177	182	62	156	65
$Ca(NO_3)_2$	178	200	60	143	54

[a]Before = before anthesis (14 days after transplanting), after = after anthesis when symptoms appeared in 50% of the plants (37, 53, and 50 days after transplanting in 1990, 1991, and 1992, respectively).

[b]The means of three or four replicates, depending on the year. Values followed by an asterisk are significantly different from their respective paired value, using Tukey's test at $P = 0.05$.

Table 6. Effect of nitrogen fertilizers on mineral composition of eggplant leaves and roots before and after anthesis in 1990 and 1992 field trials

Sampling time[a] / N form	1990 (μmol/g tissue) Leaf N	Leaf P	Leaf Mn	Roots N	Roots P	Roots Mn	1992 (μmol/g tissue) Leaf N	Leaf P	Leaf Mn	Roots N	Roots P	Roots Mn
Before												
$(NH_4)_2SO_4$	1,289[b]	119*	2.2*	634*	79*	1.7*	1,399	93	6.5*	335*	69*	1.5
$Ca(NO_3)_2$	1,229	106	1.5	335	47	1.0	1,310	86	2.9	310	47	1.4
After												
$(NH_4)_2SO_4$	11,676*	106	2.6*	575	90	1.4*	1,828*	163*	2.3	506*	76	4.0*
$Ca(NO_3)_2$	11,215	105	2.0	571	86	1.0	1,163	96	2.5	431	63	1.0

[a]Before = before anthesis (14 days after transplanting), after = after anthesis when symptoms appeared in 50% of the plants (53 days after transplanting).

[b]The means of three or four replicates. Values followed by an asterisk are significantly different from their respective paired value, using Tukey's test at $P = 0.05$.

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