THE INFLUENCE OF PLANT NUTRITION ON FUSARIUM WILT OF BROADLEAF TOBACCO

TOBACCO SCIENCE

J. A. LaMondia and T. M. Rathier[1]

The role of soil pH, calcium, and nitrogen nutrition on Fusarium wilt of broadleaf tobacco was investigated in a series of field and greenhouse experiments. Soil pH and nitrogen nutrition did not independently affect wilt severity. However, NH_4-N fertilizers were associated with increased wilt severity compared to NO_3-N at low (4.1) but not high (6.4) pH. Higher calcium availability was effective at reducing wilt severity and increasing plant shoot weight at soil pH levels of 6.2 to 6.4. Up to 13,400 kg/ha gypsum was applied annually to field plots to increase calcium availability without increasing soil pH. Increased calcium availability in field plots with low levels of *Fusarium* in soil reduced *F. oxysporum* stem infection in the first year and reduced wilt incidence during the second year. Increased calcium availability in soils heavily infested with *F. oxysporum* did not affect wilt incidence or severity. The manipulation of calcium availability in soil may assist in the management of low levels of *F. oxysporum* infestation.

Additional key words: calcium, *Fusarium oxysporum*, *Nicotiana tabacum*, pH, resistance.

INTRODUCTION

Over the past 15 years, Fusarium wilt, caused by *Fusarium oxysporum* (Schlecht.) Wr., has been the most destructive disease affecting broadleaf tobacco in Connecticut. Severe wilt incidence has been associated with tobacco cyst nematode infestation, but fumigation or oxamyl application to suppress nematodes has reduced wilt incidence and severity (13,15). However, nematode control can be expensive and may not reduce wilt when *F. oxysporum* soil densities are high. Although wilt-resistant tobacco cultivars have been developed, these cultivars are not immune to infection (14,15) and can become diseased at high inoculum levels.

A number of Fusarium wilt diseases have been managed by manipulating soil pH and macro- and microelement nutrition (5,9). Nitrogen source, lime, calcium availability, and soil pH have all been associated with wilt incidence and severity on tomato and chrysanthemum (3-5,7,8,20). Our objectives were 1) to determine the influence of soil pH, calcium, and nitrogen nutrition on Fusarium wilt of broadleaf tobacco, and 2) to evaluate nutrition as an additional wilt management tactic in field soils.

[1]Associate Plant Pathologist, Department of Plant Pathology and Ecology, and Assistant Soil Scientist, Connecticut Agricultural Experiment Station, Valley Laboratory, P. O. Box 248, Windsor, CT 06095.

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MATERIALS AND METHODS

The influence of soil pH, calcium, and nitrogen nutrition on Fusarium wilt of tobacco was investigated in three experiments using potted tobacco plants grown outdoors and under greenhouse conditions. The potting soil used in all experiments was a mix of 2:1:1 shredded peat moss:vermiculite:sand which was amended with triple super phosphate (1.8 kg/m^3), potassium sulfate (1.8 kg/m^3), and Micromax (Grace Sierra, Milpitas, CA)(0.6 kg/m^3). In the first experiment, the mix was either left unamended or amended with dolomite (15 kg/m^3) or gypsum (14 kg/m^3). Eight one-liter pots were filled with soil mix and infested with 1.2 x 10^7 colony forming units (CFU) *F. oxysporum*-infested ground straw per pot (15). The pH of the mix was 4.1 in pots without dolomite and 6.0 in pots amended with dolomite. Calcium levels (17) were trace in unamended pots and 900 ppm in pots amended with gypsum or dolomite. Two-month-old '86-4' tobacco transplants were planted into pots on 6 June 1988. 86-4 is a wilt-susceptible broadleaf tobacco cultivar. Plants were fertilized once per week for the first two weeks and twice per week for the next six weeks with 500 mL of a 400 ppm nitrogen solution using one of five nitrogen types. Nitrogen treatments consisted of sufficient amounts of NH_4SO_4, NH_4NO_3, and KNO_3 to result in equivalent rates of nitrogen with NH_4/NO_3 ratios of 100/0; 75/25; 50/50; 25/75; and 0/100. The resulting experimental design

Table 1. **The influence of soil pH, calcium, and nitrogen nutrition on Fusarium wilt rating, 86-4 *broadleaf tobacco plant size and color in outdoor pots.***

pH/Calcium ppm	NH_4/NO_3[a]	Wilt rating[b]	Color[c]	Size[d]
4.1/trace	100/0	1.0	4.5	1.8
	75/25	3.8	2.9	2.1
	50/50	2.5	3.3	3.0
	25/75	2.3	3.1	3.8
	0/100	3.8	2.4	3.0
4.1/900	100/0	0.0	4.5	3.5
	75/25	1.0	4.5	3.5
	50/50	1.5	3.4	3.3
	25/75	0.0	3.8	4.6
	0/100	0.0	3.6	4.6
6.0/900	100/0	0.0	4.3	4.4
	75/25	1.8	3.8	3.8
	50/50	0.0	3.9	4.6
	25/75	0.8	3.4	4.4
	0/100	0.5	3.4	4.9
	Source of Variation	Significance Level P =		
	pH/Calcium	0.001	0.001	0.001
	NH_4/NO_3	0.003	0.001	0.001
	Interaction	NS	NS	0.02
	Linear Contrasts	Significance Level P =		
low vs. high pH (both 900 ppm Ca)		NS	0.05	NS
low vs. high Ca (both pH 4.1)		0.05	0.001	0.001
predominantly NH_4 vs. NO_3		NS	0.001	0.001

[a] NH_4/NO_3 ratios.
[b] Wilt rating on a scale of 0-4 where 0=healthy and 4=dead.
[c] Color rating on a scale of 0-5 where 0=brown and 5=dark green.
[d] Size rating on a scale of 0-5 where 0=small and 5=large.

was a 3 x 5 factorial in a randomized complete block design with four replications.

The potting soil mix was sampled once per week to monitor pH and every two weeks for nutrient analysis. Wilt ratings (on a scale of 0 to 4 where 0 = healthy and 4 = dead)(15) were taken on 1 August and 5 August 1988. Color (0 = brown, 1 = yellow, 2 = yellow green, 3 = light green, 4 = green, and 5 = dark green) and size ratings (relative ranking on a scale of 0 to 5 where 0 = small and 5 = large) were taken on 11 August 1988.

Similar experiments were performed in the greenhouse in the fall of 1988. The potting soil was mixed with *F. oxysporum*-infested ground straw to result in 2.0 x 10^4 CFU *F. oxysporum* per cm^3 soil. The mix was amended with dolomite or gypsum to result in low pH (4.2-4.4) with low calcium (200-500 ppm) or high calcium (1,600 ppm) availability, or to result in high pH (6.2-6.4) with high calcium levels (1,600 ppm). In addition, greenhouse pots containing five liters of soil mix per pot were drenched weekly with one liter of 0.03 to 0.1 M $NaHCO_3$ (concentration adjusted according to soil test) to result in a high pH-low calcium treatment. All other soils were drenched with water alone. In the first greenhouse experiment, 2-month-old 86-4 plants were transplanted to pots on 28 September 1988. After transplanting, plants were fertilized weekly with 200 ppm nitrogen (N) with one of five NH_4/NO_3 ratios as described above in the drench solution. Fusarium wilt ratings and fresh shoot-weight measurements were taken on 14 November 1989. In the second greenhouse experiment, treatments remained the same except that all plants were fertilized with a 75/25 ratio of NO_3/NH_4 supplied by KNO_3 and NH_4NO_3. Transplants were planted on 18 November 1988, and Fusarium wilt ratings and fresh shoot-weight measurements were taken on 4 January 1989.

The effects of calcium on Fusarium wilt of broadleaf tobacco were examined in three field experiments. In the first, three rates of gypsum, 0, 560, and 2,240 kg/ha were applied to 3 m by 6 m plots (pH = 5.3) naturally infested with *F.*

Table 2. **The influence of soil pH, calcium, and nitrogen nutrition on Fusarium wilt rating and 86-4 broadleaf tobacco plant size in greenhouse pots.**

pH/Calcium ppm	NH_4/NO_3[a]	Wilt rating[b]	Fresh weight (g)
4.1/200	100/0	2.5	73.8
	75/25	3.8	47.5
	50/50	2.8	120.8
	25/75	0.0	172.8
	0/100	0.0	160.8
4.1/1600	100/0	2.0	83.3
	75/25	2.0	89.0
	50/50	0.8	181.5
	25/75	1.0	154.5
	0/100	0.8	163.5
6.4/200	100/0	1.5	53.8
	75/25	2.3	50.3
	50/50	2.0	63.0
	25/75	2.0	88.0
	0/100	3.5	50.5
6.4/1600	100/0	0.0	145.3
	75/25	0.0	195.5
	50/50	0.0	195.5
	25/75	0.0	192.5
	0/100	0.8	194.0

Source of Variation	Significance Level P =	
pH	NS	NS
Calcium	0.001	0.001
NH_4/NO_3	NS	0.001
pH x Calcium	0.02	0.001
pH x N source	0.01	0.04
Ca x N source	NS	NS
pH x Ca x N source	NS	NS

[a] NH_4/NO_3 ratios
[b] Wilt rating on a scale of 0-4 where 0=healthy and 4=dead.

oxysporum (1.7 to 4.4 x 10^3 CFU per cm^3 soil). Plots were fertilized with 150 kg N per ha using a cottonseed meal-based tobacco fertilizer (10:8:10). A preplant tank-mix application of diazinon (2.2 kg [ai]/ha) and metalaxyl (1.1 kg [ai]/ha) was incorporated into all plots 24 hours before planting. Paired rows (10 plants per row) of wilt-susceptible 86-4 and wilt-resistant 'C8' broadleaf tobacco were transplanted on 22 June 1989. There were eight replicate plots per treatment. Plots were sidedressed with 88 and 55 kg N/ha and cultivated on 7 July and 12 July, respectively. Napropamide (1.65 kg [ai]/ha) was applied between rows on 13 July. Wilt ratings were taken before harvest on 24 August 1989.

New plots were established in *F. oxysporum*-uninfested soil (pH = 6.0) in 1990. Plots (3 m by 6 m) were amended with 0, 6700, or 13400 kg gypsum per ha and treated with diazinon and metalaxyl as previously described. Plots were fertilized with 197 kg N/ha of a cottonseed meal-based fertilizer (10:8:10) before transplanting two rows of 86-4 tobacco into each plot on 19 June 1990. Rows were 1 m apart and 10 plants per row were spaced 0.6 m apart within rows. Plants were each inoculated with 1.0 x 10^7 CFU *F. oxysporum* from straw culture on 3 July 1990. The plants were sidedressed with 97 kg N per ha and cultivated on 10 July 1990. Napropamide herbicide was applied as before on 11 July. Plants were cut and stem sections were taken on 31 August. One-cm-thick stem sections from 30 cm and 120 cm above the soil surface were plated onto *Fusarium*-selective Komada media (11), and the number of infected stems was recorded after one week.

On 10 June 1991, gypsum was applied again at the same rates as the previous season to the same plots (pH = 5.5). Plots were fertilized (165 kg N/ha), treated with diazinon and

Table 3. The influence of soil pH and calcium on Fusarium wilt rating and 86-4 broadleaf tobacco plant size in greenhouse pots.

pH	Calcium ppm	Wilt rating[a]	Fresh weight (g)
4.4	500	1.6	56.6
4.4	1600	1.0	75.7
6.2	500	1.6	24.1
6.2	1600	0.4	129.7
	Source of Variation	Significance Level P =	
	pH	NS	NS
	Calcium	0.005	0.001
	Interaction	NS	0.001

[a] *Wilt rating on a scale of 0-4 where 0=healthy and 4=dead.*

metalaxyl as before and 86-4 transplants were set in plots and again inoculated with 1.0 x 10^7 CFU *F. oxysporum* from straw culture on 24 June 1991. The plants were sidedressed with 123 kg N per ha and cultivated on 1 July. Napropamide was applied as before on 2 July. Wilt ratings were taken before harvest on 7 August 1991.

Data were analyzed by analysis of variance (ANOVA). Rating data were analyzed after transformation to square root of x+1 to stabilize variance.

RESULTS

In field pot experiments, pH/calcium soil treatments significantly affected the wilt ratings, color, and size of plants (**Table 1**). Plants grown in soil that had high calcium levels had less disease than plants that had low calcium levels. There were no significant differences in disease between plants fertilized with predominantly (75% or greater) ammonia or nitrate based fertilizers. Plant color was better when pots were fertilized with ammonia, but plants were larger when they were fertilized with higher levels of nitrate.

When the experiment was performed in the greenhouse with a balanced factorial design, tobacco that was grown in pots with 1,600 ppm calcium had less wilt than plants that were grown in 200 ppm calcium, especially at high soil pH (**Table 2**). The two soil pH treatments and the nitrogen-ratio treatments alone had no significant effects on wilt severity. NH_4-N sources were associated with increased wilt severity at low pH but not at the high pH. Higher calcium levels were more effective at reducing wilt severity and increasing plant shoot weight when the pH was 6.2 to 6.4 than when the pH was 4.1 to 4.4 (**Table 2**). Fresh shoot weight was negatively correlated with wilt rating in all pot experiments. Similar results were observed when the calcium and pH treatments were repeated (**Table 3**).

The application of up to 2,240 kg/ha gypsum to field soils (pH = 5.3) naturally infested with high levels of *F. oxysporum* did not significantly increase yields or decrease Fusarium wilt incidence or severity in broadleaf tobacco (**Table 4**). Wilt-resistant broadleaf, however, had greatly reduced wilt symptom expression and fewer wilted plants than wilt-susceptible tobacco when it was grown side by side in paired rows. The reduced wilt was reflected in the higher fresh leaf weight per plot.

When up to 13,400 kg/ha gypsum was applied annually over two years in plots with low *F. oxysporum* inoculum densities, *F. oxysporum* stem infection 30 cm above the soil line was reduced in the first year and wilt incidence was reduced during the second year (**Table 5**).

DISCUSSION

The incidence and severity of Fusarium wilt in crops such as tomato and chrysanthemum have been reduced by manipulating nitrogen and calcium nutrition as well as by altering soil pH (3-5,8,20). These factors may interact, and the relationships between nutrients, pH, and wilt may be complex (7). A number of researchers found that nitrate rather than ammonia fertilization reduces *F. oxysporum* infection and symptom development (18,20). We determined that nitrogen nutrition influenced tobacco color and size, but was ineffective in reducing wilt. Ammonia fertilization of broadleaf tobacco is generally avoided in production fields as it results in poor leaf quality (19).

In our pot experiments, calcium nutrition

Table 4. **The effects of gypsum soil amendments and tobacco cultivar on Fusarium wilt rating and yield of 86-4 broadleaf tobacco in field plots - 1989.**

Gypsum (kg/ha)	Cv[a]	Wilt incidence[b]	Wilt rating[c]	No. plants harvested[d]	Fresh wt /plant (g)
0	C8	1.4	1.2	9.5	1195.6
	86-4	7.3	3.1	2.5	350.6
560	C8	0.5	0.7	9.9	1260.7
	86-4	7.3	2.7	2.6	389.0
2240	C8	2.0	1.1	9.4	1207.1
	86-4	7.9	3.0	2.0	624.4

Source of Variation	Significance Level P =			
gypsum	NS	NS	NS	NS
cultivar	0.002	0.001	0.001	0.01
interaction	NS	NS	NS	NS

[a] Cultivar: C8 = wilt-resistant, 86-4 = wilt-susceptible tobacco.
[b] Number out of ten plants with wilt symptoms.
[c] Wilt rating on a scale of 0-4 where 0=healthy and 4=dead.
[d] Number out of ten plants harvested per plot.

appeared to be the most effective of the nutritional factors tested. Poor calcium nutrition has been implicated as an important factor in the development of Fusarium wilt (3,4,8). Calcium regulates the biosynthesis of callose (10). Callose deposition in vascular tracheid pits and along the walls of contact parenchyma cells adjacent to infected vessels can be one of the first plant defense responses to invasion by vascular wilt pathogens (2).

Calcium levels may also be the most amenable to manipulation in Connecticut broadleaf tobacco production. Fusarium wilt can occur in acid, neutral, or alkaline soils (6,16), and soil pH may need to be increased to levels greater than 6.4 to have an appreciable effect on wilt (16). In our experiments, pH alone did not affect wilt incidence or severity. However, the combination of high calcium availability and higher pH resulted in the greatest disease control. Historically, black root rot of broadleaf tobacco, caused by *Thielaviopsis basicola*, has been severe in soils above pH 5.6 to 6.0 (1). Therefore, we evaluated the effects of amending field plots with gypsum to increase calcium availability without raising pH over 5.6 to 6.0.

Our results indicate that although wilt may be suppressed by manipulating calcium levels in pots, the addition of extremely large amounts of gypsum to field plots to increase calcium levels only had a minor effect on *Fusarium* infection of the plant and disease incidence at low inoculum levels. The rate of gypsum needed to reduce wilt in field plots (13,400 kg/ha) greatly exceeds the rates

Table 5. **The effects of gypsum soil amendments on *Fusarium oxysporum* infection, wilt incidence and severity of 86-4 broadleaf tobacco in field plots - 1990 and 1991.**

		1990		1991	
Gypsum (kg/ha)	ppm available Calcium	*F. oxysporum* isolations at[a] 30 cm	120 cm	Wilt incidence[b]	Wilt rating[c]
None	200	6.9	5.1	3.3	2.1
6700	910	6.5	4.3	2.0	2.0
13400	1570	5.1	3.8	1.6	1.7
Significance[d]		0.03	NS	0.02	NS

[a] Number of *F. oxysporum* isolations per eight stems.
[b] Number out of 18 plants with wilt symptoms.
[c] Wilt rating on a scale of 0-4 where 0=healthy and 4=dead.
[d] Probability of significant differences among means determined by analysis of variance.

currently used by broadleaf tobacco growers (340 to 560 kg/ha). Adverse agronomic effects, such as reduced availability of other cations, may reduce leaf quality and offset any benefits. The effects of calcium were insignificant at high disease levels in field plots.

The effects of nutrition on Fusarium wilt in field plots were small and may best be combined with other management tactics, such as fungicides (5) or wilt-resistant tobacco cultivars (14). Wilt-resistant plants are not immune, and *F. oxysporum* populations are maintained or slightly increased on wilt-resistant cultivars (12). Adequate calcium nutrition may reduce infection and slow pathogen increase in soil. The role of calcium availability on infection and subsequent inoculum potential in resistant cultivars deserves further study.

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