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Effects of temperature and nitrogen source on tomato genotypes response to *Meloidogyne incognita* infection

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Summary – The effect of water (check), Hoagland solution (HS) without nitrogen, or HS with NO_3, NH_4, or NH_4NO_3 as nitrogen sources on growth of a resistant ("VFN-8") and a susceptible ("Rutgers") tomato cultivar and their responses to an aggressive (Experiments I and II) and nonaggressive (Experiments III and IV) *Meloidogyne incognita* population was tested at 24 and 28 °C. In each experiment, 40 one-week-old seedlings per cultivar per temperature were grown in 286 g sandy loam soil contained in styrofoam cups. Half of the seedlings in Experiments I and II were each inoculated with 1000 second-stage juveniles of the aggressive nematode population and in Experiments III and IV with 500 nonaggressive population. All but Experiment III (15 days) lasted 28 days after nematode inoculation; at the end of which numbers and nematode developmental stages were determined. Both cultivars grew larger at 24 than at 28 °C and more so with NH_4 and NH_4NO_3 treatments. Nematode infection levels were similar in the susceptible cultivar regardless of plant size; whereas, larger plants had fewer nematodes in the resistant cultivar. Both NH_4 and NH_4NO_3 sources of nitrogen had fewer nematodes than NO_3 treatments in the resistant cultivar. Both nematode populations infected the resistant cultivar less than the susceptible cultivar, indicating that even if resistance breaks down, the process may be gradual. Overall, the study shows that the source of nitrogen, in addition to temperature, is a factor in the break-down of the *Mi*-gene. The inverse relationship between plant size and nematode infection in the resistant cultivar suggests that plant growth regulating (physiological) processes may be altering how the gene mediates resistance.

Résumé – ***Effets de la température et de la source d'azote sur la réaction de génotypes de tomate à l'infestation par* Meloidogyne incognita** – A été testée la réaction de cultivars de tomate sensible ("Rutgers") et résistant ("VFN-8") à des populations de *Meloidogyne incognita* agressives ou non, à 24 ou 28 °C, cultivars alimentés avec une solution de Hoagland (HS) sans azote, ou additionnée, comme source d'azote, de NO_3, NH_4, NH_4NO_3, ou avec de l'eau (témoin). Pour chaque expérience, et pour chaque cultivar, il a été utilisé 40 plants agés d'une semaine ayant poussé dans des pots en polystyrène expansé contenant 286 g de sable argileux. La moitié des plants des Expériences I et II ont été inoculés avec 1000 juvéniles de deuxième stade (J2) de la population agressive de *M. incognita*, et la moitié des plants des Expériences III et IV avec 500 J2 de la population non agressive. Ont été déterminés le nombre et le stade de développement des nématodes à la fin des expériences, qui ont toutes duré 28 jours, sauf l'Expérience III qui à duré 15 jours. La croissance des plants des deux cultivars a été plus rapide à 24 qu'à 28 °C, et meilleure avec les traitements au NH_4 ou au NH_4NO_3. Les niveaux d'infestation des plants sont semblables chez le cultivar sensible, et ce quelle que soit leur taille, alors que les niveaux d'infestation sont moins élevés chez les plants les plus grands du cultivar résistant. Les plants du cultivar résistant contiennent moins de nématodes lorsqu'ils sont traités avec NH_4 ou NH_4NO_3 qu'avec NO_3. Les deux populations ont une multiplication plus faible chez le cultivar résistant que chez le cultivar sensible, ceci suggérant que même si la résistance peut se dégrader, il s'agit là d'un processus graduel. Cette étude démontre, qu'en plus de la température, la source d'azote, peut jouer un rôle dans la supression de la résistance liée au gène *Mi*. La relation inverse entre taux de multiplication des nématodes et taille des plants du cultivar résistant suggère que des processus de régulation (physiologique) peuvent moduler le comportement du gène de résistance chez la plante.

Key-words : *Lycopersicon esculentum*, *Meloidogyne incognita*, nitrogen source, nutrition, resistance, temperature, tomato.

The development of the *Mi*-gene, which is the basis for resistance to the warm-climate root-knot nematodes in tomatoes (*Lycopersicon esculentum* L.) (Medina-Filho & Stevens, 1980), is a significant advance in the understanding of plant-nematode interactions (Williamson *et al.*, 1993). Although there are naturally occurring resistance-breaking populations of *Meloidogyne incognita* (Riggs & Winstead, 1959), the major limitation with the *Mi*-gene is that it fails at or above 28 °C (Holtzman, 1965; Dropkin, 1969; Araujo *et al.*, 1982 *a*, *b*; Haroon *et al.*, 1993). Efforts to make the *Mi*-gene stable at high temperatures have been successful in tissue culture (Ammati *et al.*, 1984) and using bridge-line breeding (Veremis & Roberts, 1994) although other desired agronomic traits were lost in the latter. However, temperature is not the only factor that results in the break-down of resistance (Dropkin & Webb, 1967; Dropkin *et al.*, 1969; Sawhney & Webster, 1975, 1979). This suggests that the factors that result in the break-down of

resistance may be affecting the gene's performance either directly or indirectly (through altering host physiology).

The effects of temperature are so complex that there are many basic (physiological and molecular) and applied research questions to be addressed. Without knowing how the gene mediates recognition of and resistance to root-knot nematodes (Williamson *et al.*, 1993) and any physiological mechanisms that may lead to the break-down of resistance at high temperatures, however, practical solutions will be difficult. A logical starting point to proceed to an in depth analysis of why the *Mi*-gene fails is to establish if there is a difference in the growth of resistant cultivars at optimum temperatures, 20 to 35 °C (Atherton & Rudich, 1986), and under commonly used agricultural inputs. Two questions that relate to temperature are: *i*) does a resistant cultivar grow similarly within the optimum ranges for tomato and *ii*) is there a relationship between plant size and the level of nematode infection? Similar growth at optimum temperature ranges but differences in the level of nematode infection and development may indicate that plant size is not a factor. A relationship between plant size and nematode infection and development, however, will suggest that plant growth regulating mechanisms may be factors in the break-down of resistance. In that case, growth conditions may need to be adjusted accordingly.

Tomato is a high fertilizer-input crop (Atherton & Rudich, 1986). The form of nitrogen is particularly important because it influences plant growth (Woolhouse & Hardwick, 1966) and plant response to a range of diseases (Hendrix & Toussoun, 1964; Huber *et al.*, 1968; Hornby & Goring, 1972; Huber & Watson, 1974). Therefore, it is logical to ask whether or not the form of nitrogen (nitrate or ammonium) used as a fertilizer is a factor in the break-down of the *Mi*-gene. If the nitrogen source is not a factor, nematode infection and development levels within the optimum temperature ranges should be similar regardless of the form of nitrogen used. If nitrogen form is a factor, some adjustments may be required in cultural practices. Thus, the objective of this study was to determine whether or not the form of nitrogen fertilizer affects the *Mi*-gene at optimum ranges of temperature and growth conditions.

Materials and methods

Plant material, growth chamber conditions and nutrient treatments

M. incognita susceptible ("Rutgers") and resistant ("VFN-8") tomato cultivars were grown at 24 and 28 °C. Plants accumulated 14 and 18 degree-days (DD-base 10 °C) per day at 24 and 28 °C, respectively (Melakeberhan *et al.*, 1989). Diurnal cycles at each temperature were set at 16 h day at 400 ± 20 µE m^{-2} s^{-1} and 8 h night cycle. Sandy loam soil was steam-sterilized and stored for 3 months. Soil texture and mineral nutrient composition determined from four homogeneous samples (Melakeberhan *et al.*, 1995) at planting were: 87 % sand, 8 % silt and 5 % clay, pH 7.0, and 28, 119, 56, 139, and 1386 kg/ha of NO_3, P, K, Mg, and Ca, respectively.

Seeds of each cultivar were separately bulk-germinated in 1 dm^3 of soil contained in plastic trays at each temperature. Germination trays were watered to saturation with tap water as required. One week after germination, 40 seedlings per cultivar per temperature (160 total per experiment) were each transplanted into 237 ml styrofoam cups containing 286 ± 7 g of soil (Fig. 1) to receive one of the following nutrient treatments daily: water (control), Hoagland solution (HS) without nitrogen (HS-N, deficient), or HS with nitrate ($HS+NO_3$), ammonium ($HS+NH_4$), or ammonium nitrate ($HS+NH_4NO_3$) as nitrogen sources (Hoagland & Arnon, 1939). The total amount of nitrogen in the respective treatments was 210 mg/l. All nutrient solutions contained similar levels of other salts. Nutrient treatments were applied daily in 10-20 ml volumes. If plants required water above the nutrient treatments, tap water was added as needed.

Experiments and nematode treatments

Two primary (I and III) and two repeat (II and IV) experiments were conducted at each temperature. One week after initiation of nutrient treatments, each set of 40 seedlings per cultivar per temperature was divided into two; half of which were inoculated with nematodes and the other half with tap water. In Experiments I and II, 20 seedlings per cultivar (five nutrient treatments × four replications) were each inoculated with 1000 24-48 h-old aggressive *M. incognita* second-stage juveniles (J2). The same number of seedlings were each inoculated with 500 non-aggressive *M. incognita* J2 in Experiments III and IV. Aggressive refers to the *M. incognita* population to which the *Mi*-gene is less effective at all temperatures, whereas non-aggressive refers to the population that is less likely to break resistance except at high temperatures. Nematodes were applied in 1-ml suspensions per plant (Melakeberhan *et al.*, 1990). Controls received 1 ml of tap water. Experiments I, II and IV were terminated at 28 days, and Experiment III at 15 days after nematode inoculation.

Observations, measurements and data analysis

At harvest, shoots were cut off and dried at 80 °C for 48 h and dry weights determined. Roots were washed free of soil and indexed for root-knot galling on a 0 (no

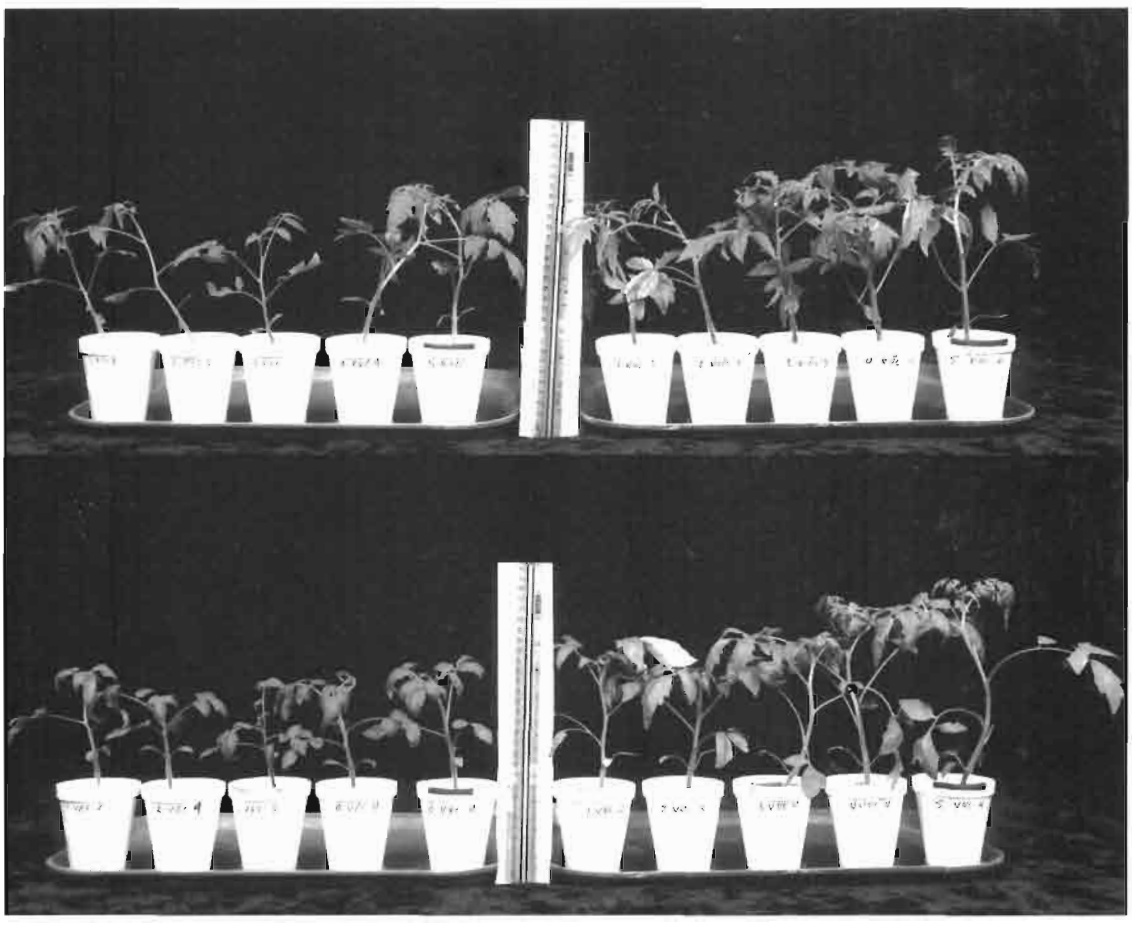

Fig. 1. *The effect of Hoagland solution (HS)-based form of nitrogen and temperature on growth of susceptible ("Rutgers", top row) and resistant ("VFN-8", bottom row) tomato cultivars grown at 28 (left of scale) and 24 °C (right of scale) in Experiment I (Nitrogen source treatments for each temperature and cultivar from left to right are water, HS - N, HS + NO_3, HS + NH_4, and HS + NH_4NO_3; the scale in between the two temperature columns is 30 cm long).*

galling) to 5 (more than 75 % of the root system galled) scale (Kinloch, 1990). Nematode population dynamics were determined microscopically from whole root systems (Melakeberhan *et al.*, 1989).

Main treatment effects of temperature and nitrogen source, and interaction effects of temperature × nitrogen source, on plant growth and number of nematodes for each developmental stage were analyzed by ANOVA (Steel & Torrie, 1980) using a general linear model. Unless statistically different, data from primary and repeat experiments were combined.

Results

AGGRESSIVE POPULATION

In Experiments I and II, 392 and 504 DD were accumulated at 24 and 28 °C, respectively.

Results from Experiments I and II were not statistically different. Therefore, data are combined. Regardless of nematode treatment, shoot dry weights from NH_4 or NH_4NO_3 treatments were generally larger ($P \leq 0.05$) than the other treatments in both cultivars (Table 1). Also, both cultivars were larger at 24 than at 28 °C (Fig. 1; Table 1). Significant interactions between nutrients and temperature were observed only in the resistant but not in the susceptible cultivar (Table 1). Generally, shoots from nematode treatments were larger than the controls.

Roots of nematode-infected plants of the resistant cultivar were more necrotic at 24 than at 28 °C, regardless of nutrient treatment. Roots of the susceptible cultivar were not necrotic. However, the numbers of adult nematodes in the NH_4 or NH_4NO_3 treatments in the resistant cultivar were fewer

Table 1. *Main effect of Hoagland solution (HS)-based form of nitrogen source, temperature, and the interaction between temperature and nitrogen on shoot dry weights (g) of a resistant (VFN-8) and susceptible (Rutgers) tomato cultivar with or without inoculation with an aggressive* Meloidogyne incognita *population at 24 and 28 °C.*

Treatments		Resistant		Susceptible	
N source[m]	Temperature	–	+	–	+
	28 °C				
Water (control)		0.26	0.34 *bc*[z]	0.28 *b*	0.31 *ab*
HS-N (deficient)		0.26	0.31 *c*	0.26 *b*	0.26 *b*
HS+NO_3		0.24	0.26 *c*	0.24 *b*	0.25 *b*
HS+NH_4		0.34	0.46 *a*	0.38 *a*	0.51 *a*
HS+NH_4NO_3		0.33	0.38 *ab*	0.30 *ab*	0.33 *ab*
	24 °C				
Water (control)		0.40 *b*	0.51 *b*	0.49 *ab*	0.45 *b*
HS-N (deficient)		0.45 *b*	0.57 *b*	0.55 *ab*	0.52 *b*
HS+NO_3		0.48 *b*	0.62 *b*	0.45 *b*	0.53 *b*
HS+NH_4		0.65 *a*	0.85 *a*	0.65 *a*	0.76 *a*
HS+NH_4NO_3		0.53 *ab*	0.83 *ab*	0.65 *a*	0.84 *a*
Temperature	28 °C	0.35 *b*	0.33 *b*	0.29 *b*	0.29 *b*
	24 °C	0.68 *a*	0.62 *a*	0.50 *a*	0.56 *a*
Nitrogen × temperature		**	**	ns	ns

+/– = Inoculated with 1000 second-stage juveniles in 1 ml of tap water suspension per plant and 1 ml tap water only, respectively.

[m] Nitrogen source refers to the form of nitrogen present in normal strength HS.

[z] Numbers are means of eight replications from experiments I and II combined. Numbers followed by no letters or the same letters in each column per temperature are not statistically different ($P \leq 0.05$) from each other according to Tukey's test.
** / ns = Interactions are significant at ($P \leq 0.01$) or not significant at ($P \leq 0.05$), respectively.

($P \leq 0.05$) than in the rest of the treatments at both temperatures. The numbers were similar in all treatments at both temperatures in the susceptible cultivar (Table 2). In the resistant cultivar, main effect of nutrient treatments showed that NH_4 and NH_4NO_3 significantly ($P \leq 0.05$) reduced the number of nematodes at 24 and at 28 °C (Table 2). However, the numbers of J2 and adults were significantly greater at 28 than at 24 °C, with significant interactions between temperature and nutrients in affecting the number of adult nematodes. In the susceptible cultivar, there were no significant interactions and only the numbers of second-stage juveniles were greater at 28 than at 24 °C (Table 2).

Galling was generally similar among nutrient treatments at both temperatures. However, galls were significantly ($P \leq 0.05$) more at 28 (4.2) than at 24 °C (3.8) in the resistant cultivar compared with 4.4 at both temperatures in the susceptible cultivar.

NON-AGGRESSIVE POPULATION

In Experiment III, a total of 210 DD and 270 DD, and in Experiment IV, 392 DD and 504 DD were accumulated at 24 and 28 °C, respectively. Plant growth in both experiments was similar to those in Experiments I and II (data not shown).

In the resistant cultivar, there was little infection at 24 °C in Experiments III and IV (Table 3). The numbers of nematodes recovered from NH_4 and NH_4NO_3 treatments at 28 °C were significantly less ($P \leq 0.05$) than the other treatments in Experiment III (Table 3). Although the trends were similar in Experiment IV, they were not statistically different. Number of nematodes were significantly greater at 28 than at 24 °C in both experiments. However, there was significant interaction between nitrogen source and temperature in affecting the number of nematodes in Experiment IV (Table 3). In the susceptible cultivar, the numbers of nematodes were lower in treatments with nitrogen than without nitrogen in Experiment III. Generally, there were fewer nematodes at 24 than at 28 °C (Table 3).

Nutrient treatments had little effect on galling in both experiments and cultivars. In the resistant cultivar, galls were fewer ($P \leq 0.05$) at 24 (0-1.0) than at 28 °C (2.8-3.9) in both experiments. In the suscep-

Table 2. *Main effect of Hoagland solution (HS)-based form of nitrogen source, temperature, and the interaction between temperature and nitrogen on number of second generation juveniles (J2) and adult females of an aggressive* Meloidogyne incognita *population of a resistant (VFN-8) and susceptible (Rutgers) tomato cultivar at 24 and 28 °C at 28 days in Experiments I and II.*

Treatments		Resistant		Susceptible[w]	
N source[m]	Temperature	J2	Adults[x]	J2	Adults
	28 °C				
Water (control)		4	234 *ab*[z]	82	464
HS-N (deficient)		15	306 *a*	86	580
HS+NO_3		15	255 *ab*	104	587
HS+NH_4		3	162 *b*	23	432
HS+NH_4NO_3		0	145 *b*	25	229
	24 °C				
Water (control)		0	99 *a*	5	437
HS-N (deficient)		0	99 *a*	4	558
HS+NO_3		1	136 *a*	0	556
HS+NH_4		0	33 *b*	0	502
HS+NH_4NO_3		0	92 *a*	0	488
Temperature	28 °C	7.2 *a*	220 *a*	64.0 *a*	458
	24 °C	0.2 *b*	92 *b*	1.6 *b*	508
Nitrogen × temperature		ns	*	ns	ns

[m] Nitrogen source refers to the form of nitrogen present in normal strength HS.

[w] Number of nematodes are based on total root system per plant. Inoculum level was 1000 second-stage juveniles per cup.

[x] The average fresh root weight per root system were 0.75 ± 0.3 g at 28 °C and 1.57 ± 0.8 g at 24 °C and 1.1 ± 0.36 g at 28 °C and 1.8 ± 0.44 g at 24 °C in the resistant and susceptible cultivars, respectively.

[z] Numbers are means of eight replications from experiments I and II combined. Numbers followed by no letters or the same letters in each column per temperature are not statistically different ($P \leq 0.05$) from each other according to Tukey's test.
*/ns = Interactions are significant or not significant at ($P \leq 0.05$), respectively.

tible cultivar, galls were more ($P \leq 0.05$) at 28 (4.3) than at 24 °C (3.8) in Experiment III, but they were similar at 28 (3.9) and at 24 °C (3.8) in Experiment IV.

Discussion

The study establishes that the form of nitrogen as a nutrient source, along with temperature, is a factor in the breakdown of the *Mi*-gene resistance. Both NH_4 and NH_4NO_3 improved the resistant cultivar's performances against *M. incognita* at both temperatures while NO_3 resulted in high nematode infection. Similar results have been reported for takeall of wheat (Huber *et al.*, 1968), *Fusarium* wilt of beans (Hendrix & Toussoun, 1964), and more recently for *M. incognita* in soybeans and tomato (Orion *et al.*, 1995). Although the recovery of more nematodes from roots treated with water, HS-N, and NO_3 than with NH_4 or NH_4NO_3 in the resistant cultivar confirms the importance of nutrition (Spiegel *et al.*, 1982; Melakeberhan *et al.*, 1988; Prakash *et al.*, 1994), why the case was different for the susceptible cultivar is unknown. Also, it is not known what the response of the resistant will be when fertilized with other sources of nitrogen.

At pH 5.5 to 6.5, tomatoes grow more rapidly with NO_3 than NH_4 fertilization and at high temperature than at low temperature (Kirkby & Mengel, 1967; Ganmore-Neuman & Kafkafi, 1980), a fact that may be attributed to the difference in rhizosphere buffering capacity of NO_3 and NH_4 (Pill & Lambeth, 1977; MacDuff & Hopper, 1986). In the present study, both the resistant and the susceptible cultivars grew significantly larger at 24 than at 28 °C. They were larger when fertilized with NH_4 and NH_4NO_3 than with NO_3, deficient and water nutrient treatments at pH 7.0, indicating that temperature and form of nitrogen are factors in altering plant growth. However, temperature and nitrogen source seemed to be independent of each other in the susceptible cultivar, whereas they significantly interacted to increase growth of the resistant cultivar (Table 1). This suggests that the

Table 3. *The number of third/fourth-stage juveniles (Preadults, Experiment III) and adults (Experiment IV) of a nonaggressive population of* Meloidogyne incognita *per root system of a resistant (VFN-8) and susceptible (Rutgers) tomato cultivar treated with Hoagland solution (HS)-based nitrogen sources at 24 and 28 °C.*

Treatments		Resistant		Susceptible[w]	
N source[m]	Temperature	Preadults[x,y]	Adults[x,y]	Preadults	Adults
	28 °C				
Water (control)		49 *ab*[z]	14.8	118 *b*	138 *a*
HS-N (deficient)		68 *a*	47.3	202 *a*	170 *a*
HS+NO_3		56 *a*	49.0	96 *b*	95 *b*
HS+NH_4		22 *bc*	14.0	101 *b*	54 *b*
HS+NH_4NO_3		8 *c*	11.0	81 *b*	47 *b*
	24 °C				
Water (control)		0	0.8	94 *ab*	50
HS-N (deficient)		1	0.3	46 *b*	40
HS+NO_3		0	0.0	95 *ab*	35
HS+NH_4		1	0.0	44 *b*	22
HS+NH_4NO_3		0	0.0	121 *a*	18
Temperature	28 °C	41 *a*	27.2 *a*	120	101 *a*
	24 °C	0.4 *b*	0.2 *b*	80	33 *b*
Nitrogen × temperature		ns	***	ns	**

[m] Nitrogen source refers to the form of nitrogen present in the normal strength HS.

[w] Number of nematodes are based on total root system per plant. Inoculum level was 500 second-stage juveniles per cup.

[x] In experiment III, the average fresh root weight per root system were 0.3 ± 0.1 g at 28 °C and 0.45 ± 0.2 g at 24 °C and 0.2 ± 0.1 g at 28 °C and 0.3 ± 0.2 g at 24 °C in the resistant and susceptible cultivars, respectively. In Experiment IV, the average fresh root weight per root system were 0.8 ± 0.4 g at 28 °C and 1.0 ± 0.3 g at 24 °C and 0.7 ± 0.2 g at 28 °C and 1.0 ± 0.3 g at 24 °C in the resistant and susceptible cultivars, respectively.

[y] Experiments III and IV were terminated at 15 and 28 days after inoculation, respectively.

[z] Numbers are means of four replications. Numbers followed by no letters or the same letters in each column per temperature are not statistically different ($P \leq 0.05$) from each other according to Tukey's test.
,*/ns = Interactions are significant at $P \leq 0.01$ and 0.001, or not significant at ($P \leq 0.05$), respectively.

resistant and susceptible cultivars differ in the way they regulate their growth process which, in turn, may affect resistance regulating processes.

Approximately 210 DD are required to reach the third juvenile stage and 400 to 500 DD are required for the start of a second generation (Melakeberhan *et al.*, 1989). In a susceptible cultivar, nematodes develop freely, whereas in a resistant cultivar, infected root tissues become necrotic and nematodes do not develop beyond the swollen third-stage juvenile (Williamson *et al.*, 1993). Hence, the higher degree of necrosis at 24 than at 28 °C in the resistant cultivar shows the influence of temperature on the level of resistance. The differences in the number of J2 between temperatures within each cultivar reflect differences in the amount of DD accumulated, whereas differences between nematode populations and cultivars reflect differences in the rate of development (Table 2).

The slight increase in shoot weight of the nematode-infected treatments compared with controls (Table 1) typifies the commonly observed initial stimulatory effects at such low infestation levels. The numbers of both nematode populations that developed in the root systems of the susceptible cultivar were greater than in the resistant cultivar at both temperatures, suggesting that either the break-down of resistance may be a gradual process or that even if resistance breaks down it is not to the level of the susceptible cultivar.

Generally both nematode populations infected the susceptible cultivar similarly at both temperatures, indicating that there is no relationship with plant size.

In the resistant cultivar, the non-aggressive population infected only at 28 °C, whereas the numbers of the aggressive population that developed in the roots of the resistant cultivar were greater at 28 than at 24 °C. This confirms that resistance does break down at high temperature. However, the inverse relationship between plant size and numbers of nematodes in the root system of the resistant cultivar suggests that possible physiological changes that lead to increased plant growth may be interfering with the performance of the *Mi*-gene.

The mechanisms by which the *Mi*-gene mediates recognition and resistance to root-knot nematodes remains unknown (Williamson *et al.*, 1993). However, there appear to be some relationships among phytohormones, nitrogen source and the break-down of resistance. For example, exogenous application of cytokinins, which play an important role in the pathogenesis and gall formation process of root-knot nematodes, has been shown to result in the break-down of the *Mi*-gene (Dropkin *et al.*, 1969; Sawhney & Webster, 1975). It is also known that NO_3 increases the concentration of cytokinins more than NH_4 does (Salam & Wareing, 1978), while NH_4 has the advantage in regulating the photosynthetic carbon flow (Mohammed & Gnanam, 1979). The lower numbers of nematodes in either NH_4 or NH_4NO_3 than in the NO_3 treatments in the resistant cultivar only, while both cultivars grew larger than in the deficient and NO_3 treatments in this study, indicate that the effect of the nitrogen source on the nematode may be direct or indirect through unidentified plant-mediated processes. Similar plant-mediated processes have been reported on insects (Leru *et al.*, 1994; Orians & Fritz, 1996), and on the suppression of giant cell formation on tomato and soybean (Orion, *et al.*, 1995).

In summary, the study shows that the source of nitrogen, in addition to temperature, is a factor in the break-down of the *Mi*-gene. It appears that the source of nitrogen may affect nematodes directly or indirectly through altering plant growth. However, it is important that other nitrogen sources (urea, etc.) should be tested under field conditions. The inverse relationship between plant size and nematode infection in the resistant cultivar suggests that plant growth regulating (physiological) processes may be altering how the gene mediates resistance.

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