Relationship Between Resistance to *Meloidogyne incognita* and a Necrotic Response to Infection by a Strain of Potato Virus Y in Tobacco

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ABSTRACT

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Cultivars of *Nicotiana tabacum* resistant to the root-knot nematode, *Meloidogyne incognita*, develop vascular necrosis when infected by strain M^SN^R of potato virus Y (PVY-M^SN^R). Young (2- to 8-cm tall) root-knot-resistant plants were more susceptible to the virus than older plants and developed necrosis under all aerial and soil temperatures tested. In older plants (17- to 30-cm tall), virus-induced necrosis was severe at 28 C, mild at 32 C, and absent at 35–40 C. The same temperature which inhibited the expression of root-knot resistance also inhibited the necrotic response to PVY-M^SN^R. The necrotic reaction to the virus was specific to root-knot resistant genotypes and did not translocate to root-knot susceptible genotypes when various combinations of these genotypes were grafted. Root-knot susceptible plants developed mild, vein-banding symptoms but no necrosis, and virus reaction was not significantly affected by plant age or temperature. The similarity in temperature sensitivity involved in both root-knot resistance and the necrotic reaction elicited by PVY-M^SN^R suggests that the basis for the association of these two responses may be due to pleiotropic effects of a single gene.

In 1963, Henderson and Troutman (6) reported a severe, vascular necrosis in tobacco (*Nicotiana tabacum* L.) plants which carried the gene for resistance to the root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood]. Necrosis was induced by a previously unreported strain of potato virus Y (PVY) (7). Later, Gooding and Tolin (5) characterized three strains of PVY in the southeastern United States based on host-specific reactions elicited in flue-cured tobacco cultivars resistant or susceptible to the root-knot nematode. The strain M^SM^R of PVY (PVY-M^SM^R) causes mild, mosaic-type symptoms on both root-knot resistant and susceptible cultivars; strain M^SN^R (PVY-M^SN^R) produces mild symptoms on root-knot susceptible and vascular necrosis on root-knot resistant cultivars; and strain N^SN^R (PVY-N^SN^R) is necrotic on both root-knot susceptible and resistant cultivars.

The basis of the association between root-knot resistance and the necrotic reaction to PVY-M^SN^R is not known. The association is so consistent that LaPrade and Henderson (8) screened breeding lines for root-knot resistance based on their reaction to inoculation with the virus, which is simpler and quicker than inoculation with the nematode. The genetic basis for the association between root-knot resistance and PVY necrosis has not been thoroughly investigated nor have the two reactions been genetically separated. All known root-knot resistant lines develop a severe, necrotic reaction to PVY-M^SN^R, but it is not known whether the association is due to extremely close linkage between two loci, or to pleiotropic effects of a single gene. A root-knot resistant cultivar that does not become necrotic when infected with PVY-M^SN^R would be of obvious value, given the widespread occurrence of this virus in North Carolina (3).

The mechanism controlling root-knot nematode resistance in tobacco and tomato is temperature sensitive (1,13). The resistance is a necrotic or hypersensitive reaction that diminishes as temperature increases. The objectives of these studies were to determine whether temperatures known to inhibit root-knot resistance also inhibit the necrotic reaction induced by PVY-M^SN^R and to determine whether the virus-induced necrosis is due to a diffusible compound or is cell-specific.

MATERIALS AND METHODS

General. Flue-cured tobacco cultivars NC 2326 and Hicks (both are root-knot susceptible and develop mild mosaic and vein-banding symptoms when inoculated with PVY-M^SN^R), NC 95 (root-knot resistant and develops necrosis when inoculated with

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PVY-M^SN^R), and Line VY 32 (root-knot susceptible and symptomless when inoculated with PVY-M^SN^R) were used in these studies.

Type isolate NC 138 of PVY-M^SN^R was used in all tests. Virus inoculum was prepared by: homogenizing systemically-infected leaves from burley tobacco cultivar Burley 21 in 0.05 M Na_2HPO_4-KH_2PO_4 buffer, pH 7.2 (1 g tissue: 5 ml buffer); filtration through cheesecloth; and adding 1 g of (600-mesh) Carborundum/100 ml filtered juice. Inoculation was performed by rubbing the two largest leaves on each plant with a cotton swab previously dipped in inoculum.

Confirmation or detection of infections by PVY was determined serologically (4) or by back inoculation to Burley 21 plants.

Aerial temperature. Experiments were conducted in the greenhouse under average daytime temperatures of 28, 32, 36, and 40 C. Seedlings of cultivars NC 95 and Hicks were transplanted 5 wk after seeding into 10-cm-diameter clay pots containing a 2:1:1 steam sterilized mixture of sandy-loam soil, sand and peat. Each pot received 30 g of slow-release fertilizer (Osmocote®) at transplanting. Plants were 2, 8, 17, or 30 cm tall when inoculated. PVY symptom development was assessed weekly for 3 wk by using the following scale: 0 = no symptoms; 1 = vein clearing, mild mottling; 2 = vein banding, severe mottling or mosaic; 3 = localized necrosis, restricted to inoculated leaf; 4 = mild, systemic necrosis; 5 = severe, systemic necrosis; and 6 = plant death. There were four replications per treatment (three plants per replication) arranged in a randomized split-plot design using temperatures as main plots and plant size as sub-plots.

To determine if the effect of temperature was reversible, plants of NC 95 were shifted from cool (28 C) to warm (36 C) temperatures, and vice versa, 3, 7, and 14 days after inoculation with the virus. Plants were 15 cm tall when inoculated with PVY-M^SN^R. There were four replications per treatment arranged in a randomized complete block design.

Soil temperature. Three experiments were conducted to determine the effect of soil temperature on the necrotic reaction induced by PVY-M^SN^R and to determine whether temperatures affecting the expression of root-knot resistance also affected the response of tobacco plants to the virus. In all experiments, constant soil temperatures were achieved by using thermostatically controlled soil-temperature tanks located in a greenhouse. Seedlings were transplanted, 6 wk after planting, into 5-cm-diameter plastic pots containing a 2:1 ratio of sand and soil.

In the first experiment, cultivars NC 95 and NC 2326 were inoculated with the virus 2 wk after transplanting and placed at 28, 32, or 35 ± 1 C soil temperatures. Average aerial temperature was 30 C. Plants were assessed for PVY damage for a period of 3 wk. A completely randomized design with six replications was used.

In the second experiment, soil temperatures again were 28, 32, and 35 ± 1 C. This experiment was repeated three times and aerial temperatures averaged 29, 33, and 35 C, respectively. Cultivars used were NC 95 and either Hicks or NC 2326. Treatments were replicated six times and consisted of inoculation with race 3 of *M. incognita* or inoculation with PVY. Nematodes were maintained on plants of *Lycopersicon esculentum* L. cv. 'Homestead'. Root-knot inoculation on tobacco was done 1 wk after transplanting using approximately 10,000 eggs/pot. Eggs were extracted by washing infected tomato roots, cutting them in 3–4 cm pieces and agitating them in 0.5% sodium hypochlorite for 3 minutes. This mixture was passed through nested sieves (200 and 400 mesh) and thoroughly rinsed to collect the eggs. Tobacco roots were indexed for nematode infection 8 wk after inoculation using the following scale: 0 = no galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; and 5 = more than 100 galls. Inoculation with PVY took place 2–3 wk after transplanting and symptoms were evaluated 3 wk after inoculation. Overall data were pooled for a combined split-plot analysis in which temperature was the whole-plot and cultivars the sub-plot. The effects of root-knot and PVY were analyzed separately for ease of computation.

Soil temperatures of 20, 25, and 30 ± 1 C were used in the third experiment. This experiment was repeated twice and average aerial temperatures were 35 and 40 C, respectively. Plants of NC 95 and NC 2326 were inoculated with PVY and disease severity was assessed 3 wk after inoculation using the scales previously described.

Grafting experiment. A grafting experiment was conducted to determine whether a diffusible substance is produced in root-knot-resistant tobacco cultivars which influences the necrotic response when infected with PVY-M^SN^R. NC 95 was the root-knot resistant genotype and NC 2326 or VY 32 the root-knot susceptible genotype. Wedge grafts were made using all possible root-knot resistant and root-knot susceptible scion-stock combinations. Self-grafts (ie, root-knot resistant scions on root-knot resistant stocks) were included to detect adverse effects from the grafting procedure. Grafts were fastened with a latex bandage and placed under a greenhouse bench for 12 days to provide shade and high humidity during the graft-healing period. Plants were returned to the bench and 4 wk after grafting all successful grafts were inoculated with the virus. Temperatures ranged from 20 to 30 C. Usually, only the scion was inoculated by rubbing the two oldest leaves as previously described. In a few cases, buds were allowed to develop from the rootstock and these were inoculated to determine if this affected the response. Disease severity was evaluated 2 wk after inoculation according to the scale described above.

RESULTS

Aerial temperatures and plant age had significant effects on the degree of necrosis induced by PVY-M^SN^R on the root-knot resistant cultivar NC 95 (Fig. 1a–c). Necrosis was more severe and proceeded fastest at the lowest temperatures. At 28 C, 2 cm plants developed systemic necrosis (index = 4) 1 wk after inoculation and died by the 2nd wk (index = 6). Eight-centimeter-tall plants did not die until the 3rd wk. Plants 17- to 30-cm tall showed localized necrosis (index = 3) on the inoculated leaves 1 wk after inoculation, mild systemic necrosis (index = 4) by the 2nd wk, and severe, systemic necrosis (index = 5) by the 3rd wk. These older plants did not die even at the coolest temperature. At 32 C, necrosis was slightly less severe in all plant sizes. At 36 and 40 C, the younger plants (2 and 8 cm) developed mild systemic necrosis but did not die. The older plants (17–30 cm) did not develop necrosis at all but showed vein banding and mottling symptoms (index = 2).

No significant differences in symptom development were observed when Hicks was inoculated with PVY-M^SN^R (Fig. 1d–f). Symptoms consisted of vein-banding and mottling.

The effect of temperature was temporary. Plants which remained in the cool temperature (28 C) for the duration of the experiment (Fig. 2a) developed severe necrosis and died by the 3rd wk. Those plants kept only in the warm environment (36 C) only developed vein-banding symptoms. Plants shifted from the cool to the warm temperatures 3 days after inoculation (Fig. 2b) developed necrotic local lesions but no systemic necrosis. When plants were shifted from a warm to a cool environment 3 days after inoculation (Fig. 2b), necrosis proceeded without any effect from the warm pre-treatment. Fig. 2c and d show the same trend, ie, once placed in a cool environment necrosis proceeded to an extent depending upon the age of the plant at the time of transfer. Conversely, despite development of necrosis under cool temperatures, once the plants were shifted to a warm temperature, necrosis was arrested.

Soil temperatures had a similar effect on PVY-disease development as aerial temperatures (Fig. 3). Plants were approximately 12-cm tall when inoculated with PVY and daily average aerial temperature was 30 C. As in the aerial temperature experiments, severe necrosis followed by plant death was observed in plants of cultivar NC 95 at 28 C, mild systemic necrosis occurred at 32 C, and no necrosis developed at 35 C. Plants of NC 2326 showed vein-banding at all temperatures and these symptoms developed slower at the higher temperatures. These results are opposite from those obtained in the aerial temperature studies in which symptoms on the root-knot susceptible cultivar Hicks developed slower at the cool temperatures. This is because very high soil temperatures (35 C) retarded plant growth in general, whereas plants grew quite well in similar aerial temperatures.

The effects of soil temperature in combination with different

aerial temperatures on the response of cultivars NC 95, and NC 2326 or Hicks, to both PVY and the root-knot nematode are shown in Fig. 4. As in previous tests, the necrosis induced by PVY-M^SN^R on plants of cultivar NC 95 was most severe at low soil/aerial temperature combinations. Necrosis occurred in plants of cultivar NC 95 to various degrees when either aerial or soil temperature was cool enough to favor development of necrosis, eg, mild systemic necrosis occurred at 35 C soil temperature, 29 C aerial temperature. No necrosis was observed when both aerial and soil temperatures were 35 C. Hicks and NC 2326 developed mild symptoms at all soil/aerial temperature combinations.

Significant differences in the degree of root galling induced by the root-knot nematode at different soil temperatures occurred in both root-knot resistant and root-knot susceptible cultivars (Fig. 4). NC 95 was completely resistant at 28 C soil temperature, ie, no galls were observed. The galling index was 3 at 32 C and 4 at 35 C. Thus, there is a correlation in the response of NC 95 to those two pathogens; the same temperatures which inhibited the expression of root-knot resistance also inhibited the necrotic response to the virus.

Cultivars Hicks and NC 2326 were equally susceptible to root-knot (index = 5) at 28 and 32 C soil temperatures. At 35 C the degree of galling decreased (index = 4). This may also be explained by the fact that this soil temperature is suboptimal for tobacco plant growth and the root systems were less developed than at 32 or 28 C.

Cool soil temperatures (Fig. 5) induced development of the necrotic reaction in NC 95 despite high aerial temperatures (eg, 20 C soil and 40 C aerial), but this necrosis remained in the lower 4- to 10-cm portion of the stem where soil temperatures still exerted their influence. Upper leaves of NC 95 in the hot aerial environment did not become necrotic even when soil temperature was 28 C. These results provide further evidence that when either soil or aerial temperatures are conducive to necrosis, necrosis develops, ie, the stimulating effects of relatively cool temperatures on disease development override the inhibitory effects of hot temperatures. There were no differences due to temperatures on cultivar NC 2326.

Viral necrosis in grafted plants developed only in the root-knot resistant portion of the graft (Table 1) regardless of whether scion or rootstock was inoculated. The substance(s) responsible for the necrotic reaction is not diffusible or translocatable to root-knot susceptible genotypes. Plants of cultivars NC 2326 developed typical vein-banding symptoms and VY 32 showed no symptoms although positive serological reactions revealed presence of the virus. Grafts in which the rootstock portion was root-knot resistant often showed wilting of the scion. Necrosis at the base of the stem apparently interfered with water translocation.

DISCUSSION

The vascular necrosis induced by PVY-M^SN^R on root-knot resistant cultivars is most severe in young plants. This is consistent with a previous report stating that PVY in general causes greatest yield losses when infection occurs early (12). This necrotic reaction is also temperature-sensitive. The degree of necrosis is greatest at cool temperatures and decreases as temperatures increase. Although we did not conduct field studies we can extrapolate from these results to predict that damage from this strain of PVY under field conditions should be directly correlated with the stage of plant maturity when infection occurs. Also, damage should be directly

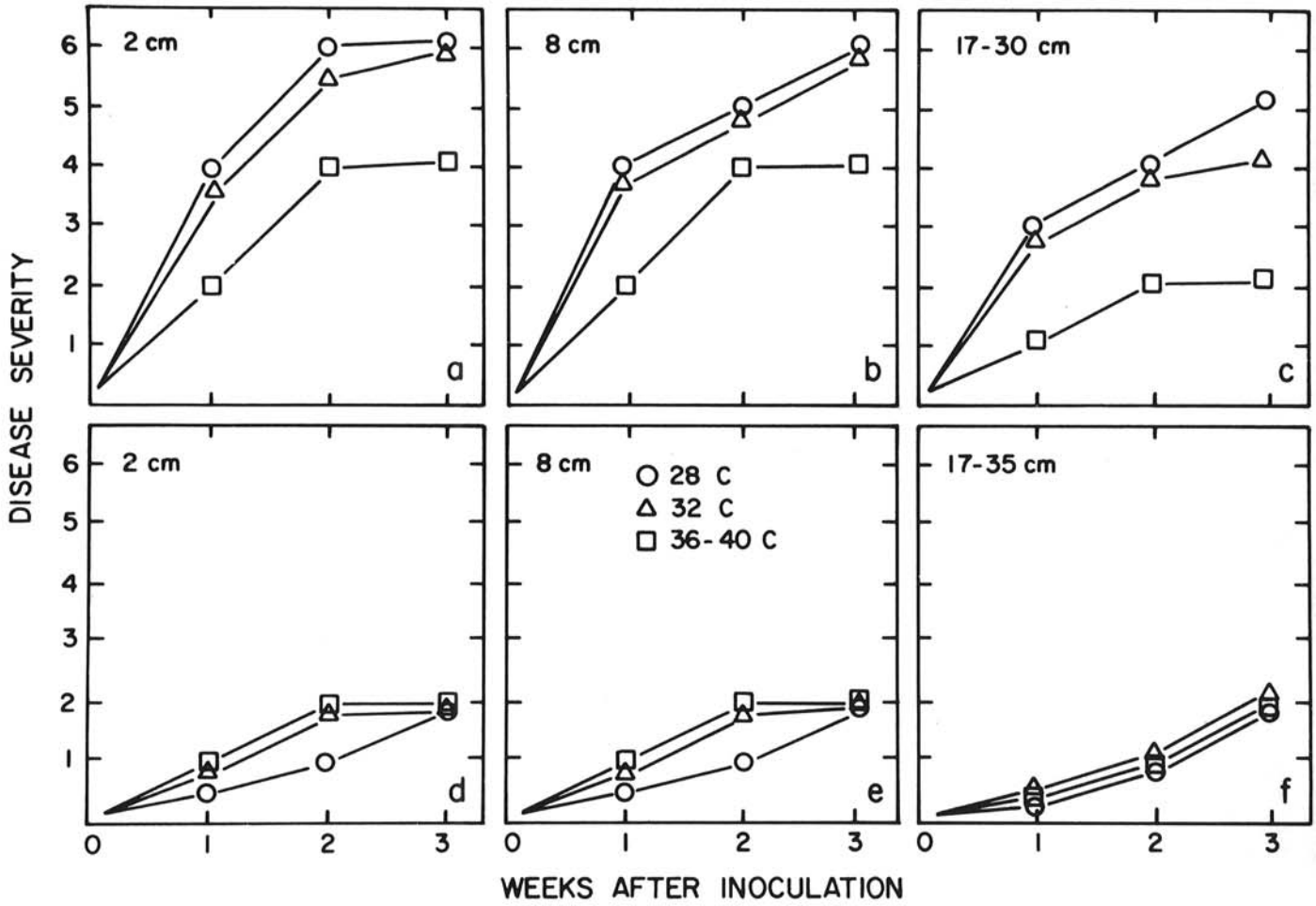

Fig. 1. Response of tobacco cultivars inoculated with strain M^SN^R of potato virus Y at different growth stages under different aerial temperature regimes. a–c = NC 95. d–f = Cultivar Hicks. (Disease severity index: 0 = no symptoms; 1 = vein clearing, mild mottling; 2 = vein banding, severe mottling or mosaic; 3 = localized necrosis, restricted to inoculated leaf; 4 = mild, systemic necrosis; 5 = severe, systemic necrosis; and 6 = plant death.)

correlated with temperatures occurring after infection.

Induction of the necrotic reaction can be arrested by the transfer of plants from a cool to a hot environment as long as the plants remain at the high temperature; when plants are again subjected to a cool temperature, necrosis resumes. This reaction is similar to the formation of local lesions in certain *Nicotiana* species or breeding lines carrying the *N. glutinosa* factor (N) for resistance to tobacco mosaic virus (9). Plants carrying the N factor develop mosaic and vein banding at high temperatures (36 C), but develop necrotic local lesions when the temperature is lowered to 24 C or below. In fact, the response of root-knot resistant tobacco cultivars to PVY-M^SN^R is similar to the necrotic local lesions involved in resistance (hypersensitivity) to many viruses in many plant species. Inoculated leaves develop necrotic local lesions, but with PVY-M^SN^R these lesions do not localize the virus and soon the virus (and hence the necrosis) becomes systemic. However, the necrotic reaction does appear to have an inhibitory effect on virus replication. Virus titer was lower in plants that developed necrosis than in plants that developed vein banding.

Resistance to the root-knot nematode often involves a necrotic reaction at the cellular level. Larvae penetrate the roots of resistant plants, but the cells surrounding the larvae become necrotic and die soon after the larvae become sedentary. This prevents establishment of a feeding relationship and resistance is expressed. The same or a very similar necrotic reaction may be involved in response to the virus. The main difference lies in the manner in which these two pathogens infect the plant—the nematode is confined to the root system whereas the virus is systemic throughout the plant. Evidently, the necrotic reaction is not rapid or inhibitory enough to localize the virus.

Root-knot resistance has been reported to be temperature-sensitive and to be reversed toward a susceptible reaction under

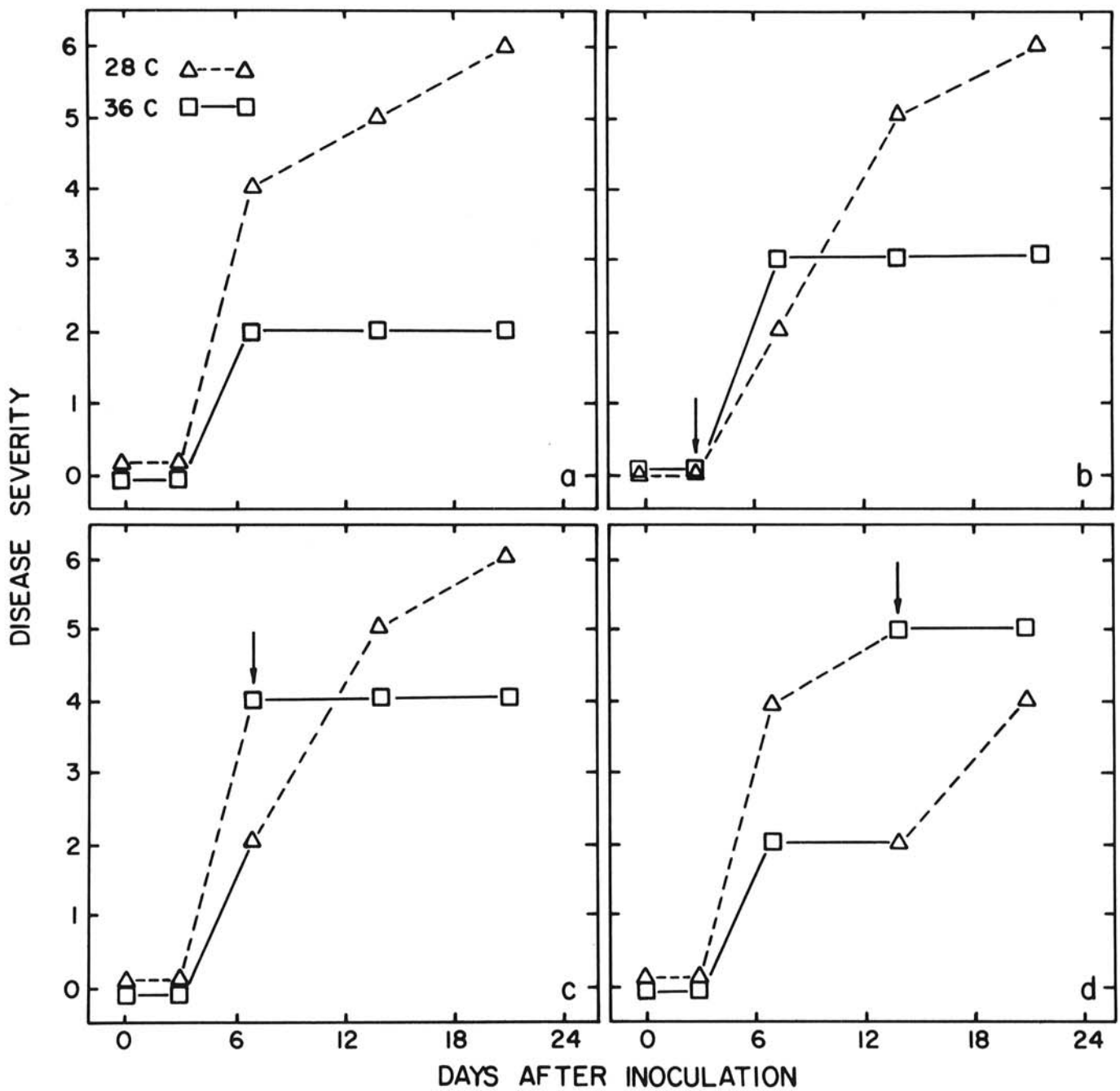

Fig. 2. Response of tobacco cultivar NC 95 inoculated with the M^SN^R strain of potato virus Y at 28 or 36 C. Arrow indicates time of temperature shift. (Disease severity index: 0 = no symptoms; 1 = vein clearing, mild mottling; 2 = vein banding, severe mottling or mosaic; 3 = localized necrosis, restricted to inoculated leaf; 4 = mild, systemic necrosis; 5 = severe, systemic necrosis; and 6 = plant death.)

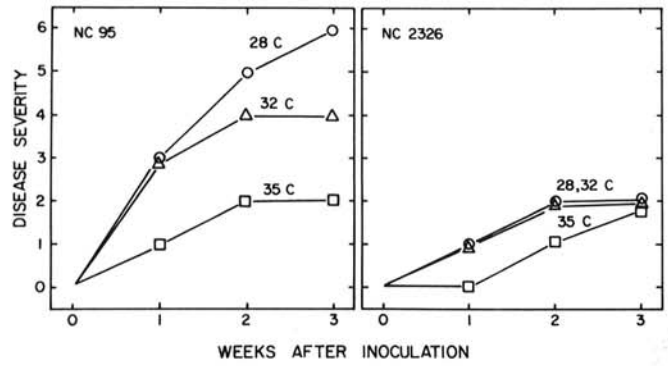

Fig. 3. Response of tobacco cultivars inoculated with strain M^SN^R of potato virus Y under different soil temperature regimes. (Disease severity index: 0 = no symptoms; 1 = vein clearing, mild mottling; 2 = vein banding, severe mottling or mosaic; 3 = localized necrosis, restricted to inoculated leaf; 4 = mild, systemic necrosis; 5 = severe, systemic necrosis; and 6 = plant death.)

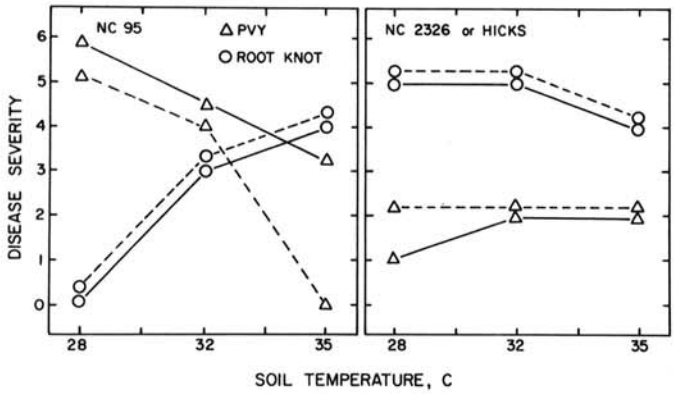

Fig. 4. Response of tobacco cultivars inoculated with the M^SN^R strain of potato virus Y (PVY) or the root-knot nematode (*Meloidogyne incognita*) under different combinations of soil and aerial temperatures. (PVY disease severity index: 0 = no symptoms; 1 = vein clearing, mild mottling; 2 = vein banding, severe mottling or mosaic; 3 = localized necrosis, restricted to inoculated leaf; 4 = mild, systemic necrosis; 5 = severe, systemic necrosis; and 6 = plant death. Root-knot disease severity index: 0 = no galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; and 5 = more than 100 galls.) —— = 29 C aerial temperature. ------ = 33, 35 C aerial temperature.

TABLE 1. Response of grafted plants with different root-knot resistant (NC 95) and root-knot susceptible (VY 32 and NC 2326) rootstock-scion combinations inoculated with the M^SN^R strain of potato virus Y (PVY). Inoculation was performed 4 wk after grafting

Rootstock	Scion	Grafts (no.)	Reaction to PVY 2 wk after inoculation: Rootstock	Scion
Grafted entry:				
NC 95	VY 32	32	Necrosis	No symptoms;[a] scion shows wilting
NC 95	NC 2326	22	Necrosis	Vein banding
NC 95	NC 95	15	Necrosis	Necrosis
VY 32	NC 95	35	No symptoms[b]	Necrosis
NC 2326	NC 95	20	No symptoms[b]	Necrosis
VY 32	VY 32	10	No symptoms[b]	No symptoms
NC 2326	NC 2326	12	Vein banding	Vein banding
Nongrafted controls				
NC 95		5	Necrosis	
VY 32		5	No symptoms	
NC 2326		5	Vein banding	

[a] Serological reaction was positive indicating presence of virus.
[b] Rootstocks remained green throughout experiment. Serological tests were also positive.

relatively high soil temperatures (1,13). Dropkin (1) and Slana and Stavely (13) found that the loss of resistance is gradual over a range of temperatures with changes toward greater susceptibility with rising temperatures. Our studies agree with these findings. We also found that the same temperatures which inhibit root-knot resistance also inhibit a necrotic reaction to the virus. The degree of inhibition between the two was very similar. At 28 C, root-knot resistance was completely expressed and the necrotic response to the virus was severe; at 32 C when root-knot resistance began to "break," the necrotic reaction to the virus was intermediate; and at 35 C, when root-knot resistant plants became susceptible, the necrotic reaction to the virus disappeared. Therefore, these two reactions appear to be regulated by the same, or very similar, temperature-controlled gene or mechanism. If one thinks of the two responses as two necrotic reactions, then the two behave in parallel fashion; ie, both reactions are inhibited by the same temperatures. The degree of necrosis (hypersensitivity) involved in root-knot resistance would be difficult to quantify, but presumably there is a gradual decline in the degree of necrosis as plants become more susceptible. Evidently, temperature exerts its effect directly on the host since the response is the same regardless of which pathogen was used for inoculation.

Root-knot resistance has also been reported to be inhibited by relatively high concentrations of cytokinins (2). In these studies, the necrosis induced by PVY-M^SN^R could not be reversed by foliar application of kinetin. The effect of cytokinin may be limited to the root since synthesis of that growth regulator normally takes place there.

Further evidence that these two reactions are of similar nature was found by grafting experiments (Table 1). When leaves of a root-knot susceptible cultivar are inoculated with a suspension of root-knot nematode eggs, galls develop on the leaf surface; but when the eggs are injected into the leaves of root-knot-resistant cultivars, no galls develop (10). Thus, the expression of root-knot resistance is not confined to the roots. The resistance factor is an attribute of every cell of a root-knot-resistant genotype and the necrosis incited by the nematode takes place in the leaves as well. The necrotic reaction to the virus was found in this study to be of a

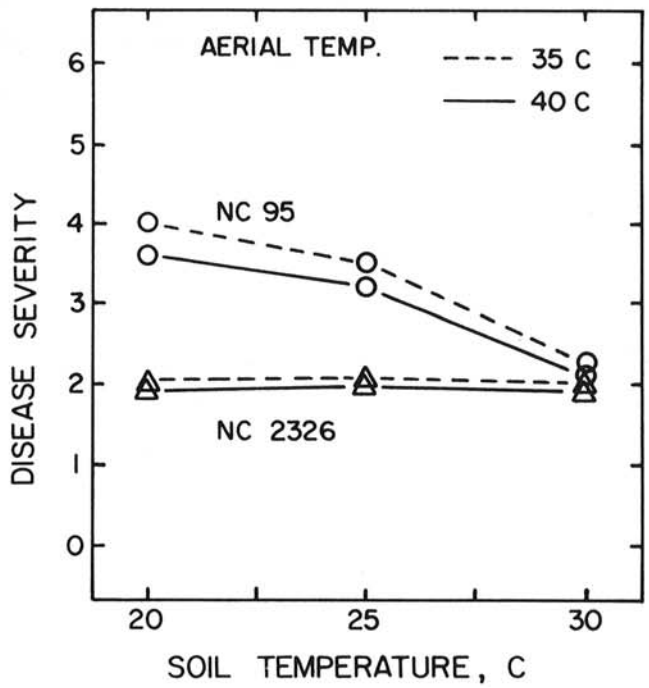

Fig. 5. Response of tobacco cultivar NC 95 or NC 2326 3 wk after inoculation with strain M^SN^R of potato virus Y under different combinations of soil and aerial temperatures. (Disease severity index: 0 = no symptoms; 1 = vein clearing, mild mottling; 2 = vein banding, severe mottling or mosaic; 3 = localized necrosis, restricted to inoculated leaf; 4 = mild, systemic necrosis; 5 = severe, systemic necrosis; and 6 = plant death.)

similar nature, ie, it is a cell-specific response expressed only in the cells of root-knot-resistant genotypes. Whatever substance is involved in the necrosis is not translocatable to cells of root-knot susceptible genotypes. The biochemical basis for the reaction is unknown but preliminary electrophoretic studies revealed the presence of an additional peroxidase isozyme in plants of NC 95 infected with PVY-M^SN^R as compared with infection by PVY-M^SM^R (*unpublished*).

The data presented here support the hypothesis that the necrotic response of root-knot resistant cultivars to strain M^SN^R of PVY is due to a pleiotropic effect of the gene conditioning root-knot resistance. This conclusion is further supported by results from genetic studies (11).

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