Influence of NO_3/NH_4 Ratio, N, K, and pH on Root Rot of *Viola* × *wittrockiana* Caused by *Thielaviopsis basicola*

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ABSTRACT

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Fertility treatments were applied every 2 days in three factorial experiments: (i) N (52, 105, 158 µg of N per ml) × NO_3/NH_4 ratio (1:0, 3:1, 1:1, 1:3); (ii) NO_3/NH_4 ratio (3:1, 1:3) × K (113, 213, 313 µg of K per ml); and (iii) pH range (4.6 to 4.8 and 6.2 to 6.4) × NO_3/NH_4 ratio (1:0, 2:1, 1:3) to pansy plants grown in sand culture. Inoculum (noninoculated and inoculated with *T. basicola*) was a factor in all three experiments. The NO_3/NH_4 ratio was the dominant nutritional factor that reduced disease. Disease incidence, presence on root segments, was lowest with high NH_4 (1:3 NO_3/NH_4 ratio) and a low level of K (113 µg/ml) when the NO_3/NH_4 ratio was 1:3, compared with other NO_3/NH_4 ratios. pH had no effect on disease incidence in sand medium (cation exchange capacity [CEC] = 0.225), presumably because element availability from sand surfaces into substrate solution was low. High disease incidence corresponded with reductions in plant growth. Plant growth of noninoculated plants was greatest at the 3:1 NO_3/NH_4 ratio. Plant growth of inoculated plants was greatest at the 3:1, 2:1, or 1:1 NO_3/NH_4 ratios with disease being moderate at the 2:1 and 1:1 ratios, compared with other ratios. Plant growth was not affected by pH or K at the levels tested.

Additional keyword: black root rot

Black root rot caused by *Thielaviopsis basicola* (Berk. & Broome) Ferraris (= *Chalara elegans* Nag Raj & Kendrick) has caused severe crop loss of pansy (*Viola* × *wittrockiana* Gams) and other bedding plants grown in the southeastern U.S. (14,15). The fungus is a common soil inhabitant of peat bogs and, thus, a contaminant of peat-based media (11,32). Characteristic symptoms are necrotic lesions on the tap and lateral roots (28,29). Appearance of symptoms, combined with melanized aleuriospores in and on necrotic tissue, is exemplified by the common name black root rot.

Thielaviopsis basicola has been most devastating to pansies during summer greenhouse production. An optimal temperature for pansy production of 21/15.5°C day/night cannot be achieved in greenhouses during July, August, and September in the southeastern U.S. (7). While a temperature range for disease development on pansies has not been determined, *T. basicola* is generally most severe at temperatures suboptimal to the host (1,16). Production timing is necessary to meet fall market demand for outdoor planting of fall annuals. The fungicides currently recommended are not consistently effective. There is a need for cultural controls that can be integrated with greenhouse production practices.

Several soil factors, including a pH that is ≤5.6, low phosphorus (P), low base saturation, high ammonia (NH_3), and high aluminum (Al), have been reported to reduce severity of black root rot (3,5,6,8,18, 19,21,25,30). The effect of pH on disease development by *T. basicola* may be due to pH influencing other factors that affect disease (3,19). The release of cations from the medium/soil column and absorption by roots are partly determined by pH, ionic strength, and relative elemental concentrations. While most field and greenhouse research indicates that high acidity reduced black root rot severity on different plants, the mechanism(s) involved are not fully elucidated and may involve a number of factors.

Nitrogen form and/or concentration have been reported to influence survival of fungal pathogens in soil, change dynamics of soil microbiota, and alter plant resistance (13,22,26). Nitrogen and pH are interrelated at several levels. Ammonification and nitrification are reduced at low pH values. Root uptake of elements such as ammonium and nitrate acidify or neutralize soil pH, particularly in the rhizosphere.

Plant growth of many species is reduced with NH_4 as the N source (9,17,24). NH_4 can inhibit the absorption of K but this effect is variable by plant species and cultivar (4,9). While not due to inhibition, K and pH can counter the effects of NH_4 and enhance plant growth (2,9,23,31,33).

The present studies examined the relationship of NO_3/NH_4 ratio and several other soil chemical factors to black root rot caused by *T. basicola* in sand, a relativity inert substrate. Objectives were to determine (i) the effect of N concentration and form, (ii) the relationship between K and NO_3/NH_4 ratio on disease and growth of pansies, and (iii) the influence of NO_3/NH_4 ratio under two pH ranges.

MATERIALS AND METHODS

Plant production. Approximately 25 pansy seed, of Universal Mixed (Park Seed Co., Greenwood, SC) in the N concentration study and Universal Orange in the K concentration and pH studies, per section of an 18 cell plastic insert, were sown onto autoclaved sand. Seeds were germinated in low light (0.23 µE s^{-1} m^{-2}) at 22°C and misted two to three times daily. At 95% germination, seedling trays were moved to a 20°C growth chamber and grown under 16-h light 65 cm below a bank of 12 Sylvania Excel Line 40 watt incandescent and 24 Sylvania 95 watt Cool White Deluxe High Output fluorescent bulbs for 35 to 42 days. Seedlings were watered with deionized water one to three times daily and fertilized with a half rate of modified Hoagland's solution once weekly (12). At the five- to seven-leaf stage of growth, seedlings were moved to the greenhouse (average day/night temperatures 22/15°C, natural photoperiod), roots were gently separated, and plants transferred into 10-cm-diameter pots filled with autoclaved sand and watered with deionized water. Experiments were carried out from 2 March to 23 April 1993 (NO_3/NH_4 ratio × N), from 4 March to 26 April 1994 (NO_3/NH_4 ratio × K), and from 12 April to 1 June 1994 (pH × NO_3/NH_4 ratio). Plants were watered daily as needed, alternating between 150 ml of deionized water and 120 ml of the appropriate fertilizer treatment.

White sand medium had a pH of 5.7, cation exchange capacity (CEC) of 0.225 meq per 100 ml of sand, and bulk density of 1.63 g/cm^3. Percent separates (by weight) of oven dried sand listed by the smallest screen size it passed through were 61% (40 mesh = 0.42 mm openings), 19% (30 = 0.60 mm), 12% (20 = 0.84 mm), 4% (18 = 1.00 mm), 3.8% (10 = 2.00 mm), and 0.2% (8 = 2.38 mm).

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Experimental design and nutrient treatments: NO_3/NH_4 ratio × N (N experiment). The experiment was a 2 × 4 × 3 factorial in a completely randomized design. Treatments consisted of two inoculum levels (noninoculated and inoculated), four NO_3/NH_4 ratios (1:0, 3:1, 1:1, 1:3), and three concentrations of N (52, 105, 158 mg of N per liter^{-1}). Treatments were replicated four times. Essential elements were provided by using a half-strength modified Hoagland's solution (105 µg of N, 15 µg of P, 113 µg of K, 100 µg of Ca, 25 µg of Mg, 0.25 µg of B, 0.01 µg of Cu, 0.25 µg of Fe, 0.005 µg of Mo, 0.25 µg of Mn, and 0.0025

Table 1. The effect of the NO_3/NH_4 ratio on pansy plants noninoculated and inoculated with *Thielaviopsis basicola*

		N concentration (ppm)													
		52					105					158				
		NO_3/NH_4 ratio														
Variable	Inoc.[a]	1:0	3:1	1:1	1:3	Si.[b]	1:0	3:1	1:1	1:3	Si.	1:0	3:1	1:1	1:3	Si.
Interaction[c]																
Root weight (g)	–	6.7[d]	7.1	4.6	2.2	L**	3.7	11.5	5.1	2.7	LQ**	2.5	6.6	5.1	1.6	LQ**
Root weight (g)	+	2.1	2.9	4.0	1.6	Q*	0.9	3.9	4.2	2.7	Q**	0.8	4.6	3.2	1.1	Q**
Shoot weight (g)	–	11.2	13.7	11.2	6.4	NS	17.6	23.9	19.0	10.2	LQ**	21.3	38.9	21.8	10.4	LQ**
Shoot weight (g)	+	5.9	9.1	8.4	4.0	NS	4.6	16.3	15.2	8.6	Q**	6.0	26.7	9.9	4.9	Q**
Flowers (no.)	–	4.0	5.8	4.2	2.5	NS	6.0	5.2	5.2	4.2	NS	8.8	16.0	4.5	5.8	L**
Flowers (no.)	+	2.2	4.2	2.5	3.0	NS	1.0	6.5	5.2	3.8	NS	3.2	13.2	4.0	3.0	Q**
No interaction[e]																
Disease incidence (%)	+	76.6[f]	75.4	31.3	19.7	L**										
Root discolored (%)	–	0.2	21.5	46.2	64.6	L**										

[a] Plants noninoculated (–) or inoculated (+) with *T. basicola*.
[b] Significance pertains to preceding row of numbers across NO_3/NH_4 ratio values within each N concentration. Orthogonal contrasts were used to determine relationship of the line through values. No significant difference (NS) between values, or a significant linear (L), quadratic (Q), or linear-quadratic (LQ) relationship between values. Significance at 5% (*) or 1% (**) level of probability.
[c] Variables with an interaction between N concentration and NO_3/NH_4 ratio.
[d] All disease and growth measurements taken on a per plant (= replication) basis 4 weeks after inoculation. Means were calculated from four replications per treatment.
[e] Variables with no interaction between N concentration and NO_3/NH_4 ratio.
[f] Means were calculated from 12 replications, which include measurements from all three N concentrations.

Table 2. Analysis of variance for the effects of inoculum, NO_3/NH_4 ratio, and N concentration on disease and growth measurements of pansy plants[a]

Treatment	Inoc.[b]	df	Roots discolored (%)	Disease incidence	Root weight	Shoot weight	Flower no.
Model		11/23[c]	2,698.8**[d]	0.310**	26.77**	284.00**	45.14**
Source							
Inoculum (Inoc)[b]		1	...	...	186.59**	1204.81**	68.34*
N concentration (N conc)[e]		2	52.7	0.001	1.59	626.59**	119.01**
Inoc × N conc		2	...	...	0.59	112.15*	8.47
NO_3/NH_4 ratio[f]		3	9,524.4**	1.046**	87.82**	852.10**	119.37**
Inoc × NO_3/NH_4		3	...	...	25.73**	68.20*	15.37
N conc × NO_3/NH_4		6	168.1	0.044	8.99**	168.31**	46.66**
Inoc × N conc × NO_3/NH_4		6	...	...	5.03	9.53	5.12
Orthogonal contrasts							
Linear N conc	–	1	30.0	...	0.83	1,233.52**	171.12**
Quadratic N conc	–	1	75.3	...	1.91	6.44	16.67
Linear N conc	+	1	...	0.000	0.37	202.31**	66.12*
Quadratic N conc	+	1	...	0.002	1.27	34.46	1.04
Linear NO_3/NH_4	–	1	2,8492.6**	...	73.34**	585.94**	67.20*
Quadratic NO_3/NH_4	–	1	25.5	...	163.21*	884.49*	31.69
Linear NO_3/NH_4	+	1	...	2.766**	1.48	16.12	0.42
Quadratic NO_3/NH_4	+	1	...	0.032	63.03**	865.41**	126.75**
Linear NO_3/NH_4 at 52 ppm N	–	1	8,466.6**	...	50.56**	56.55	7.20
Quadratic NO_3/NH_4 at 52 ppm N	–	1	45.5	...	8.35	54.46	12.25
Linear NO_3/NH_4 at 105 ppm N	–	1	12,300.8**	...	16.79**	147.26**	5.51
Quadratic NO_3/NH_4 at 105 ppm N	–	1	4.0	...	102.44**	227.87**	0.06
Linear NO_3/NH_4 at 158 ppm N	–	1	8,000.0**	...	13.14*	496.02**	84.05*
Quadratic NO_3/NH_4 at 158 ppm N	–	1	0.0	...	83.10**	843.12**	36.00
Linear NO_3/NH_4 at 52 ppm N	+	1	...	0.616**	0.09	8.51	0.05
Quadratic NO_3/NH_4 at 52 ppm N	+	1	...	0.007	10.73*	56.98	2.25
Linear NO_3/NH_4 at 105 ppm N	+	1	...	1.335**	6.80	23.40	9.80
Quadratic NO_3/NH_4 at 105 ppm N	+	1	...	0.025	20.58**	308.69**	49.00
Linear NO_3/NH_4 at 158 ppm N	+	1	...	0.884**	0.04	79.82	20.00
Quadratic NO_3/NH_4 at 158 ppm N	+	1	...	0.056	35.27**	666.69**	121.00**
Error		36/72[c]	338.0	0.045	2.40	24.49	12.52

[a] Mean squares are for partial sums of squares and orthogonal contrasts determined by using the SAS general linear model procedure (6th ed., SAS Institute Inc. Cary, NC).
[b] Plants were noninoculated (–) or inoculated (+) with *Thielaviopsis basicola*.
[c] Model and error terms had 11 and 36 df for percent roots discolored and disease incidence and had 23 and 72 df for root and shoot weights and flower number.
[d] Significance at the 5% (*) or 1% (**) level of probability.
[e] Nitrogen concentrations were 52, 105 and 158 ppm of N per liter of solution.
[f] NO_3/NH_4 ratios were 1:0, 3:1, 1:1, and 1:3.

μg of Zn per ml) (12). Nitrogen was supplied as $(NH_4)_3SO_4$ and/or $Ca(NO_3)_2$ with or without KNO_3. Equal Ca and K levels were maintained in all nutrient solutions with the addition of $CaCl_2$ and K_2SO_4. Only SO_4 and Cl varied among treatments.

NO_3/NH_4 ratio × K (K experiment). The experiment was a 2 × 2 × 3 factorial in a completely randomized design. Treatments consisted of two inoculum levels (noninoculated and inoculated), two NO_3/NH_4 ratios (3:1, 1:3) at 105 μg of N per ml, and three concentrations of K (113, 213, 313 μg of K per ml). Treatments were replicated four times. Essential elements were provided as previously described. Nitrogen was supplied as $(NH_4)_3SO_4$ and/or $Ca(NO_3)_2$ with or without KNO_3. Levels of K were adjusted with the addition of KCl and K_2SO_4. Levels of Ca were adjusted with the addition of $CaCl_2$. Only SO_4 and Cl varied among treatments.

pH × NO_3/NH_4 ratio (pH experiment). The experiment was a 2 × 2 × 3 factorial in a completely randomized design. Treatments consisted of two inoculum levels (noninoculated and inoculated), two pH ranges (4.6 to 4.8, 6.2 to 6.4), and three NO_3/NH_4 ratios (1:0, 2:1, 1:3) at 105 mg of N per liter^{-1}. Treatments were replicated four times. Essential elements were provided as previously described. Nitrogen was supplied as $(NH_4)_3SO_4$ and/or $Ca(NO_3)_2$ with or without KNO_3. Equal Ca and K levels were maintained in all nutrient solutions with the addition of $CaCl_2$ and K_2SO_4. Only SO_4 and Cl varied among treatments.

A 0.1 N KCl extraction was used to analyze Al in sand at the start and end of the experiment. Each treatment was analyzed separately.

Inoculum production. An isolate of *T. basicola* obtained from diseased pansy roots was grown on V8 agar under continuous light (32 μE s^{-1} m^{-2}) 13 cm below one 15 watt cool white fluorescent lamp for 14 to 21 days at 20°C. Endoconidia were obtained by flooding cultures with sterilized deionized water, rubbing the agar surface with a rubber policeman, then filtering through four layers of cheesecloth. Spore concentrations as determined with a hemacytometer were adjusted to 5 × 10^5 endoconidia per liter. Plants were grown under the appropriate fertilizer regime for 3 weeks, at which time plants were inoculated with 100 ml of inoculum. Inoculum was poured onto the medium's surface and allowed to percolate into the sand.

Data collection. Four weeks after inoculation, roots were washed free of sand, placed individually into a plastic bag, and set in a cooler with ice. Plants were refrigerated until removal to measure fresh root and shoot weights. One gram of roots, representative of the whole root system, was stored in a preservative solution (950 ml of ethanol 95%, 90 ml of lactic acid 85% USP, and 950 ml of deionized water) for subsequent disease indexing.

Roots of the 1-g subsample were cut into approximately 3-mm lengths and evenly distributed in the bottom half of a 100 × 15 mm disposable petri dish. This was set on top of a transparency imprinted with 2.54-cm spaced grid lines and set in the top half of a Pyrex 100 × 15 mm glass petri dish. Presence or absence of *T. basicola* was assessed with 20× magnification at a minimum of 25 root-line intercepts per line for four lines; thus, totaling greater than 100 intercepts per sample (10). A root-line intercept consisted of approximately 0.4 mm length of root with the midpoint over a grid line. Disease incidence was calculated by the number of intercepts with disease present divided by the total number of intercepts.

A tan discoloration of cortical cells, caused by NH_4 toxicity, was observed on noninoculated plants. The percent root system exhibiting discoloration was calculated in the same manner and method as disease incidence. The physiological discoloration was not measured on *T. basicola* inoculated plants because black root rot symptoms were more prominent. Black root rot was distinctive from physiological discoloration.

Statistical analysis. The data were analyzed using the SAS general linear model procedure (GLM, 6th ed., SAS Institute Inc., Cary, NC). The source of variation for the three experiments included the main effects and their interactions. When initial analysis revealed significant interactions between main effects for disease and growth variables, orthogonal contrast statements were used to calculate probability of linear and/or quadratic response(s). Contrasts included the effects of the NO_3/NH_4 ratio within each inoculum, the effects of N concentration within each inoculum, and the effects of the NO_3/NH_4 ratio for each N concentration within each inoculum. Orthogonal contrasts were used similarly in all experiments.

RESULTS

NO_3/NH_4 ratio × N. Disease incidence decreased linearly with increased NH_4 and was not affected by N concentration (Tables 1 and 2). Fresh root and shoot weights and the number of flowers were reduced by *T. basicola*.

Fresh root weight of noninoculated and inoculated plants responded curvilinearly to the NO_3/NH_4 ratio and was not affected by N concentration (Tables 1 and 2). The maximum fresh root weight of noninoculated plants was at the 3:1 NO_3/NH_4 ratio (Table 1). Although the fresh root weight response of noninoculated plants to NO_3/NH_4 ratio was similar for inoculated plants, a high disease incidence at the 1:0 and 3:1 NO_3/NH_4 ratios caused a large difference between root weights of noninoculated and inoculated plants (Table 1). The influences of disease and nutrition resulted in greater fresh root weights of inoculated plants at the 3:1 and 1:1 NO_3/NH_4 ratios than at other NO_3/NH_4 ratios (Table 1).

Fresh shoot weight of noninoculated and inoculated plants responded curvilinearly to NO_3/NH_4 ratio at 105 and 158 μg of N per ml and was not affected by NO_3/NH_4 ratio at 52 μg of N per ml (Tables 1 and 2). Fresh shoot weight of noninoculated and inoculated plants increased linearly with increased N concentration and the curvilinear response to varying NO_3/NH_4 ratios was steeper with increased N concentration (Tables 1 and 2). The maximum fresh shoot weights were at the 3:1 NO_3/NH_4 ratio regardless of interactions (Table 1).

The number of flowers of noninoculated and inoculated plants increased with increased N concentration and was only affected by NO_3/NH_4 ratio at 158 μg of N per ml with the maximum flower number at the 3:1 NO_3/NH_4 ratio (Tables 1 and 2).

Root discoloration of noninoculated plants increased with increased NH_4 and was not affected by N concentration (Table 1).

NO_3/NH_4 ratio × K. Disease incidence and fresh root and shoot weights decreased with increased NH_4 (Tables 3 and 4). There was no disease or plant growth response due to varying K. Fresh root and shoot weights were reduced by infection with *T. basicola* (Tables 3 and 4).

A graph of disease incidence plotted against K (not shown) revealed that disease incidence increased with increased K at the 1:3 NO_3/NH_4 ratio and did not respond to K at the 3:1 NO_3/NH_4 ratio. Also, variability was greater at the 3:1 ratio. The large

Table 3. The effect of NO_3/NH_4 ratio on pansy plants noninoculated and inoculated with *T. basicola*

Variable	Inoculation[a]	NO_3/NH_4 ratio 3:1	NO_3/NH_4 ratio 1:3
Disease incidence (%)	+	71.5[b]	28.2**[c]
Root weight (g)	–	12.7	2.6**
Root weight (g)	+	2.4	1.9**
Shoot weight (g)	–	14.7	7.5**
Shoot weight (g)	+	7.5	5.6**
Flowers (no./plant)	–	3.0	1.8
Flowers (no./plant)	+	2.3	1.1
Roots discolored (%)	–	0.0	55.0**

[a] Plants noninoculated (–) or inoculated (+) with *T. basicola*.
[b] All disease and growth measurements taken on a per plant (= replication) basis 4 weeks after inoculation. Means were calculated from all K concentrations, which totaled 12 replications.
[c] Significance at the 1% (**) level of probability.

Table 4. Analysis of variance for the effects of inoculum, NO_3/NH_4 ratio, and K concentration on disease and growth measurements of pansy plants[a]

Treatment	Inoc.[b]	df	Roots discolored (%)	Disease incidence	Root weight	Shoot weight	Flower no.
Model		5/11[c]	4,540.0**[d]	0.239**	89.84**	56.42**	3.19
Source							
Inoculum (Inoc)[b]		1	...	...	359.54**	253.62**	6.02
NO_3/NH_4 ratio[e]		1	1,8150.0**	1.130**	332.95**	247.13**	17.52**
Inoc × NO_3/NH_4 ratio		1	...	...	279.93**	81.95**	0.02
K concentration (K conc)[f]		2	1,138.0**	0.017	4.29	14.00	2.44
Inoc × K conc		2	...	...	1.64	2.05	0.77
NO_3/NH_4 ratio × K conc		2	1,138.0**	0.015	1.68	1.15	1.90
Inoc × NO_3/NH_4 ratio × K conc		2	...	...	0.29	1.80	0.65
Orthogonal contrast							
Linear K conc at 25% NH_4	–	1	0.0	...	...	...	...
Quadratic K conc at 25% NH_4	–	1	0.0	...	...	...	...
Linear K conc at 75% NH_4	–	1	3,200.0**	...	...	...	...
Quadratic K conc at 75% NH4	–	1	1,350.0**	...	...	...	...
Error		18/36[c]	142.0	0.025	2.98	6.39	2.10

[a] Mean squares are for partial sums of squares and orthogonal contrasts determined by using the SAS general linear model procedure (6th ed., SAS Institute Inc. Cary, NC).
[b] Plants were noninoculated (–) or inoculated (+) with *Thielaviopsis basicola.*
[c] Model and error terms had 5 and 11 df for percent roots discolored and disease incidence and had 18 and 36 df for root and shoot weights and flower number.
[d] Significance at the 5% (*) or 1% (**) level of probability.
[e] NO_3/NH_4 ratios were 3:1 and 1:3.
[f] Potassium concentrations were 133, 233, and 333 ppm of K per liter of solution.

Table 5. The effect of K at two NO_3/NH_4 ratios on pansy plants noninoculated and inoculated with *T. basicola*

		NO_3/NH_4 ratio							
		3:1				1:3			
		K concentration (ppm)							
Variable	Inoc.[a]	133	233	333	Sig.[b]	133	233	333	Sig.
Disease incidence (%)	+	75.8[c]	65.5	73.3	NS[d]	22.5	25.9	36.1	L*[d]
Roots discolored (%)	–	0.0	0.0	0.0	NS	82.5	40.0	42.5	LQ**[d]

[a] Plants were noninoculated (–) or inoculated (+) with *T. basicola.*
[b] Significance pertains to preceding row of numbers across K values within each NO_3/NH_4 ratio. Orthogonal contrasts were used to determine relationship of the line through values.
[c] All disease and growth measurements taken on a per plant (= replication) basis 4 weeks after inoculation. Means were calculated from four replications per treatment.
[d] No significant difference (NS) between values, or a significant linear (L) or linear-quadratic (LQ) relationship between values. Significance at the 5% (*) or 1% (**) level of probability.

Table 6. Analysis of variance for the effects of K concentration at the 1:3 NO3/NH4 ratio on the percentage of root discoloration and disease incidence measurements of pansy plants[a]

Treatment	Inoc.[b]	df	Root discolored (%)	Disease incidence
Model		2	2,275.0**[c]	0.020*
Source				
K concentration (K conc)[d]		2	2,275.0**	0.020*
Orthogonal contrasts				
Linear K conc	–	1	3,200.0**	...
Quadratic K conc	–	1	1,350.0**	...
Linear K conc	+	1	...	0.037**
Quadratic K conc	+	1	...	0.003
Error		9	...	0.003

[a] Mean squares are for partial sums of squares and orthogonal contrasts determined by using the SAS general linear model procedure (6th ed., SAS Institute Inc. Cary, NC).
[b] Plants were noninoculated (–) or inoculated (+) with *Thielaviopsis basicola.*
[c] Significance at the 5% (*) or 1% (**) level of probability.
[d] Potassium concentrations were 133, 233, and 333 ppm of K per liter of solution.

variability within treatments (between replications) for K concentrations at the 3:1 NO_3/NH_4 ratio produced a large error sum of square. The large error sum of squares could erroneously result in a small *F* value for K levels at the 1:3 NO_3/NH_4 ratio, where the variability within treatments was small. When K level for the 1:3 ratio was tested separately, disease incidence increased linearly and physiological discoloration decreased curvilinearly with increased K (Tables 5 and 6). Plant growth responses were still not affected by K (data not shown).

Roots of noninoculated plants fertilized with a 3:1 NO_3/NH_4 ratio were white and those fertilized with a 1:3 NO_3/NH_4 ratio exhibited a tan discoloration of cortical cells (Tables 3 and 4). The discoloration was greatest at 113 µg of K per ml and lowest at the 213 and 313 µg of K per ml concentrations (Table 5).

pH × NO_3/NH_4 ratio. Disease incidence decreased linearly as NH_4 was increased and was not affected by pH (Tables 7 and 8). Fresh root and shoot weights were significantly reduced by infection with *T. basicola.*

Fresh root and shoot weights responded curvilinearly to the varying NO_3/NH_4 ratios for both noninoculated and inoculated plants (Tables 7 and 8). The maximum root and shoot weights occurred at the 2:1 NO_3/NH_4 ratio. Root and shoot weights were not affected by pH.

Flower number was not affected by *T. basicola* or pH (Table 8). The number of flowers decreased linearly as NH_4 was increased for noninoculated plants at pH 4.7 and 6.2, and inoculated plants at pH 4.7 (Tables 7 and 8).

Discoloration of root cortical cells of noninoculated plants increased with increased NH_4. The discoloration was not affected by pH (Tables 7 and 8).

Al averaged 15.2 and 5.3 µg/ml at the start and finish of the experiment, respectively. The loss of Al from sand surfaces was consistent for all NO_3/NH_4 ratios and pH values (data not shown).

DISCUSSION

The NO_3/NH_4 ratio was the dominant nutritional factor that reduced disease. Disease incidence was lowest with high NH4 but plant growth was also inhibited. Growth of inoculated plants was affected by nutrition and disease responses to NO_3/NH_4 ratio. The maximum root and shoot weight and flower number of noninoculated plants was at the 3:1 NO_3/NH_4 ratio.

Table 7. The effect of NO_3/NH_4 ratio at two pH values on pansy plants noninoculated and inoculated with *T. basicola*

		pH							
		4.7[a]				6.2			
Variable	Inoc.[b]	1:0	2:1	1:3	Sig.[c]	1:0	2:1	1:3	Sig.
Disease incidence (%)	+	68.9[d]	40.5	12.2	L**[e]	65.0	43.3	8.1	L**
Root weight (g)	–	4.2	5.8	1.2	LQ**	4.1	6.9	1.2	LQ**
Root weight (g)	+	1.3	2.6	1.2	Q*	1.5	2.6	1.4	Q*
Shoot weight (g)	–	11.1	12.2	4.4	LQ*	10.1	11.9	3.1	LQ**
Shoot weight (g)	+	5.3	7.5	3.9	Q*	5.3	8.6	4.3	Q*
Flowers (no.)	–	4.3	4.0	2.3	L*	3.6	3.0	1.7	L*
Flowers (no.)	+	3.0	2.5	1.3	L*	2.5	4.3	1.8	Q**
Roots discolored	–	0.0	3.3	53.3	LQ*	0.0	3.3	36.7	L*

[a] Disease and growth measurements not significantly different between pH treatments; listed separately to note similarity between values.
[b] Plants were noninoculated (–) or inoculated (+) with *T. basicola*.
[c] Significance pertains to preceding row of numbers across NO3/NH4 ratio values within each pH. Orthogonal contrasts were used to determine relationship of the line through values. Significant linear (L), quadratic (Q), or linear-quadratic (LQ) relationship between values.
[d] All disease and growth measurements taken on a per plant (= replication) basis 4 weeks after inoculation. Means were calculated from four replications per treatment.
[e] Significance at the 5% (*) or 1% (**) level of probability.

Table 8. Analysis of variance for the effects of inoculum, pH, and NO_3/NH_4 ratio on disease and growth measurements of pansy plants[a]

Treatment	Inoc.[b]	df	Roots discolored (%)	Disease incidence	Root weight	Shoot weight	Flower no.
Model		5/5/11[c]	1,592.2**[d]	0.261**	12.28**	36.20**	3.78*
Source							
Inoculum (Inoc)[b]		1	...	...	48.35*	90.66**	4.02
pH[e]		1	138.8	0.002	0.51	0.34	0.10
Inoc × pH		1	...	...	0.04	4.87	4.76
NO_3/NH_4 ratio[f]		2	3,772.2**	0.648**	34.22**	132.11**	12.55**
Inoc × NO_3/NH_4 ratio		2	...	...	12.99**	30.43*	1.12
pH × NO_3/NH_4 ratio		2	138.8	0.003	0.35	0.81	0.79
Inoc × pH × NO_3/NH_4		2	...	...	0.60	0.13	1.45
Orthogonal contrasts							
Linear NO_3/NH_4	–	1	6,075.0**	...	23.60**	140.65**	12.00**
Quadratic NO_3/NH_4	–	1	1,469.4*	...	53.20**	95.01**	1.00
Linear NO_3/NH_4	+	1	...	1.290**	0.06	5.34	6.25*
Quadratic NO_3/NH_4	+	1	...	0.006	7.69**	59.72**	8.33*
Error		12/18/30[c]	238.9	0.03	0.71	6.34	1.30

[a] Mean squares are for partial sums of squares and orthogonal contrasts determined by using the SAS general linear model procedure (6th ed., SAS Institute Inc. Cary, NC).
[b] Plants were noninoculated (–) or inoculated (+) with *Thielaviopsis basicola*.
[c] Model and error terms had 5 and 11 df for percent roots discolored, 5 and 18 df for disease incidence, and 11 and 30 df for root and shoot weights and flower number.
[d] Significance at the 5% (*) or 1% (**) level of probability.
[e] pH levels were 4.7 and 6.2.
[f] NO_3/NH_4 ratios were 1:0, 2:1, and 1:3.

However, growth of inoculated plants was reduced at the 3:1 NO_3/NH_4 ratio because of high disease incidence, which resulted in no difference between plant growth at the 3:1 and 1:1 NO_3/NH_4 ratios (Table 1).

Growth and disease responses to high levels of NH_4-N have been documented with other plants and pathogens (17,22,24, 26). Many related factors could be influencing *T. basicola* and/or *V.* × *wittrockiana* to explain effects mediated by nitrogen and several possible explanations are reported in the literature. *T. basicola* spore viability can be reduced with NH_3 and germination inhibited with Al (6,20). While NH_3 is toxic at ≥200 µg/ml, NH_4 is the preferred N source for growth of *T. basicola* (6,27). In these studies, a reduction in disease did not necessarily correspond with a high concentration of NH_4; for example, the 2:1 and 1:3 NO_3/NH_4 ratio treatments, at 105 µg N per ml, provided 37 and 79 µg of N per ml as NH_4.

The ability of Al to inhibit spore germination can vary considerably and depends on nutrient levels, pH, solubility, and possibly other factors (20). The pH of all fertilizer solutions, in the N and K experiments, ranged from 4.2 to 4.5; thus, all treatments were acidified. Plant uptake of NO_3 and NH_4 altered pH of the soil solution, which became more neutral (6.5) or remained acidic (4.2) with application of 1:0 or 1:3 NO_3/NH_4 ratio fertilizer, respectively. Uptake of 100% NO_3 would take ≥12 h before soil solution pH would increase. Plant growth and disease responses due to NO_3/NH_4 ratio were confounded with the subsequent pH change of the soil solution.

In the pH study, disease and plant growth responses to NO_3/NH_4 ratios were not affected by pH. In this study, pH was not buffered but was adjusted daily. Changes to soil solution pH would only occur with uptake of NO_3 at a pH of 4.7 and of NH_4 at a pH of 6.2. If plant growth and disease responses, in the N and K experiments, were strictly due to changes in soil solution pH that affected elemental availability such as that of Al, then there should be significant interaction between NO_3/NH_4 ratio and pH, in the pH study. In fact, disease and growth measurements were nearly identical for both pH treatments. Also, there were no differences in the amount of Al released from the sand due to pH or NO_3/NH_4 ratio. Most of the solubilized Al could have been released in the 3 weeks prior to inoculation with remaining Al inconsequential at the high inoculum level used in these studies.

Previous research reported reduced disease at a pH that was ≤5.6 (3,8,18,19,25, 30). The lack of response to pH may be due to the lack of influential factors in sand, which had a high base saturation (100% with Ca, Mg and K), low CEC (0.225), and negligible levels of exchangeable Al. In field

soils, particularly clay, Al hydrolysis can be a major factor in the buffering capacity of soil solutions. The pH would affect ammonification, nitrification, P form and solubility, Al, Cu, Mn, and Fe solubility, and K, Ca, and Mg availability.

Increased K decreased NH4 toxicity symptoms substantially and increased disease incidence slightly but only at the 1:3 NO_3/NH_4 ratio (Table 3). Growth and disease responses to interactions between NH_4 and N or K have been documented with other plants and diseases (2,22). A number of interactions between NH_4, K, and pH could alter the influence of NO_3/NH_4 ratio on *T. basicola* and/or *V.* × *wittrockiana*. In plants, exogenous NH_4 inhibits NO_3 and K uptake. Conversely, high K can moderate negative effects from NH_4 on NO_3 uptake without altering NH_4 uptake (23). A mutual interference of NH_4 and K uptake can occur at a pH of 5.0 and 4.0 (9).

These studies show that N form influences disease with a 56% decrease in disease from a 1:0 to a 1:3 NO_3/NH_4 ratio of plants grown in sand. It was not known whether N form reduced endoconidia viability, limited penetration, or reduced colonization in root tissue. Different results may occur in a peat-based medium that is commonly used by the bedding plant industry and with aleuriospores as the inoculum source. The more that soil factors and interactions are understood, the easier nutritional information can be adapted to different media and utilized as a component for integrated pest management programs.

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