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Effects of *Meloidogyne arenaria* infection on *M. incognita*-resistance in tobacco

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Summary – Roots of tobacco resistant to *Meloidogyne incognita* host races 1 and 3 were separated into two portions using three different split-root techniques. One portion of each plant root was inoculated with eggs of *M. arenaria* and the other root portion with *M. incognita*. Eggs of *M. arenaria* and *M. incognita* were applied to separate root portions either simultaneously or *M. incognita* inoculation was delayed. After 45 to 60 days in the greenhouse, root portions were rated for galling and egg mass number. *M. arenaria* infection of one root portion did not systemically predispopse the other portion to *M. incognita* infection. Plants with intact root systems were maintained at 25, 28, 31, or 35 °C and inoculated simultaneously with eggs of both species. *M. incognita*-resistance failed at temperatures above 28 °C. *M. arenaria* infection did not alter the *M. incognita*-resistance phenotype at different temperatures.

Résumé – ***Influence de l'infestation par* Meloidogyne arenaria *sur la résistance à* M. incognita *chez le tabac*** – Le système racinaire de plants de tabac résistants à *Meloidogyne incognita* races 1 et 3 est séparé en deux parties en utilisant trois techniques différentes. Une partie est inoculée avec des œufs de *M. arenaria* et l'autre avec des œufs de *M. incognita*. Les œufs de *M. arenaria* ou de *M. incognita* sont placés sur l'une ou l'autre partie du système racinaire, soit simultanément, soit successivement, mais en décalant l'inoculation de *M. incognita*. Après 45 à 60 jours de croissance en serre des plants infestés, l'importance de l'infestation et le nombre de masses d'œufs sur les deux parties du système racinaire sont évalués. L'infestation d'une partie des racines par *M. arenaria* ne prédispose pas systématiquement l'autre partie à l'infestation par *M. incognita*. Des plants de tabac résistants à *M. incognita*, à système racinaire intact, sont inoculés simultanément avec des œufs des deux espèces et maintenus à 25, 28, 31 ou 35 °C. La résistance à *M. incognita* disparaît aux températures supérieures à 28 °C. Quelle que soit la température, l'infestation par *M. arenaria* n'a pas d'effet sur le phénotype résistant à *M. incognita*.

Key-words : *Meloidogyne*, tobacco, resistance, susceptibility, interaction, split-root, temperature.

Root-knot nematodes (*Meloidogyne* spp.) are serious pests of field, vegetable, and horticultural crops (Franklin, 1979; Lamberti, 1979; Sasser, 1979). They are considered the major pest of tobacco throughout the world with an estimated annual yield loss of approximately 15 % (Schneider, 1991). *Meloidogyne arenaria* and *M. incognita*, often in mixed populations, are responsible for substantial flue-cured tobacco yield losses in the southeastern U.S. (Fortnum *et al.*, 1984; Barker, 1989; Gooden *et al.*, 1991). Management systems typically include the use of nematicides, cultural practices, and host-resistance (Todd, 1981; Schneider, 1991). Current resistant cultivars are derivatives of the tobacco cultivar NC 95 and confer protection only against *M. incognita* races 1 and 3. Eisenback (1983), Tedford (1986), Tedford *et al.* (1986), and Ibrahim (1987) indicated that resistance to *M. incognita* failed when tobacco plants were also infected by *M. arenaria*. Eisenback (1983) and Ibrahim (1987) attributed the loss of resistance to a systemic host reaction observable in split-root tobacco plants. Tedford (1986) and Tedford *et al.* (1986) observed this phenomenon under high infection pressure of *M. arenaria* in field experiments.

Host plant resistance has been widely used to suppress *M. incognita* in many southeastern tobacco producing areas. Surveys of root-knot nematode infested fields revealed that almost 60 % of detected *Meloidogyne* populations in South Carolina's tobacco production area were mixed *M. arenaria*/*M. incognita* populations (Fortnum *et al.*, 1984). Cultivation of susceptible tobacco cultivars in fields with mixed infestations of *M. arenaria* and *M. incognita* would allow reproduction of both species. On the other hand, the wide spread use of *M. incognita*-resistant tobacco is likely to increase *M. arenaria* proportions in mixed infestations. Large *M. arenaria* proportions, however, may result in failure of *M. incognita*-resistance in tobacco as observed by Tedford *et al.* (1986). Besides leading to increased damage due to parasitism of both species, such a loss of resistance would invalidate projects aimed at deliberate-

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Table 2. *Egg mass (EM) and root galling indices (GI) of split-root* Meloidogyne incognita*-resistant NC 95 tobacco plants with roots parted without cutting (method 2) and grown in soil infested with eggs of* M. arenaria*-Govan (MA) and 2 weeks later with eggs of* M. incognita *(MI) in different combinations.*

Treatment (× 10^3)		MA root half		MI root half	
MA	MI	EM	GI	EM	GI
0	0	0.0 *c*	0.0 *c*	0.0 *a*	0.0 *a*
0	*15*	*0.0 c*	0.0 *c*	0.0 *a*	0.0 *a*
0	*30*	*0.0 c*	0.0 *c*	0.0 *a*	0.0 *a*
15	*0*	*3.8 b*	3.3 *b*	0.0 *a*	0.0 *a*
30	*0*	*4.3 b*	3.5 *b*	0.0 *a*	0.0 *a*
15	*15*	*4.3 c*	4.0 *a*	0.0 *a*	0.0 *a*
15	*30*	*4.3 b*	4.0 *ab*	0.0 *a*	0.0 *a*
30	*15*	*4.0 b*	4.0 *ab*	0.0 *a*	0.0 *a*
30	*30*	*5.0 a*	4.8 *a*	0.0 *a*	0.0 *a*

Within columns, parameter means with the same letter are not significantly different ($P = 0.05$). Viability of *M. incognita* inoculum was verified by inoculating susceptible tomato or tobacco plants.

egg masses were *M. incognita* (Table 5). Equal proportions of both species were found in mixed inoculations at 35 °C. No *M. incognita* infections were detected in mixed infestations below 31 °C.

Discussion

Infection with neither *M. arenaria* isolate did affect *M. incognita*-resistance in split-root tobacco plants. Even when present 2 or 3 weeks prior to *M. incognita, M. arenaria* was without apparent influence on plant defense against *M. incognita.* Data from the temperature water bath experiment indicated that *M. arenaria* did not influence the temperature sensitivity of *M. incognita*-resistance. *M. incognita* started to reproduce between 28 and 31 °C, regardless of the presence or absence of *M. arenaria.* Slana (1978) similarly found that *M. incognita*-resistance in NC 95 became ineffective at soil temperatures above 30 °C.

Our split-root experiments show that *M. arenaria* parasitism did not elicit a systemic host reaction inducing detectable susceptibility to *M. incognita.* Moreover, mixed *M. arenaria*/*M. incognita* infestations of resistant tobacco with intact roots (water bath experiment) did not result in a loss of resistance to *M. incognita* either. Therefore, *M. incognita*-resistance is stable also in the immediate vicinity of *M. arenaria* infections.

Use of *M. incognita*-resistant tobacco appears appropriate for control of *M. incognita* race 3 even in fields where this race of *M. incognita* coexists with *M. arena-*

Table 3. *Egg mass (EM) and root galling indices (GI) of* Meloidogyne incognita*-resistant NC 95 tobacco plants with roots divided into proximal (top) and distal (bot.) root portions (method 3) and grown in soil infested with eggs of* M. arenaria*-Govan (MA) and with eggs of* M. incognita *(MI) in different combinations and time intervals (2 or 3 weeks) after* M. arenaria *infestation.*

Treatment (× 10^3)						Ma root half		MI root half	
MA		MI (2 weeks)		MI (3 weeks)		EM	GI	EM	GI
top	bot.	top	bot.	top	bot.				
0	0	0	0	0	0	0.0 *f*	0.0 *e*	0.0 *b*	0.0 *a*
	15	0				4.0 *abc*	2.6 *bcd*	0.0 *b*	0.0 *a*
15			0			4.5 *a*	4.3 *a*	0.0 *b*	0.0 *a*
	30	0				2.6 *de*	1.6 *d*	0.0 *b*	0.0 *a*
30			0			2.3 *e*	2.5 *cd*	0.0 *b*	0.0 *a*
	0	15				0.0 *f*	0.0 *e*	0.3 *a*	0.0 *a*
0			15			0.0 *f*	0.0 *e*	0.3 *a*	0.0 *a*
	15	15				3.3 *cd*	2.3 *cd*	0.3 *a*	0.0 *a*
15			15			4.5 *a*	4.8 *a*	0.0 *b*	0.0 *a*
	30	15				4.3 *ab*	4.0 *ab*	0.0 *b*	0.0 *a*
30			15			4.5 *a*	4.5 *a*	0.0 *b*	0.0 *a*
	0			15		0.0 *f*	0.0 *e*	0.0 *b*	0.0 *a*
15					15	4.3 *ab*	4.3 *a*	0.0 *b*	0.0 *a*
	30			15		4.0 *abc*	3.5 *abc*	0.0 *b*	0.0 *a*
30					30	4.5 *a*	4.5 *a*	0.0 *b*	0.0 *a*

Within columns, parameter means with the same letter are not significantly different ($P = 0.05$). Viability of *M. incognita* inoculum was verified by inoculating susceptible tomato or tobacco plants.

Table 4. *Egg mass (EM) and root galling (GI) indices of* Meloidogyne incognita-*resistant tobacco plants grown at controlled soil temperatures and infested with eggs of* M. arenaria *(MA) and 3 weeks later with eggs of* M. incognita *(MI) in different combinations.*

Treatment (× 10^3)			EM	GI
Temperature	MA	MI		
25 °C	0	0	0.0 *c*	0.0 *b*
	10	0	4.0 *b*	3.8 *a*
	0	10	0.0 *c*	0.0 *b*
	10	10	4.5 *a*	3.8 *a*
28 °C	0	0	0.0 *b*	0.0 *b*
	10	0	3.8 *a*	3.8 *a*
	0	10	1.3 *b*	1.0 *b*
	10	10	4.5 *a*	4.0 *a*
31 °C	0	0	0.0 *c*	0.0 *b*
	10	0	3.0 *b*	2.8 *a*
	0	10	4.5 *a*	3.0 *a*
	10	10	4.0 *ab*	3.0 *a*
35 °C	0	0	0.0 *b*	0.0 *b*
	10	0	4.3 *a*	3.0 *a*
	0	10	3.8 *a*	2.8 *a*
	10	10	4.5 *a*	3.3 *a*

Within columns of each temperature, parameter means with the same letter are not significantly different ($P = 0.05$).

Table 5. *Species proportions of* Meloidogyne arenaria *(MA) and* M. incognita *(MI) as determined by DNA hybridization assays in* M. incognita-*resistant NC 95 tobacco after infestation with 10 000 eggs of each species and incubation at controlled soil temperatures.*

Temperature	MA	MI
25 °C	100 % *a*	0 % *b*
28 °C	100 % *a*	0 % *b*
31 °C	66 % *ab*	34 % *ab*
35 °C	53 % *a*	47 % *a*

Within columns, parameter means with the same letter are not significantly different ($P = 0.05$).

ria. Observations made by Eisenback (1983), Tedford (1986), Tedford *et al.* (1986), and Ibrahim (1987) that *M. arenaria* is capable of inducing susceptibility to *M. incognita* are not confirmed by our experiments with the selected nematode isolates.

M. arenaria isolates differ widely in their capability to incite damage and elicit physiological changes in a given host (Carpenter & Lewis, 1991; Noe, 1992; Ibrahim & Lewis, 1993). Other *M. arenaria* isolates may have behaved differently in these experiments. In particular, the two known host races of *M. arenaria* may differ in their potential to predispose resistant tobacco cultivars to *M. incognita* infection, although Ibrahim (1987) observed a loss of *M. incognita*-resistance with a *M. arenaria* race 2 isolate, i.e., the same race we used in our experiments. On the other hand, different *M. incognita* isolates could have benefitted from physiological changes elicited by *M. arenaria.* Eisenback (1983) and Ibrahim (1987) both used race 1 *M. incognita* isolates for their studies, whereas we used race 3. However, *M. arenaria* race 2 and *M. incognita* race 3, as used in our experiments, are the predominant *Meloidogyne* species and races in South Carolina.

Certain management practices aim at shifting mixed root-knot nematode populations away from *M. arenaria* (Fortnum & Currin, 1993) since *M. incognita* races 1 and 3 can be controlled by resistant cultivars and appear to be more susceptible to some nematicides (Barker *et al.*, 1981; Nordmeyer & Dickson, 1985). Such strategies are useful in the design of long term rotation schemes to control nematode infestations in a sustainable agriculture framework. This approach would be jeopardized in case of loss of *M. incognita*-resistance in fields with mixed *M. arenaria*/*M. incognita* infestations. Judging from the presented results, this concern may be unfounded. If induced susceptibility occurs, the frequency is so low that it is hard to detect in the mass of *M. arenaria* reproduction.

Increased incidences of mixed *Meloidogyne* populations in the southeastern U.S. flue-cured tobacco producing area mandate the development of new concepts for root-knot nematode management. Knowledge of peculiarities of mixed infestations, particularly the influence of resistant *vs* susceptible crops in rotations and the effects of nematicidal treatments, is of prime importance for managing polyspecific *Meloidogyne* infestations.

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