Influence of the Tobacco Cyst Nematode (*Globodera tabacum*) on Fusarium Wilt of Connecticut Broadleaf Tobacco

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ABSTRACT

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The influence of *Globodera tabacum* on wilt of broadleaf tobacco caused by *Fusarium oxysporum* was examined under field microplot and greenhouse conditions. At high inoculum densities, *F. oxysporum* alone readily killed plants regardless of *G. tabacum* infection, but at lower *F. oxysporum* densities, *G. tabacum* densities above about 25 juveniles per cubic centimeter of soil increased wilt in tobacco both susceptible and resistant to *Fusarium*. The nematode alone did not cause wilt symptoms. Fusarium wilt symptoms on resistant tobacco lines were much less severe than on susceptible lines, despite the fact that *F. oxysporum* was isolated from stems of up to 80% of resistant plants. In a split-root system experiment, *G. tabacum* increased wilt in a localized rather than systemic manner. These data suggest that the incidence of Fusarium wilt in production fields can be decreased by reducing *G. tabacum* populations below threshold levels.

Over the past decade, Fusarium wilt has increased in importance to become the most destructive disease of broadleaf tobacco in Connecticut. Circumstantial evidence for the involvement of the tobacco cyst nematode (*Globodera tabacum* (Lownsbery & Lownsbery) Behrens) with this increase in disease severity was similar to previous associations of *Meloidogyne* spp. with Fusarium wilts (13). Severely affected commercial tobacco fields have been associated with *G. tabacum* infestations, and incidence of Fusarium wilt has been reduced by fumigation with dichloropropene and methyl isothiocyanate (139 L/ha) (G. S. Taylor, *unpublished*).

The causal agent of Fusarium wilt, *Fusarium oxysporum* (Schlecht.) Wr., is widespread in tobacco-growing areas, but serious losses are generally restricted to isolated regions. The reasons for the limited distribution of severely affected areas are not well understood, but other plant pathogens may be involved (9). Root-knot nematodes (*Meloidogyne* spp.) have been shown to increase the incidence and severity of Fusarium wilt on tobacco as well as other crops (12). Predisposition of tobacco to Fusarium wilt occurred when *F. oxysporum* and *Meloidogyne* spp. were inoculated simultaneously but was greatest when the fungus was added 3–4 wk after nematode infection (11). It has been suggested that the specialized, nutrient-rich feeding cells induced by the nematode contribute to this interaction (10). Cyst nematodes, seden-

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tary endoparasites that produce similar feeding cells, have also been implicated in disease complexes (3,5,14,15).

The circumstantial association between *G. tabacum* infestations and production fields with a high incidence of wilt and the previous implication of other sedentary endoparasitic nematodes in similar disease complexes led us to investigate the role of the nematode in this disease syndrome. This paper is a report of the effects of *G. tabacum* on Fusarium wilt severity for broadleaf tobacco lines both susceptible and resistant to Fusarium under greenhouse and field conditions.

MATERIALS AND METHODS

Microplots were constructed using Rubbermaid waste cans 37.5 cm top diameter, 30 cm bottom diameter, and 45 cm deep. Holes of the above diameter and 37.5 cm deep were dug in a Merrimac fine sandy loam soil (71.8% sand, 23.0% silt, 5.2% clay, and 2.2% organic matter, pH 6.2) typical of Connecticut Valley tobacco soils in rows 1 m apart and 2.4 m apart within each row. Soil used in the microplots was removed, fumigated with methyl bromide (1 kg/20 m^2 of soil, 15–20 cm deep between two sheets of plastic), and replaced in the microplots. Subsoil and topsoils were treated separately.

F. oxysporum was grown for 2 days in 1.5 L potato-dextrose broth on a shaker, then added to 0.5 kg straw that had been cut in a Waring Blendor for 1 min and autoclaved twice for 1 hr at 121 C. After 2 wk of growth, the infested straw was mixed thoroughly and used as inoculum.

G. tabacum cysts to be used for inoculum were extracted from field soil, soaked overnight in water, and surface-sterilized in 0.5% NaOCl plus surfactant for 10 min to allow inoculation of nematodes without contaminating fungi.

Microplots were left uninoculated or infested with either of two levels of each pathogen in a factorial experiment with five replicates. Levels of *F. oxysporum* were none, 20 g, or 50 g of infested straw per plot. Nematode levels were 0, 1.8×10^5, or 9.0×10^5 encysted juveniles in eggs per plot (0, 5.6, and 28.0 juveniles per cubic centimeter of soil, respectively). Soil was fertilized according to commercial recommendations, mixed to 20 cm deep, and susceptible tobacco transplants (CT86-3) were placed in the center of each plot on 16 June 1986. Susceptible tobacco transplants were also transplanted into border rows and between plots within rows at 60-cm centers to simulate field conditions.

Plants were rated weekly for wilt symptoms by a subjective rating scale of 0–4, where 0 = healthy plants, 1 = stunted or off-color plants, 2 = plants with one symptomatic leaf, 3 = plants with more than one symptomatic leaf, and 4 = dead plants. Plots with plants that died before 24 July were replanted 16 or 23 July with the *Fusarium*-resistant broadleaf line CT86-8.

Susceptible and resistant tobacco plants were harvested 26 August and 26 September, respectively. Soil within each microplot was mixed, and 10 soil cores 2 cm in diameter and 20 cm deep were removed to determine *F. oxysporum* final populations by dilution plating on Komada's medium (7). Cysts extracted from microplot soil with a modified Fenwick can were crushed to determine *G. tabacum* juveniles per cubic centimeter of soil. Shoot dry weight and *F. oxysporum* infection of stem tissues 2–3 cm above the soil line were determined at harvest.

The greenhouse study involved two *Fusarium*-susceptible (CT86-4 and MD-Wilson) tobacco cultivars and two resistant (CT86-8 and MD-609) cultivars left uninoculated or inoculated with 1×10^6 or 1×10^7 *F. oxysporum* microconidia and 5,000 or 20,000 *G. tabacum* juveniles per 400-cm^3 pot in a factorial experiment. Both pathogens were inoculated to the surface of the pot, and moist soil was added to prevent desiccation. Roots were neither disturbed nor wounded.

Tobacco plants of one susceptible (CT86-4) and two *Fusarium*-resistant lines (CT86-6, CT86-8) were grown in the greenhouse in 2-L capacity pots containing 1 L of artificial potting mix (60% peat moss and 40% vermiculite) and 1 L of fumigated microplot field soil. Each pot was amended with 30 g of *F. oxysporum*-infested straw as previously described. Either 0, 3,200, 16,000, or 56,000 *G. tabacum* juveniles and eggs released by crushing cysts were added in suspension around plants immediately after transplanting. There were four replicates of each treatment. Plants were rated weekly as before, and shoot dry weight was determined at the end of the experiment.

The effects of *G. tabacum* on Fusarium wilt of tobacco were also investigated using a split-root system approach. Roots of CT86-4 tobacco seedlings were washed free of soil and split equally into two adjacent 10-cm pots containing artificial potting mix and fumigated field soil as previously described. Plants were left uninoculated or inoculated 2 wk after transplanting with 2.0×10^5 *F. oxysporum* microconidia and/or 17,000 juveniles and eggs of *G. tabacum* in the same or different pots. Plants were rated weekly for symptom expression, and shoot dry weight was determined as before.

RESULTS

Fusarium-susceptible broadleaf tobacco plants in field microplots were severely wilted in the presence of either level of *F. oxysporum*. All plants inoculated with high *Fusarium* levels died by harvest (Table 1). Because of this, there were no significant differences between nematode inoculum levels. Plants infected with both organisms tended to die more quickly. All plants infected with high levels of *F. oxysporum* alone died within 8 wk of transplanting, whereas plants inoculated with the same *Fusarium* level and low or high nematode levels died within 5 and 4 wk, respectively. *F. oxysporum* population densities at harvest were 2.2×10^3, 3.1×10^4, and 7.1

Table 1. Effects of *Globodera tabacum* (*G. t.*) and *Fusarium oxysporum* (*F. o.*) on wilt of broadleaf tobacco susceptible and resistant to *Fusarium* in field microplots

		Susceptible CT86-4			Resistant CT86-8	
F. o.	*G. t.*	Wilt rating[a]	Dry wt	% *F. o.* isolations[b]	Wilt rating	% *F. o.* isolations
0	0	0.2	173.1	...	...	...
0	Low	0.6	157.5	...	...	...
0	High	0.0	159.4	...	...	...
Low[c]	0	3.8	11.1	100	1.0	50
Low	Low	3.8	29.0	100	1.6	50
Low	High	4.0	0.0	100	2.2	60
High[d]	0	4.0	0.0	100	0.8	75
High	Low	4.0	0.0	100	1.4	80
High	High	4.0	0.0	100	2.8	80

Analysis of variance

Source (wilt)	Susceptible	Resistant
F. o.	0.0001	NS
G. t.	NS	0.001
*F. o.***G. t.*	NS	NS
Linear contrasts (resistant tobacco)		
With *G. t.* vs. without *G. t.*		0.001
High *G. t.* vs. low *G. t.*		0.01
High *F. o.* vs. low *F. o.*		NS

[a] Wilt rating: 0 (healthy) to 4 (plant dead).
[b] Isolation of *F. o.* from stem tissue.
[c] *F. o.* dilution plate count (Komada) at harvest: $X = 5 \times 10^4$.
[d] *F. o.* dilution plate count (Komada) at harvest: $X = 1 \times 10^5$.

$\times 10^4$ per cm^3 of soil for 0, low, and high inoculum levels, respectively. *G. tabacum* final population densities determined by sampling were 1.6, 6.6, and 19.9 juveniles per cm^3 of soil for 0, low, and high inoculum levels, respectively.

When *Fusarium*-resistant tobacco plants were transplanted into these microplots, higher *G. tabacum* inoculum levels increased the expression of wilt symptoms (Table 1). *F. oxysporum* was isolated from 50–80% of resistant tobacco plant stems and 100% of susceptible tobacco plants in microplots inoculated with the fungus.

The previous experiment was repeated in the greenhouse, using lower *F. oxysporum* inoculum densities. These data indicated a significant interaction between *G. tabacum* and *F. oxysporum* resulting in increased wilt severity in susceptible tobacco varieties (Table 2). At these pathogen densities, the *Fusarium* resistant tobacco lines CT86-8 and MD-609 did not show wilt symptoms regardless of *G. tabacum* inoculum level.

G. tabacum inoculum level significantly affected wilt severity and plant dry weight of one *Fusarium*-susceptible and two resistant tobacco lines inoculated with *F. oxysporum* (Table 3). Each tobacco line responded differently to inoculation with *F. oxysporum* and various initial nematode densities, but wilt rating increased and shoot dry weight decreased with increasing *G. tabacum* levels for all lines. Shoot dry weight was negatively correlated ($r = -0.91$, $P = 0.001$) with wilt rating.

G. tabacum and *F. oxysporum* in the same half of a tobacco plant split-root system increased disease severity over either *Fusarium* alone or the two pathogens in different halves of the same root system (Table 4). Shoot dry weight was negatively correlated ($r = -0.89$, $P = 0.001$) with wilt rating. Percentages of dead plants were 40, 60, and 100 for the two pathogens in separate halves of the same root system, *F. oxysporum* alone, or both pathogens in the same half of a paired root system, respectively.

DISCUSSION

Fusarium wilt was sporadic in Connecticut broadleaf tobacco until fields became infested with *G. tabacum* despite the fact that Fusarium wilt was described in Connecticut in 1943 (2). *F. oxysporum* alone can readily kill tobacco plants, as evidenced in these experiments, but severe symptoms rarely occur in production fields in the absence of the nematode. This observation indicates that the *F. oxysporum* population densities used in these experiments may be higher or more effective as inoculum than those that occur in the field. The *F. oxysporum* levels used in these experiments were similar to densities used by other researchers (1). Conditions in the sterile potting mix or fumigated soil may have reduced competition or increased virulence of the wilt fungus. *G. tabacum* population densities were within the range of reported field densities in all experiments (8).

The subjective wilt rating of 0 (healthy) to 4 (plant death) was an acceptable method of estimating disease severity. Wilt rating was correlated with shoot dry weight in these experiments.

Early pathogenicity experiments indicated that Connecticut broadleaf cultivars were resistant to Fusarium wilt (2). Recent pathogenicity tests of grower broadleaf lines indicate that most are susceptible to Fusarium wilt (G. S. Taylor, *unpublished*), and a comparison of CT86-4 with the *Fusarium*-susceptible Maryland cultivar Wilson indicates no difference between the two cultivars. The difference in susceptibility of Connecticut broadleaf over time may be due to changes in the fungus or in the host or to the protocol for pathogenicity testing.

Despite the fact that *F. oxysporum* alone resulted in plant death, wilt severity

Table 2. Effects of *Globodera tabacum* (*G. t.*) and *Fusarium oxysporum* (*F. o.*) on wilt of broadleaf tobacco susceptible and resistant to *Fusarium* under greenhouse conditions

		Wilt rating[c] 10 wk after inoculation			
		Susceptible		Resistant	
F. o.[a]	*G. t.*[b]	MD Wilson	CT86-4	MD 609	CT86-8
0	0	0.0	0.2	0.0	0.0
0	Low	0.0	0.0	0.0	0.0
0	High	0.0	0.0	0.0	0.0
Low	0	0.2	0.0	0.0	0.0
Low	Low	0.0	0.0	0.0	0.0
Low	High	0.8	0.6	0.0	0.0
High	0	0.6	0.0	0.0	0.0
High	Low	0.8	0.8	0.0	0.0
High	High	1.4	2.8	0.0	0.0

Analysis of variance

Source (wilt)	Susceptible	Resistant
G. t.	0.007	NS
F. o.	0.001	NS
Host (MD vs. CT)	NS	NS
*G. t.***F. o.*	0.04	NS
Linear contrasts (susceptible tobacco)		
Uninoculated vs. others		0.001
With *F. o.* vs. without *F. o.*		0.01
High nematode vs. low and none		0.01
High nematode vs. low		0.01
Nematode and fungus vs. fungus alone		0.05
High nematode + fungus vs. low nematode + fungus		0.01
Low fungus + nematode vs. high fungus + nematode		NS

[a] *F. o.* inoculum densities: 0, 1×10^6, and 1×10^7 per pot.
[b] *G. t.* inoculum densities: 0, 25, and 100 juveniles per cubic centimeter of soil.
[c] Wilt rating: 0 (healthy) to 4 (plant dead).

Table 3. Effects of *Globodera tabacum* (*G. t.*) inoculum density on wilt of broadleaf tobacco susceptible and resistant to *Fusarium* in *Fusarium*-infested soil under greenhouse conditions

	Susceptible		Resistant			
	CT86-4[a]		CT86-6		CT86-8	
G. t. juveniles/cm^3	Wilt[b]	Wt	Wilt	Wt	Wilt	Wt
0.0	3.5	17.6	0.2	52.5	0.7	54.0
1.6	3.5	28.1	0.5	50.5	1.5	50.2
8.0	3.7	19.7	0.5	54.1	1.2	48.5
28.0	4.0	3.3	1.2	44.3	2.5	29.0

Analysis of variance

Source	Wilt	Wt
Host	0.0001	0.0001
G. t.	0.005	0.0001
Host**G. t.*	NS	NS
	LSD wilt = 0.50	LSD wt = 6.51
	MSE wilt = 0.56	MSE wt = 82.37
Correlation of wilt severity rating with dry wt: $r = 0.91$		

[a] CT-4 *Fusarium*-susceptible and CT-6 and CT-8 *Fusarium*-resistant.
[b] Wilt rating: 0 (healthy) to 4 (plant dead).

Table 4. Interactions of *Globodera tabacum* (*G. t.*) and *Fusarium oxysporum* (*F. o.*) in the same or different halves of a tobacco split-root system under greenhouse conditions

Pot 1	Pot 2	Final wilt rating[a]	Dry wt (g)	Percent dead plants
...	...	0.0	43.2	0
G. t.[b]	...	0.0	39.9	0
G. t.	*F. o.*[c]	3.0	23.9	40
...	*F. o.*	3.6	16.5	60
G. t. + *F. o.*	...	4.0	10.1	100

Orthogonal contrasts	*P*
Check vs. other treatments	0.001
With *F. o.* vs. without *F. o.*	0.001
F. o. alone vs. *F. o.* and *G. t.* in separate pots	NS
Both pathogens in same pot vs. *F. o.* alone or both in separate pots	0.05
LSD wilt = 0.8, LSD wt = 13.2.	
MSE wilt = 0.4, MSE wt = 99.8.	

[a] Wilt rating: 0 (healthy) to 4 (plant dead).
[b] Eighty-five juveniles per cubic centimeter of soil.
[c] One thousand microconidia per cubic centimeter of soil.

was increased in the presence of *G. tabacum*. This phenomenon was most pronounced with high nematode numbers, low fungal population densities, and *Fusarium*-resistant tobacco lines. Interaction experiments using lower *F. oxysporum* inoculum levels than those used in the field microplot experiment mirrored the situation encountered in production fields. Fusarium wilt symptoms were minor in the absence of *G. tabacum* and increased in severity with increasing nematode density. It appears that *G. tabacum* may act to reduce the inoculum levels of *F. oxysporum* required to cause significant disease losses and that a certain threshold density of the nematode is required to result in this effect.

The interaction between root-knot nematodes and Fusarium wilt of tomato may be so specific that nematodes are required for disease expression on resistant plants (6). The interaction between *G. tabacum* and Fusarium wilt of tobacco also appears to be specific, although the expression of wilt symptoms on resistant plants is quite mild, even in the presence of the nematode, with plants often outgrowing early symptoms. Resistance in Connecticut broadleaf tobacco was derived from crosses with the *Fusarium*-resistant Connecticut cultivar C2 (16). *Fusarium* resistance in tobacco appears to be conditioned by several genes (4). This resistance in Connecticut broadleaf tobacco lines does not exclude infection by the pathogen, because *F. oxysporum* can be isolated from stems of up to 75% of asymptomatic plants inoculated with *F. oxysporum* alone.

G. tabacum suppresses plant growth and yield of Connecticut shade tobacco (8), but it did not affect the growth of Connecticut broadleaf tobacco in these experiments.

A split-root system approach was used to further examine wilt in the presence or absence of both pathogens. It appears that *G. tabacum* acts to increase wilt in a localized rather than systemic manner. Results indicate that both *G. tabacum* and *F. oxysporum* present in the same pot increase disease expression over either *F. oxysporum* alone or the two pathogens infecting separate halves of the same root system.

Because *F. oxysporum* was isolated from stems of tobacco both susceptible and resistant to wilt in the absence of the nematode, *G. tabacum* may do more than simply allow ingress of the fungus through wounds or feeding sites. The mechanism of this interaction, currently unknown, may be similar to that of other *Fusarium* spp.-sedentary endoparasitic nematode interactions (9). Localized physiological differences or root exudate changes resulting from nematode invasion and feeding may affect the virulence of *F. oxysporum*, either by overcoming host response to fungal infection or by decreasing the number of inoculum propagules necessary to cause systemic infections and wilt.

Management of the disease complex can be achieved by control tactics aimed at the fungus, such as the planting of *Fusarium*-resistant tobacco, or by tactics aimed at decreasing *G. tabacum* numbers below threshold levels, or combinations of both. The quantification of these threshold population densities for both pathogens will be an important tool for future management programs.

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