JOURNAL OF NEMATOLOGY

VOLUME 4 JANUARY 1972 NUMBER 1

Nature of Sweet Potato Resistance to Meloidogyne incognita and the Effects of Temperature on Parasitism[1]

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Abstract: Penetration, rate of development, and total population of *Meloidogyne incognita* in roots of susceptible 'Allgold' and resistant 'Nemagold' sweet potatoes increased with temperature 24-32 C. Rate of larval penetration in 'Allgold' was significantly higher than in 'Nemagold' after 48 hr of root exposure at 24, 28, and 32 C. At 24, 28, and 32 C (16 hr) day and 20 C (8 hr) night temperature the life cycle of *M. incognita* required 42, 32, and 28 days in 'Allgold', and 44, 33, and 31 days in 'Nemagold'; mature females in the first generation were 40, 40, 40, and 10, 22, 20 respectively. The correlation between the length of time roots were allowed to grow in the soil prior to inoculation and number of larvae recovered from the roots after inoculation was positive for 'Allgold' and negative for 'Nemagold'. Therefore, a root exudate repellent to *M. incognita* larvae is proposed as a hypothetical basis for resistance to *M. incognita* in sweet potatoes. *Key Words: Ipomoea batatas*, Root observation box.

Temperature effects on the life cycle of root-knot nematodes and upon resistance expression in some host plants has been well-documented (1, 5, 8, 9, 10, 17). Each root-knot nematode species has its own temperature range requirement for development though the optima vary for different host-parasite combinations. Tyler (17) found the minimum larva to larva life cycle of the root-knot nematode on tomato was 25 days at 27 C. At 16.5 C, however, the required time was increased to 87 days. Holtzman (8) reported that penetration and development of *Meloidogyne incognita* in roots of resistant tomato varieties increased at higher temperatures. Similarly, Dropkin (5) found that increased temperature reduced the resistance of 'Chief' soybean to *M. incognita acrita*.

The nature of resistance in plants to root-knot nematodes is not completely documented for each plant species and warrants further attention. Resistance has been attributed to failure of larvae to penetrate the host plant (14), inability of larvae to establish a nutritive relationship with the plant and their subsequent departure from roots (13), retardation or failure of larvae to develop to maturity (8, 9), plant hypersensitivity (7), and secretion of toxic or repellent chemical compounds (3, 9, 18).

This study was conducted to determine the effect of temperature on the penetration and life cycle of *Meloidogyne incognita* (Kofoid and White) Chitwood on resistant 'Nemagold' and susceptible 'Allgold' sweet potatoes (*Ipomoea batatas* [L.] Lam.), and further define the nature of resistance in the 'Nemagold' sweet potato.

MATERIALS AND METHODS

Infective larvae of *M. incognita* were obtained from a colony originally established by the late Dr. F. B. Struble (Oklahoma State University, Stillwater, Okla.) from a single egg mass taken from 'Allgold' sweet potato and maintained by periodic transfer to greenhouse-grown 'Rutgers' tomato. Galled roots were comminuted, placed on cheesecloth over 32 or 60-mesh sieves, the sieves placed in containers and tap water added to cover the bottom of the sieves and saturate the cheesecloth. Every 24 hrs for 4 days larvae were collected, refrigerated at 5 C, and fresh water was added beneath the sieves. Preliminary studies showed equal penetration and development of freshly hatched larvae and those stored 4 days at 5 C. The studies were conducted in three controlled environment chambers unless otherwise indicated. Photoperiods of 16 hr (2000 ft-c) were used with 24, 28, and 32 C day temperatures and an

Received for publication 19 June 1970.

[1] Portion of an M.Sc. thesis of the senior author. Journal Article No. 2023, Agricultural Experiment Station, Oklahoma State University, Stillwater 74074.

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8 hr, 20 C night period. The experiments were repeated three times for each temperature regime. In each experiment, cultivars were replicated five times at each temperature.

Cuttings of 'Allgold' (susceptible) and 'Nemagold' (resistant) sweet potato cultivars were rooted separately in water for 10 days prior to inoculation. Rooted cuttings were placed singly, in individual 210 ml styrofoam cups which were partially filled with methyl bromide-fumigated soil. The plants were inoculated by pouring 500 larvae in 5 ml water over each root system. Then roots were covered with soil and the planted cups distributed to the controlled environment chambers.

PENETRATION STUDIES: In penetration studies, the roots were exposed to larvae for periods of 1, 2, 4, 6, 8, and 10 days at three temperature levels. The whole root system of each plant after exposure to nematodes was washed and stained according to the method of McBeth et al. (11). The whole stained root systems were placed between two microscope slides, crushed, and examined with a stereoscopic scope. The number of nematodes in roots were counted and the rate of penetration was calculated.

DEVELOPMENT AND LIFE CYCLE EXPERIMENTS: Since the results of penetration studies indicated that differences in number of larvae entering roots of resistant and susceptible cultivars were not significant until 48 hr exposure to nematodes, the plants in this study were exposed to nematodes for 48 hr in the greenhouse. The roots then were washed free of soil to remove unattached larvae, replanted in 210-ml cups containing sterilized soil and placed in controlled environment chambers. Every 24 hr, one complete root system of each variety from each temperature was carefully removed from the soil, washed, stained, and examined as described in penetration studies. Developmental stages of the nematode were identified (A, B, C, D, and E) according to the method of Christie (2). Only individuals easily distinguishable from females were counted as males.

ROOT EXUDATE EXPERIMENT: In determining the presence and effect of root exudates, the standard sliding front root observation box (16) was modified by use of a lift-off glass front as shown in Figure 1. With this modification, injury and disturbance of the roots was avoided when the glass was removed and then replaced after inoculation. Each box measured 38X25X4½ cm with an opening of 23X23 cm for the placement of the glass.

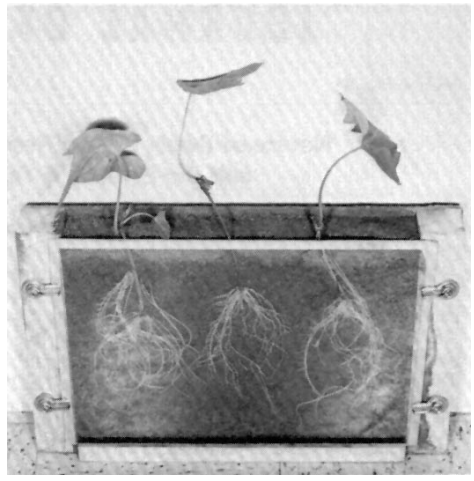

FIG. 1. Modified root observation box with soil and rooted cuttings in place.

'Allgold' and 'Nemagold' cuttings for an entire study were rooted simultaneously in water to avoid discrepancies in the age of individual cuttings. The two cultivars were rooted in separate containers to avoid contamination by root diffusate of the other variety. After an initial period of 48 hr, the plants of each variety were transplanted to steam-sterilized soil (1:1 mixture of loam and sand which had been passed through a 32-mesh screen) in separate root observation boxes at 48 hr intervals. The glass slide was replaced, covered to exclude light and boxes were inclined at a 45 degree angle to encourage root growth against the glass. The plants were allowed to grow in the soil for 10, 8, 6, 4, 2, 1, and 0 days prior to inoculation. The plants which were given the 0 day treatment were inoculated immediately upon transplanting and simultaneously with all other treatments. At the time of inoculation, all plants were 12 days old. The experiment was conducted in the greenhouse with a temperature variation of 27 ± 1 C during the 48 hr interval following inoculation. Inoculation consisted of application of a water droplet containing 25 larvae to the soil surface 5 mm from each of 25 root apices. After 48 hr a 5-cm section of each inoculated root, including the root tip, was excised, stained, and observed to determine the incidence of larval penetration. This experiment was repeated three times.

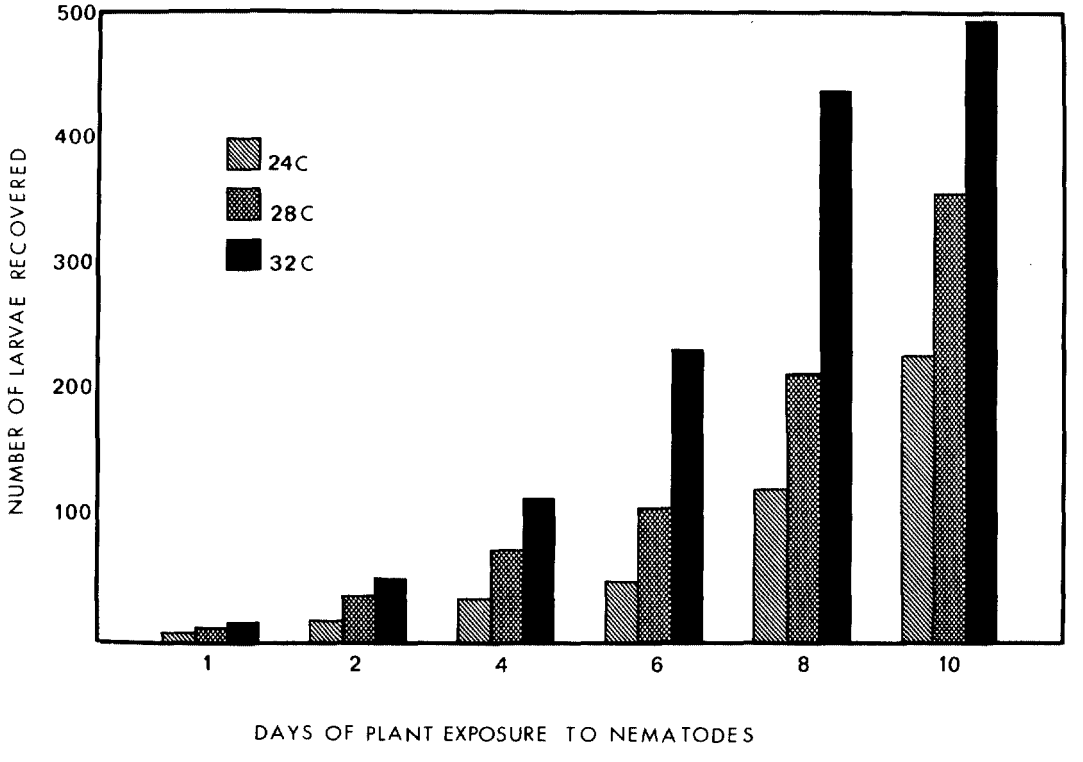

FIG. 2. Effect of temperature on penetration of 'Allgold' sweet potato roots by *Meloidogyne incognita* larvae.

RESULTS AND DISCUSSION

Rates of nematode penetration increased with increasing temperature in roots of both cultivars. Although there was a direct relationship between the increase of temperature and penetration within varieties, the rate of penetration was significantly higher in 'Allgold' (Fig. 2) than in 'Nemagold' (Fig. 3) at all three temperatures after 48 hr exposure to inoculum.

After 6 days the number of larvae which had penetrated 'Allgold' roots were 53, 105, 233 compared to 30, 41, and 70 in 'Nemagold' at 24, 28, and 32 C, respectively. After 10 days this increased to 228, 357, and 495 larvae in 'Allgold' and 45, 72, and 172 in 'Nemagold' at 24, 28, and 32 C, respectively. Fig. 2 and 3 illustrate the pattern of increased penetration at the three temperature levels in both cultivars.

Temperature effects on *M. incognita* penetration in resistant 'Nemagold' closely paralleled the report by Holtzman (8) in resistant tomato cultivars. It is evident from these studies that soil temperature should be controlled by plant breeders screening sweet potato lines for resistance to *M. incognita*.

Equal penetration of root-knot nematode larvae in roots of both resistant and susceptible sweet potato lines was reported by Dean and Struble (4) and Shibuya (15). Radewald (12), however, found significantly lower rates of penetration in resistant sweet potato lines. Our results indicated no significant difference in penetration until after 48 hr exposure of roots to nematodes. After 48 hr nematode penetration of resistant roots increased less rapidly than in the susceptible plants.

From our results, we postulate: (i) that after a short period of time from the initial contact the physiology of the resistant plant is altered to the disadvantage of the nematode, or (ii) that the initial contact and penetration of the nematode immediately stimulate a chemical production or a defense reaction in resistant plants which may cause a reduction in future penetration rate.

The results of the development and life cycle studies indicated that larvae of *M. incognita* remained vermiform in roots of 'Allgold' and 'Nemagold' for approximately 5 days following inoculation at 24 C and 4 days at 28 and 32 C. Enlargement and sedentary feeding began on the 6th day at 24 C and on

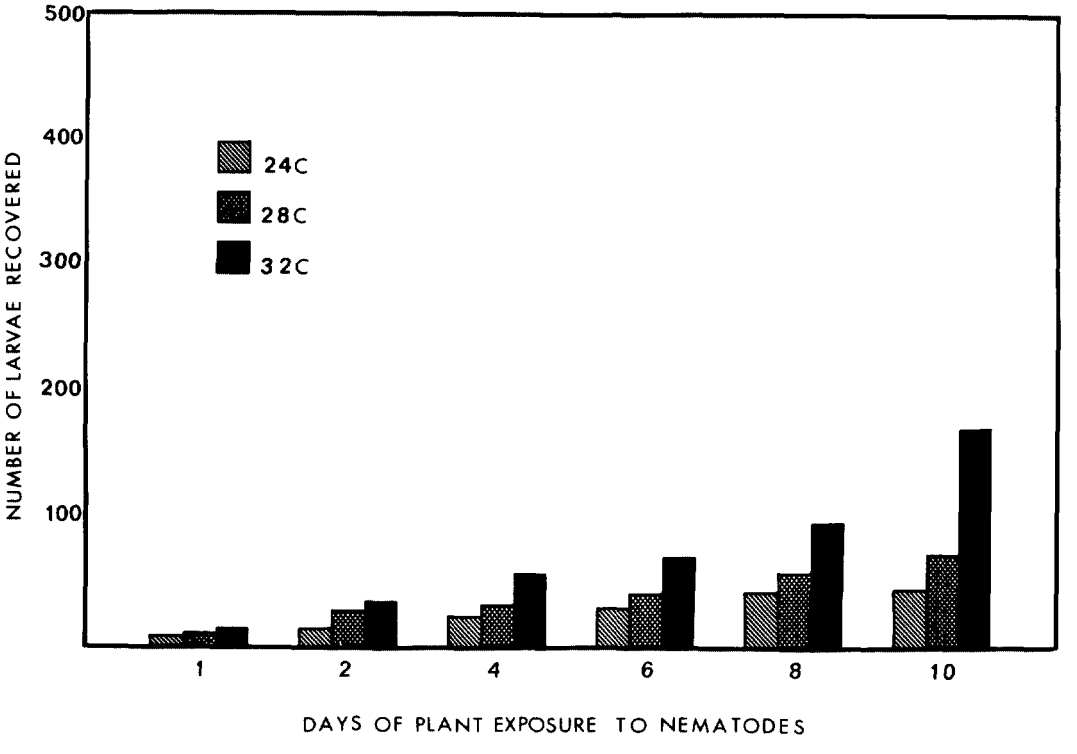

FIG. 3. Effect of temperature on penetration of 'Nemagold' sweet potato roots by *Meloidogyne incognita* larvae.

TABLE 1. Developmental stages of *Meloidogyne incognita* in roots of susceptible ('Allgold') and resistant ('Nemagold') sweet potato cultivars at 16 hr (2000 ft-c) 24, 28, 32 C day and 8 hr, 20 C night temperature.

Temp. Levels °C	'Allgold'							'Nemagold'						
	Days after inoculation	No. nematodes in each stage[2] A	B	C	D	E	Male	Days after inoculation	No. nematodes in each stage[2] A	B	C	D	E	Male
24-20	8	22	11	0	0	0	0	9	29	1	0	0	0	0
	12	3	20	6	0	0	0	13	2	18	6	0	0	0
	16	0	11	23	13	0	0	19	0	1	10	13	0	0
	23	0	0	16	23	8	0	23	1	5	6	9	4	1
	42[1]	0	0	0	8	40	0	44[1]	0	2	4	9	10	0
28-20	8	13	31	0	0	0	0	9	25	3	0	0	0	0
	10	1	38	1	0	0	0	14	0	25	4	0	0	0
	14	0	7	32	1	0	0	15	0	7	21	13	0	0
	20	0	6	4	31	2	0	19	0	4	4	12	1	0
	32[1]	0	0	1	6	40	0	26	0	5	5	6	14	1
								33[1]	0	0	1	1	22	0
32-20	8	18	20	0	0	0	0	9	27	3	0	0	0	0
	15	2	30	9	0	0	0	12	10	15	4	0	0	0
	16	0	20	13	2	0	0	16	1	1	12	18	0	0
	19	0	3	8	30	4	0	19	0	3	2	14	10	0
	28[1]	0	0	0	3	40	0	31[1]	0	0	4	8	20	0

[1] Initiation of second generation.

[2] A = larvae from initial growth to conical tail; B = hemispherical posterior with terminal spike to just before final molt; C = final molt to almost grown; D = fully grown but no eggs laid; E = egg-laying females (after Christie, 2).

the 5th day at 28 and 32 C. Developmental group B was first observed the 8th day following inoculation in 'Allgold' and on the 9th day in 'Nemagold' under all temperature regimes (Table 1). Rate of nematode development in roots of both cultivars increased with increasing temperature, but 'Nemagold' supported fewer nematodes and smaller mature females than 'Allgold'. These findings support the results of Dean and Struble (4), who found that only a few *M. incognita* larvae matured and oviposited in three resistant sweet potato lines they tested. However, we found that a higher proportion of the nematodes recovered from 'Nemagold' roots did reach maturity when soil temperature was increased.

Oviposition occurred in both varieties after 23 days at 24 C and 19 days at 32 C. At all three temperatures individual females deposited fewer eggs in 'Nemagold' than in 'Allgold'. Hatching was more rapid in both varieties at higher temperatures; at 24, 28, and 32 C, hatch required 19, 12, or 9 days in 'Allgold' and 21, 14, or 12 days in 'Nemagold'. Group E was still increasing in both cultivars and all three temperature regimes when experiments were terminated. Males were recovered only from 'Nemagold' roots grown at 24 and 28 C.

Effect of increased temperature on resistance breaking in tomato was reported by Dropkin (6). The results of our studies paralleled those of Dropkin's experiment. The development of *M. incognita* was retarded by low temperatures in 'Nemagold' roots. The total number of larvae which had developed to more advanced stages was significantly higher in 'Allgold' than in 'Nemagold' during any given period or temperature level except at 32 C after 16 days, when the total number of nematodes in advanced stages in 'Nemagold' surpassed those in 'Allgold' (Table 1). Nevertheless, the life cycle was completed more rapidly in 'Allgold' at this temperature.

Hypersensitive cell necrosis in 'Nemagold' was higher at lower temperatures. Necrosis

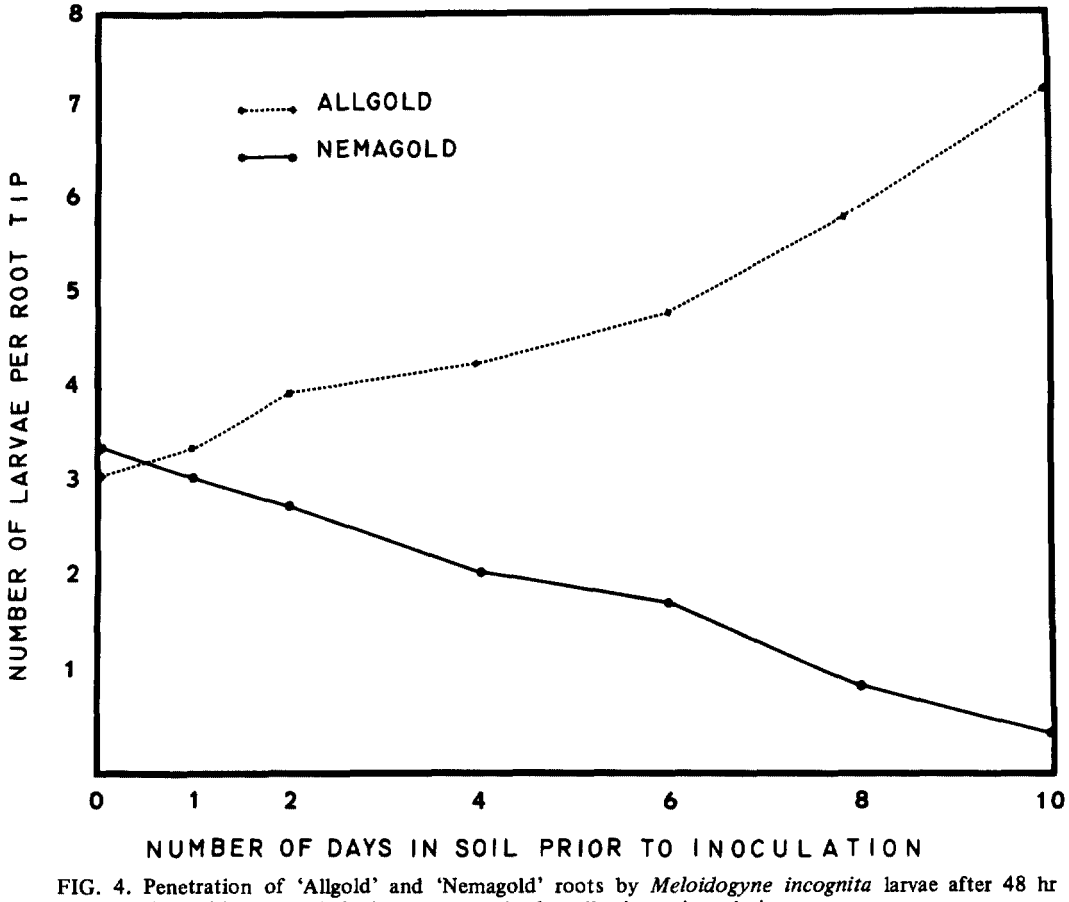

FIG. 4. Penetration of 'Allgold' and 'Nemagold' roots by *Meloidogyne incognita* larvae after 48 hr exposure as affected by the periods the roots were in the soil prior to inoculation.

occurred rapidly in 'Nemagold' and was confined to tissues surrounding the larvae and the site of larval penetration at the root tip. This reaction may result either in (i) a failure of the nematode to establish a nutritive relationship with the host and subsequent departure from the roots, or (ii) death and disintegration of the nematode. Reactions of this type may account for the presence of fewer nematodes in roots of resistant 'Nemagold' even though initial penetrations were approximately equal.

The results of root exudate experiments are illustrated in Fig. 4. No statistical differences in the penetration rate existed between cultivars up to 48 hr of presence of roots in soil. This finding parallels the results of penetration studies. Those roots which were in the soil 6 days prior to inoculation, however, contained 4.8 and 2.1 larvae per root tip for 'Allgold' and 'Nemagold', respectively. This penetration level was increased to 7.3 for 'Allgold' and reduced to 0.4 per root for 'Nemagold' in roots which had been in the soil 10 days prior to inoculation. Positive correlation of time and penetration for 'Allgold' and negative for 'Nemagold' strongly suggests that the exudates of resistant 'Nemagold' are toxic or repellent, and may preclude or reduce larval contact with roots. Further, the negative correlation between penetration and time noted for 'Nemagold' suggests that exudates are somewhat persistant in the soil and that their accumulation in the rhizosphere of established plants is responsible for reduced larval penetration of roots.

On the basis of these studies, and the work of other investigators, it appears that nematode resistance in sweet potatoes may be based on one or a combination of the following factors: (i) the production of nematode repellent exudates which would preclude or reduce larval contact with the root; (ii) failure of larvae to penetrate the plant; (iii) inability of larvae to establish a nutritive relationship with the plant due to hypersensitive plant reaction or nutrient deficiency and (iv) the probable post-infection production of inhibitory chemicals.

Our results demonstrated that temperature is an important factor governing the expression of sweet potato resistance to *M. incognita*. It is important to investigate the possibility of production of repellent root exudates by resistant cultivars and to investigate the possibility of transferring the gene(s) governing this character to other agronomically suitable cultivars.

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