Effect of Nutrition on Deformation Disease in *Gypsophila paniculata* Mother Plants

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ABSTRACT

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Deformation disease of *Gypsophila paniculata* mother plants reduces cutting yields as much as 50% but does not kill the mother plants. In preliminary experiments, fertigation of *G. paniculata* mother plants with a 20:20:20 compound fertilizer (N, P, K, plus microelements) at an N concentration of 720 mg/liter reduced the expression of deformation disease compared with the conventional N concentration of 360 mg/liter. The current study determined which component of the compound fertilizer reduced the disease. Experiments were carried out in 10-liter buckets packed with naturally infested 0- to 8-mm black tuff (Scoria = crushed volcanic stones). Irrigation was applied once a day at 0.5 liter per bucket with the nutrient under test being added at 1.0 liter per bucket via the water once a week. Treatments included: (i) four levels of 20:20:20 fertilizer; (ii) four levels of each of N, P, K, and Fe-Zn-Mn mixture (ME) corresponding to their concentrations in the compound fertilizer; (iii) different N sources (20:20:20, $(\text{NH}_4)_2\text{SO}_4$, KNO_3 , NH_4NO_3 , urea); and (iv) three pHs of the irrigation water at each of three NO_3^- and NH_4^+ application levels. Increasing the 20:20:20 fertilizer concentration reduced the

disease level from 82 to 96% with N at 180 mg/liter to 6 to 10% with N at 720 mg/liter. When either P, K, or ME was the sole variable, increasing concentrations had no significant effect on the disease, but elevating a mixed source nitrogen concentration from 0 to 180, 360, and 720 mg/liter (as 50% urea, 40% NH_4NO_3 , and 10% KNO_3) significantly reduced deformation similar to that observed with increasing concentrations of the 20:20:20 fertilizer. Among tested N sources, NH_4^+ was the most effective in reducing the disease (almost to zero at an N concentration of 360 mg/liter). Low disease incidence (0 to 10%) was always associated with effluent pH of 6 or lower. Irrigating with acidified water (pH 5.5) in the presence of N, as NH_4^+ at 180 mg/liter, additionally reduced disease from 56% under tap water (pH 7.8) irrigation to 11%. Similar acidification in the presence of NO_3^- N at 180 mg/liter was ineffective in reducing the disease, but water basification in the presence of NO_3^- N reduced disease incidence from 93 to 38% 90 days after planting. The N, P, K, Fe, and Zn concentrations in gypsophila cuttings were similar under the three tested levels of NH_4^+ , NO_3^- , and 20:20:20, whereas the concentration of Mn increased with increasing N. The Mn concentration in cuttings was inversely correlated with disease and is probably an important factor to understanding the physiological background of the deformation disease.

Additional keywords: Fe, fertilizer, K, Mn, N, nitrogen forms, P, pH, Zn.

The effects of plant nutrition in reducing disease incidence caused by soilborne pathogens have been widely reported (4,6,8,10–13,21,22,29,34). Huber and Watson (13) observed that the incidence of several cortical and root diseases was reduced by NO_3^- N and enhanced by NH_4^+ N, but the responses in terms of mitigation of all diseases evaluated were inconsistent. Woltz and Magie (34) reported that high levels of N, regardless of source, increased Fusarium corm rot of gladiolus caused by *F. oxysporum* f. sp. *gladioli*. Spiegel and Netzer (29) demonstrated that muskmelon plants fertilized with NH_4^+ N showed more wilt caused by *F. oxysporum* f. sp. *melonis*, alone or in combination with the root-knot nematode *Meloidogyne javanica*, than those fertilized with NO_3^- N applications; they also found that the K level had very little effect on these wilts. Schneider (25) related the inhibition of Fusarium yellows of celery in greenhouse and field experiments to NO_3^- N and adding KCl reduced the disease further. Engelhard et al. (6) reported that NO_3^- N decreased Fusarium wilt in tomato in comparison with NH_4^+ N and that liming the soil to a pH of 6.0 to 7.5, in the absence of organic matter or NH_4^+ N, decreased Fusarium wilt. The effects of soil pH and NO_3^- N were additive in reducing Fusarium wilt in tomato and other crops (15). Huang and Kuhlman (10) reported that N-fertilizers $\text{Ca}(\text{NO}_3)_2$,

NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$, or urea affected colonization of pine stem segments by *Rhizoctonia solani* to various degrees; however, whereas $(\text{NH}_4)_2\text{SO}_4$ suppressed the incidence of damping-off more effectively than other N-fertilizers, NH_4NO_3 enhanced it.

In Israeli nurseries, rooted cuttings of *Gypsophila paniculata* L. (baby's breath) planted in fumigated 0- to 8-mm tuff (Scoria) are used as mother plants to produce rooted cuttings for flower production. Harvesting of cuttings begins 4 to 5 weeks after planting (April to May) and continues until September to October. Although nurseries have traditionally relied on substrate fumigation, usually metham-Na, to manage soilborne diseases, occasionally this treatment is not successful. A disease of unknown etiology in mother plants reduced the growth rate of cuttings and caused chlorotic leaves. As the disease severity increased, there was loss of apical control; this induced a broom-like growth of new cuttings that reduced the yield of cuttings as much as 50%. We named this disease, deformation disease. In nurseries, disease incidence in mother plants appeared after several cycles of harvesting cuttings; usually 2 to 3 months after planting. Weekly harvesting of cuttings was one of the factors that enhanced expression of the disease. In our experimental study of organisms that might cause deformation disease, we eliminated the possible involvement of mites, nematodes, viruses, and phytoplasmas. When a naturally infested substrate was fumigated with either a fungicide or a bactericide before a new planting cycle of baby's breath mother plants, we were able to control the disease. Although we were unable to prove that specific organisms were

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involved, we had indirect proof for the involvement of both fungi and bacteria (1,23). Involvement of organisms such as fungi, phytoplasmas, bacteria, mites, and aphids that may cause such abnormal growth has been widely reported in the literature. However, the only example to be proved was the involvement of *Crinipellis pernicios*a in the appearance of witches'-broom disease in cacao (*Theobroma cacao*) (7). In preliminary experiments in which baby's breath mother plants were fertilized weekly, we found that a twofold increase of the conventional concentration of a compound 20:20:20 fertilizer (raising the N concentration from 360 to 720 mg/L) mitigated the incidence of this disease. The objective of the current study was to investigate the effects of the compound fertilizer and its components (N, P, K, or microelements) on the incidence of deformation disease in *G. paniculata* mother plants planted in naturally infested substrates.

MATERIALS AND METHODS

Baby's breath rooted cuttings were planted in pairs in 10-liter buckets packed with naturally infested 0- to 8-mm black tuff (Scoria = crushed volcanic stones) or with steam-treated tuff in the control treatments. Tuff has a high pH-dependent surface charge, and therefore, strongly buffers the solution pH (27). In all treatments (Table 1), nutrients were added manually with the water (1 liter per bucket) once a week. On the other days of the week, fresh or pH-treated water was applied via two 1-liter/h emitters at a rate of 0.5 liter per bucket.

All experiments took place from June 1 to October 15 between 1998 and 2002 in a nethouse that provided 10% shade. The daily mean outside air temperatures (maximum–minimum) during June, July, August, September, and October were 29.7 to 19.5, 31.9 to 22.2, 32.4 to 23, 31.3 to 20.9, and 28.5 to 17.5°C, respectively, with year-to-year fluctuations around the monthly mean not exceeding 7%. Each treatment was replicated five times in a random block design, with each replicate comprising five buckets and two plants per bucket. Disease assessment and harvesting of cuttings were done weekly beginning 30 to 35 days after planting. Disease assessment recorded disease incidence and disease severity of mother plants. Disease severity was rated on a 0 to 3 scale. Level 0 corresponds to healthy cuttings with long, dark green

leaves. At levels 1, 2, and 3, the growth rates of cuttings are reduced slightly, moderately, or severely, respectively, with enhanced leaf chlorosis as reduced growth rate. At levels 2 and 3, apical control was lost, and 2 or 3, or more than 3 cuttings, respectively, emerged from each bud.

The ionic composition (mg/liter) of the fresh water was 58 Ca, 28 Mg, 110 Na, and 220 Cl; the HCO₃ concentration was 4.5 mM; the electric conductivity (EC) and pH were 1.1 and 7.8, respectively. The Fe, Zn, and Mn concentrations in solution were determined by atomic absorption, K determined by flame photometry, and P, NO₃, and NH₄ determined with an auto-analyzer (32). To determine element concentrations in cuttings, plant tissue was oven dried at 65°C, ground, and digested with H₂SO₄ + H₂O₂ for N and P analysis and with HNO₃ + HClO₄ for cation analysis (9). Ion concentrations in water were determined as in the digest solutions. Summary of experiments and treatments appears in Table 1.

Statistical analysis. Analysis of variance was conducted on all data and the mean separation was provided by Duncan's multiple range test. Stepwise multiple regression analysis was used to correlate between disease incidence and growth factors. All analyses were performed using SAS version 6 (SAS Institute, Cary NC).

RESULTS

Effect of compound fertilizer (20:20:20) concentrations on disease incidence. Disease incidence was first observed 34 days after planting and reached its maximum 20 to 27 days later. Increasing the fertilizer N concentration from 0 to 180, 360, and 720 mg/liter reduced disease incidence from ~90% to ~50, 40, and ~10% on day 64 (mean of the two reported experiments) and similar effects were recorded on intermediate dates (Table 2). Even though experiment 1 was conducted between June and early August and experiment 2 conducted between August and early October, the effects of the treatments followed very similar trends, indicating that the seasonal effect was negligible. The correlation between disease incidence and disease severity was insignificant (*r*² values vary between 0.59 and 0.67), which indicated that the relationship between the two was not related. Since the effects of treatments on disease incidence were better defined

TABLE 1. Summary of experiments and treatments over time

Tested factor	Table no. (year)	Treatment description
Concentration of a 20:20:20 + ME fertilizer ^u	2 (1998a) (1998b)	N at 0, 180, 360, and 720 mg/liter (repeated)
Concentration of individual nutrients ^v	3 (1999a)	N at 0, 180, 360, and 720 mg/liter ^w KH ₂ PO ₄ at 0, 80, 160, and 320 mg/liter KCl-K at 0, 150, 300, and 600 mg/liter Microelements: Fe at 0.45, 0.90, 1.80, and 3.60 mg/liter ^x
N source × N concentration	4 (1999b)	N at 180 and 720 mg/liter as 20:20:20 N at 180 and 720 mg/liter as KNO ₃ N at 180 and 720 mg/liter as NH ₄ NO ₃ N at 180 and 720 mg/liter as urea N at 180 and 720 mg/liter as (NH ₄) ₂ SO ₄
	4 (2000)	N at 180 and 720 mg/liter as 20:20:20 N at 180, 360, and 720 mg/liter as KNO ₃ N at 180 and 720 mg/liter as urea N at 180, 360, and 720 mg/liter as (NH ₄) ₂ SO ₄
N source × irrigation water pH ^y	5 (2001)	pH 5.5, 7.8 ^z , and 8.2; N at 180 mg/liter as (NH ₄) ₂ SO ₄ pH 5.5, 7.8 ^z , and 8.2; N at 180 mg/liter as KNO ₃ pH 5.5, 7.8 ^z , and 8.2; N at 180 mg/liter as 20:20:20
	6 (2002)	pH 7.8 ^z and 8.6; N at 180, 360, and 720 mg/liter as KNO ₃ pH 5.5 and 7.8 ^z ; N at 180, 360, and 720 mg/liter as (NH ₄) ₂ SO ₄

^u N composition: 50% urea, 40% NH₄NO₃ and 10% KNO₃.

^v Other nutrient concentrations as in 20:20:20 + ME at 720 mg/liter N.

^w N composition as in 20:20:20 + ME.

^x Microelements: Fe/Zn/Mn ratio = 0.05:0.0125:0.025 as EDTA.

^y Treated-water pH prior to irrigation (applied in each irrigation).

^z Fresh water pH prior to irrigation (applied in each irrigation).

than their effect on disease severity data, only disease incidence was reported.

Effects of individual fertilizer ingredients on deformation disease. Elevating only the concentration of nitrogen (comprised of 50% urea-N, 40% NH_4NO_3 -N, and 10% KNO_3 -N) in the irrigation water significantly reduced the disease. At the highest N concentration, disease incidence was reduced to a similar level (16.7%), slightly higher than the 20:20:20 fertilizer. Elevating the concentrations of other fertilizer ingredients (P, K, or ME) had no significant effect on disease incidence (Table 3).

Effects of nitrogen source on deformation disease and substrate leachate pH. Disease incidence declined with increasing N regardless of the source; however, the effectiveness of the sources declined in the order of $\text{NH}_4 > \text{urea} > 20:20:20 > \text{NH}_4\text{NO}_3 > \text{NO}_3$ (Table 4). As the NH_4 and NO_3 contents in the N solution increased, the leachate pH decreased, the effect being stronger with NH_4 than with NO_3 . Since the pH was measured in leachate collected 2 days after the N source was added to the bucket, and in light of the high pH buffering capacity of the growth substrate (27), it is safe to conclude that the effects of the treatments on the actual pH at the root surface immediately after fertilization exceeded the above-mentioned effects on leachate pH.

When the leachate pH under low N fertigation was reduced from 7.5 to 6.5, disease incidence in the urea and 20:20:20 fertilizer treatments was reduced from 70 to 49% and 93 to 54%, respectively (Tables 4 and 5). The pH had a negligible effect in the presence of NO_3 or NH_4 . Under high N, decreasing the pH from 6.1 to 5.6 reduced disease incidence with all N sources (Table 4).

Effect of irrigation water pH on deformation disease. To elucidate the specific effect of pH on deformation disease, plants were irrigated daily with acidified (pH 5.5) or fresh (pH 7.8) water at the lowest N concentration (180 mg/liter) (Table 5). In the presence of nitrate, acidified water had no effect on the disease compared with untreated fresh water, although the leachate pH was reduced to 6.3. Disease incidence was lower in plants fertilized with ammonium in untreated fresh water than with nitrate and fresh water, although the time-averaged leachate pH values were quite similar in both cases (7.4 and 7.9, respectively). Acidified water used in combination with NH_4 decreased disease incidence further than the untreated fresh water irrigation over the entire growth period (128 days), although it was minimal when the leachate pH dropped below 6. When acidified water was used with 20:20:20, disease incidence was reduced only if the leachate pH dropped to 6 or less (Table 5).

Raising the daily irrigation water pH from 7.8 to 8.6 decreased the disease 30 to 67% when the N source was nitrate (Table 6). Nitrate concentration in the water had only a small effect on dis-

ease reduction. Elevation of the water pH had no effect on disease incidence when the N source was NH_4 , partly because the substrate pH could not be effectively raised (data not presented). When the daily water pH was lowered to 5.5 with NH_4 as the N source, disease incidence was reduced further compared with that with fresh water, when NH_4 was added at 180 and 360 mg/liter, but not when it was added at 720 mg/liter when the disease incidence was the same as that with fresh or acidified water (Table 6).

Effects of nitrogen source and concentration on N, P, K, and ME contents in *Gypsophila* cuttings. Increasing the NO_3 concentration in irrigation water had no significant effect on N, P, K, and Ca concentrations in tissues of the cuttings, but it reduced the concentration of Mg and increased Fe, Zn, and Mn in dried tissue. Increasing the NH_4 -N concentration in the water had insignificant effects on the tissue N and P concentrations but increased tissue microelements, particularly Mn. The impact on Mn could plausibly be attributed to the pronounced reduction in leachate pH. Increasing the 20:20:20 fertilizer concentration affected nutrient contents proportionally to the NH_4 so that the overall effect was

TABLE 3. Effect of four rates of N, P, K, or microelement mixture (ME) constituting the 20:20:20 fertilizer on deformation disease in *Gypsophila paniculata* mother plants 64 days after planting compared with the complete 20:20:20 fertilizer

Concentration in water ^w	Disease incidence (%)				
	Ingredient				Fertilizer
	N	P	K	ME	
None	98.5 a ^x	26.6	6.7	35.0	93.4 a
Low ^y	82.8 a	23.4	2.8	50.0	93.4 a
Medium	61.3 b	43.4	5.2	34.2	58.4 b
High	16.7 c	10.0	4.0	30.8	11.6 b
Analysis of variance (ANOVA)					
Mean	64.8	25.9	4.7	37.5	43.8
F	41.2	1.2	0.23	1.1	10.8
PR > F	0.0001	0.36	0.87	0.39	0.0001
LSD ^z	18.0	36.3	11.2	23.3	40.2

^w N, P, K, and Fe concentrations at the low level were 180, 80, 150, and 0.45 mg/liter, respectively. The concentrations in the medium and high levels were, respectively, twice and four times the low level. N sources in the N experiment were in the same proportions as in the 20:20:20 fertilizer mixture.

^x Values in a given column marked with the same letter are not significantly different from each other according to Duncan's multiple range test at the $P = 0.05$ probability level.

^y When N, P, K, or microelement was absent or at a reduced concentration, other constituents were present at the highest concentration. When 20:20:20 fertilizer was absent, irrigation was with fresh water only.

^z LSD = least significant difference.

TABLE 2. Effects of compound fertilizer (20:20:20 + ME)^w rate (expressed as N concentration in water) on disease incidence in two successive experiments in *Gypsophila paniculata* mother plants planted in naturally infested tuff

N (mg/liter)	Disease incidence (%)									
	Experiment 1 ^x					Experiment 2 ^x				
	34	42	49	57	64	34	42	49	57	64
0	62.1 a ^y	65.4 a	69.3 a	84.7 a	86.3 a	81.5 a	81.7 a	91.3 a	98.3 a	96.3 a
180	40.5 a	31.8 b	33.3 b	47.0 b	35.9 b	33.7 b	36.2 b	63.0 b	76.2 ab	65.5 b
360	36.1 ab	31.7 b	26.8 bc	35.4 b	30.3 b	29.8 b	31.8 b	48.0 b	69.5 b	48.8 b
720	9.8 b	5.7 c	9.2 c	6.0 c	6.9 c	2.2 c	4.2 c	2.8 c	6.7 c	11.2 c
Analysis of variance (ANOVA)										
Mean	37.1	33.6	34.6	43.3	39.8	36.8	38.5	51.3	62.7	55.5
F	5.6	10.2	15.6	21.4	40.0	18.1	22.2	21.5	24.6	16.6
PR > F	0.009	0.0006	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD ^z	29.6	24.9	20.8	22.9	17.2	25.2	22.2	25.9	25.8	28.3

^w 20% N (50% urea-N, 40% NH_4NO_3 -N, 10% KNO_3 -N), 20% P_2O_5 , 20% K_2O , and 0.05% Fe, Zn, and Mn.

^x Data collected at 34, 42, 49, 57, and 64 days after planting.

^y Values in a given column marked with the same letter are not significantly different from each other according to Duncan's multiple range test at the $P = 0.05$ probability level.

^z LSD = least significant difference.

less pronounced because of the NO₃ present in the compound fertilizer (Table 7). To quantify the relationship between growth factors and disease incidence (DI [%]), a multiple regression model between DI and measured leachate pH (within the pH range of 5.6 to 7.6) and nutrient concentrations in the plant tissue (Table 7) was calculated (Table 8). The regressions between growth factors and DI (Table 8) showed that leachate pH accounted for 87% of the total variability in disease incidence in the pH range of 5.6 to 7.6 (equation 1), and no other variable met the $P = 0.150$ criterion of the model. When the solution pH was omitted from the multiple regression model, tissue Mn (T_{Mn}) in the range 63 to 301 mg/kg was the factor best correlated with DI (equation 2). The second entrée into the model was (T_{Mn})² that accounted for another 13% of the total variability in DI.

DISCUSSION

In the current study, a twofold increase in the currently recommended fertilizer 20:20:20 dose reduced deformation disease incidence in *G. paniculata* mother plants growing in a naturally infested tuff (Scoria). The same effect could be achieved by increasing only the N concentration in the water. Increasing only the P, K, or microelement concentration had no effect on deformation disease. The effects of specific nutrients and their application rate on the incidence of various plant diseases are well documented (3,22,24). Mineral nutrients reduce disease incidence either by direct toxic effects on the pathogen, by reducing susceptibility

of the host, or by enhancing the population of antagonistic members of the soil microflora (13). Tenuta and Lazarovits (30) reported that nitrogenous amendments, such as meat and bone meal, kill sclerotia of the soilborne plant pathogen *Verticillium dahliae* through the ammonia and nitrous acid generated and that the effect depended on soil pH. In general, sufficient ammonia was generated only in the presence of NH₄ and a pH above 8, whereas nitrite or nitrous acid was produced in the presence of NH₄ when the soil pH was below 6 (2,8,18,19,21,24,26,28,31,33). In our current study, NH₄ reduced disease incidence more efficiently than urea, NH₄NO₃, or NO₃. The high efficacy of NH₄ could, as inferred above, be attributed either to direct pathogen toxification by NH₃ or nitrite or to indirect effects that involve enhanced crop resistance to pathogens, especially as a result of the increased uptake of Mn (Table 7) (4,5,11,12). The observed reduction in pH is consistent with existing knowledge on the effects of nitrification and NH₄ uptake on the release of protons (14,20). A pH reduction was observed under NO₃ nutrition also (Table 4), but in this case it was attributed to the accompanying K⁺ that can replace the exchangeable H⁺ and increase the cation-anion charge balance in the plant, with consequent H⁺ excretion into the soil solution (20).

The finding that disease incidence at any NH₄ concentration could be reduced by acidifying the irrigation water (Table 6) supports the hypothesis that solution pH was a predominant factor in reducing disease incidence. Notably, even when there was relatively little change in pH, a considerable difference in disease was observed between plants supplied with ammonium or nitrate; this

TABLE 4. Effects of nitrogen source on the rate of disease incidence (DI) and respective pot leachate pH values^w in *Gypsophila paniculata* mother plants 56 days after planting

Nitrogen source ^x	Experiment 1 (1999)				Experiment 2 (2000)					
	N (180 mg/liter)		N (720 mg/liter)		N (180 mg/liter)		N (360 mg/liter)		N (720 mg/liter)	
	DI (%)	pH	DI (%)	pH	DI (%)	pH	DI (%)	pH	DI (%)	pH
20:20:20	93.4 a ^y	7.6	16.6 bc	6.1	54.0 ab	6.4 b	—	—	0 b	5.5 b
NO ₃	70.0 ab	7.7	25.0 a	6.2	65.8 a	6.7 a	54.2 bc	6.3 d	3.4 a	5.8 a
NH ₄ NO ₃	64.7 b	7.5	20.0 b	6.1	—	—	—	—	—	—
Urea	70.2 ab	7.7	16.2 c	6.2	49.0 b	6.3 bc	—	—	5.0 a	5.7 a
NH ₄	28.0 c	7.4	2.2 d	6.0	29.4 c	6.3 bc	0 d	6.0 e	0 b	5.4 b
Analysis of variance (ANOVA)										
Mean	65.3	7.6	15.6	6.1	49.6	6.5	—	—	2.8	5.7
<i>F</i>	14.2		14.2		16.6	39.3	—	—	7.8	12.8
<i>PR</i> > <i>F</i>	0.001		0.001		0.001	0.006	—	—	0.05	0.009
LSD ^z	25.6		8.2		12.2	0.25	—	—	2.6	0.24

^w The leachate pH was recorded 2 days after fertilization (which was applied once weekly, followed by 6 days of irrigation with fresh water). The pH levels of the irrigation solutions with the various fertilizers in treatments were 6.5 with the 20:20:20 fertilizer, 5.8 with NO₃, 6.3 with urea and 6.3 with NH₄.

^x In the zero-N control treatment, disease percentage and pH were 93.7 and 8.4, respectively, in experiment 1 (1999) and 100 and 7.1, respectively, in experiment 2 (2000).

^y Values in a column with the same letter are not significantly different from each other according to Duncan's multiple range test at the $P = 0.05$ probability level.

^z LSD = least significant difference.

TABLE 5. Effects of acidified irrigation solution and nitrogen source on disease incidence (DI) in *Gypsophila paniculata* mother plants during the growing season

Nitrogen source ^x	Irrigation water pH	57 Days		100 Days		128 Days	
		DI (%)	Leachate pH	DI (%)	Leachate pH	DI (%)	Leachate pH
20:20:20	7.8	70.0 ab ^y	8.0 a	95.0 a	7.6 a	95.0 a	7.6 b
20:20:20	5.5	82.5 ab	6.8 c	57.5 b	6.0 d	50.0 b	5.8 d
NO ₃	7.8	82.5 ab	7.8 a	100.0 a	7.9 a	100.0 a	7.9 a
NO ₃	5.5	87.5 a	6.3 d	97.5 a	6.4 c	97.5 a	6.2 c
NH ₄	7.8	65.0 ab	7.5 b	50.0 b	6.9 b	55.0 b	7.4 b
NH ₄	5.5	45.0 b	6.5 d	12.5 c	5.8 d	10.0 c	5.9 d
Analysis of variance (ANOVA)							
Mean		72.1	7.15	68.9	6.8	67.9	6.8
<i>F</i>		1.9	43.4	24.4	59.3	57.3	136
<i>PR</i> > <i>F</i>		0.46	0.0001	0.0001	0.0001	0.001	0.0001
LSD ^z		38.3	0.30	23.4	0.37	42	0.26

^x N concentration in the water (applied once a week) was 180 mg/liter.

^y Values in a column with the same letter are not significantly different from each other according to Duncan's multiple range test at the $P = 0.05$ probability level.

^z LSD = least significant difference.

difference increased as pH declined (Table 4). This indicates that the impact of reduced pH on disease could be fully expressed in the presence of NH_4 but not of NO_3 (e.g., at pH ~6 and N at 360 mg/liter, disease incidence in the presence of NO_3 or NH_4 was 54.2 or 0, respectively, Table 4). This may be related to the fact that at soil pH <7, the second stage of nitrification is inhibited (16), causing nitrite accumulation in the system (21). The biocidal

effect of nitrite (21) may provide additional mitigation that cannot be achieved in the presence of nitrate. The decreased pH might also have a directly adverse effect on pathogenic bacteria, otherwise it would not be effective in the presence of nitrate. Alternatively, it might mediate the dissolution of sparsely soluble minerals, particularly P and Mn (17), and thus increase their uptake by plants. The present data shows that NH_4 did not stimulate N, P,

TABLE 6. Effects of pH of basified irrigation water and nitrogen source and concentration on disease incidence (DI) in *Gypsophila paniculata* mother plants during the growing season

N source ^x	N (mg/liter)	Irrigation water pH	48 Days		61 Days		77 Days		90 Days	
			DI (%)	Leachate pH	DI (%)	Leachate pH	DI (%)	Leachate pH	DI (%)	Leachate pH
NO_3	180	7.8	75.6 ab ^y	7.3 c	82.5 ab	7.6 bc	89.8 ab	7.5 d	93.5 a	7.6 c
NO_3	180	8.6	33.0 c	8.0 a	61.2 bc	8.2 a	56.4 bc	8.3 a	37.6 e	8.3 a
NO_3	360	7.8	85.0 a	7.2 c	94.6 a	7.3 c	97.1 a	7.2 e	94.6 a	7.4 cd
NO_3	360	8.6	51.4 b	7.8 ab	45.3 c	8.1 ab	45.6 c	8.0 b	49.2 d	8.2 ab
NO_3	720	7.8	57.8 b	7.1 c	70.1 b	7.2 c	68.4 b	7.0 e	75.3 b	7.1 d
NO_3	720	8.6	25.0 cd	7.6 b	25.4 cd	7.7 b	26.2 d	7.8 c	8.3 f	8.0 b
NH_4	180	7.8	33.6 c	6.5 d	49.9 c	6.5 e	54.1 c	6.4 f	56.2 c	6.4 e
NH_4	180	5.5	5.0 d	6.1 e	17.5 d	5.9 f	17.9 de	5.7 g	10.7 f	5.8 f
NH_4	360	7.8	10.0 d	5.8 f	17.5 d	5.9 f	47.2 cd	5.6 g	12.3 f	5.4 g
NH_4	360	5.5	2.9 d	5.4 g	5.0 de	5.0 g	13.3 e	5.0 h	0 g	5.0 h
NH_4	720	7.8	4.0 d	4.8 h	0 e	4.5 h	10.0 e	4.3 i	0 g	4.2 i
NH_4	720	5.5	2.9 d	4.7 h	0 e	4.7 h	5.7 e	4.5 i	0 g	4.3 i
Analysis of variance (ANOVA)										
Mean			33.0	6.5	39.1	6.5	44.3	6.4	36.5	6.5
F			7.5	10.5	10.8	93.5	9.0	134.8	17.3	212.6
PR > F			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD ^z			14.8	0.16	13.7	0.18	14.0	0.16	11.9	0.14

^x N concentration in water (applied once a week) as indicated.

^y Values in a column with the same letter are not significantly different from each other according to Duncan's multiple range test at the $P = 0.05$ probability level.

^z LSD = least significant difference.

TABLE 7. Content of N, P, K and microelements in *Gypsophila paniculata* mother plant cuttings as a function of N source and concentration in the irrigation water^w, the corresponding leachate pH^x, and disease incidence

N source	N (mg /liter)	Macro and microelement content								Leachate pH	Disease incidence (%)
		N (g/100 g)	P (g/100 g)	K (g/100 g)	Ca (g/100 g)	Mg (g/100 g)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)		
NO_3	180	4.5 bcd ^y	0.66 ab	4.2 abc	1.8 ab	0.65 ab	197 cd	78 d	65 d	6.7	65.8
NO_3	360	4.8 bc	0.68 ab	4.3 abc	1.9 ab	0.59 ab	250 bcd	103 bcd	115 cd	6.5	54.2
NO_3	720	4.7 bcd	0.68 ab	4.0 bcd	1.8 ab	0.47 c	353 ab	118 ab	202 b	5.8	3.4
NH_4	180	4.3 d	0.67 ab	4.7 a	1.6 ab	0.65 ab	309 abc	104 bcd	151 bc	6.2	29.4
NH_4	360	4.4 cd	0.59 b	4.2 bc	1.8 ab	0.60 ab	268 bcd	106 bc	184 b	5.7	0
NH_4	720	4.7 bcd	0.63 ab	3.8 cd	1.6 b	0.55 bc	428 a	135a	301 a	5.6	0
20:20:20	180	4.5 cd	0.64ab	3.5 d	1.8 ab	0.62 ab	161 d	83 cd	63 d	7.6	93.4
20:20:20	360	5.3 a	0.69 ab	4.6 ab	2.1 a	0.69 a	165 d	75 d	105 cd	6.8	58.4
20:20:20	720	4.9 ab	0.71 a	4.4abc	1.4 b	0.56 bc	281 bcd	100 bcd	179 b	6.1	11.6
Analysis of variance (ANOVA)											
Mean		4.7	0.66	4.2	1.8	0.60	268	100	152		
F		4.8	1.3	4.0	1.7	3.3	3.9	4.6	13.1		
PR > F		0.0006	0.78	0.002	0.36	0.007	0.002	0.0009	0.0001		
LSD ^z		0.46	0.104	0.64	0.52	0.12	148	30	110		

^w Tissue samples and pH values were taken from plants in treatments in Table 4 for NO_3 and NH_4 and from Table 3 for 20:20:20.

^x Leachate pH was measured the second day after fertilization.

^y Values in a column with the same letter are not significantly different from each other according to Duncan's multiple range test at the $P = 0.05$ probability level.

^z LSD = least significant difference.

TABLE 8. Multiple regression model between disease incidence (DI [%]) and measured leachate pH and nutrient concentrations in plant tissue in *Gypsophila paniculata* mother plants^w

Equation	X ^x	Coefficient values			F			R ²
		a1 ^y	a2 ^y	b ^y	a1 ^z	a2 ^z	b ^z	
1	pH	46.78 ± 6.95	...	261.1 ± 44.3	45.2**	...	34.8***	0.87
2	T _{Mn}	-0.377 ± 0.076	...	92.4 ± 12.8	24.5**	...	52.4***	0.78
3	T _{Mn}	-1.028 ± 0.028	0.00187 ± 6.4E-4	138.6 ± 18.1	20.5**	8.6*	58.8***	0.91

^w The general regression equation is $\text{DI} = a1X + a2X^2 + b$ where X is defined in the table.

^x Range in pH is 5.6 to 7.6 and in T_{Mn} (Mn concentration in plant tissue) is 63 to 301 mg/kg.

^y ± Standard error.

^z *, **, and *** = significant difference at $P = 0.05$, 0.01, and 0.001, respectively.

and K uptake, but it significantly elevated the Mn concentration in the tissues of cuttings (Table 7). Increasing the NH_4 concentration in irrigation water enhanced this effect, plausibly by further depressing substrate solution pH.

In the experimental growth substrate used in the current study, the pH of the leachate could be elevated above that of fresh water (pH 7.8) only in the presence of NO_3 , which naturally stimulates OH^- release by roots (20). When the leachate pH exceeded 8, disease incidence significantly declined and the impact was enhanced as the nitrate concentration increased (Table 6). When nitrate increases, the ratio between the OH^- content in the rhizosphere and the entire substrate volume increases; therefore, similar leachate pH values may be associated with enhancing disease reduction. This presumption would be true if pathogen activity were more effective in the rhizosphere than in bulk soil.

We suggest that the mechanism by which high pH (>7.8) reduces disease involves ammonia release in the soil. The tuff substrate includes a small quantity of organic matter and at a pH >8, the NH_4 released via mineralization is sufficient to produce toxic NH_3 at a concentration sufficient to kill or inhibit soil microorganisms (2).

The regressions between growth factors and DI (Table 8) showed that the two factors that best correlated with DI were substrate solution (leachate) pH (equation 1) and tissue Mn concentration (equations 2 and 3). The equations indicate that leachate pH was the main factor suppressing DI. The high correlation between T_{Mn} and DI cannot answer the question whether Mn was instrumental in suppressing the disease or not.

To summarize, we suggest that the following factors and mechanisms determine disease incidence: (i) increasing the application rates of NO_3 and NH_4 reduced DI because of the reduction in substrate pH; (ii) the reduced pH (from 7.8 to 5.6) increased the uptake and tissue concentration of Mn and this could have reduced DI when pH was within the above range; (iii) decreasing soil pH also caused nitrite accumulation that further suppressed the disease, the fact that this effect was NH_4 -specific explains why, under a similar pH, NH_4 was more effective than NO_3 in reducing the DI; and (iv) increasing the substrate (leachate) pH from ~7.5 to 8.3 (possible only at low and intermediate NO_3 concentrations) caused release of ammonia. This requires the presence of a small concentration of NH_4 such as could be obtained from tuff or root debris. The biocidal effect of NH_3 was obtained despite a decrease in plant Mn concentration stemming from the high pH. The wide range of effects that were carefully controlled and monitored in this study is consistent with the diversity of the published data on crop resistance to root pathogens under a variety of environmental conditions.

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