Influence of salt concentration on infectivity and development of *Meloidogyne incognita* on tomato

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SUMMARY

In greenhouse studies, infectivity and development of *M. incognita* on tomato was impaired by increasing soil solution concentrations (ECe 1.5, 2.5, 3.5, 5.0 mmhos/cm) of NaCl, $CaCl_2$ or combinations of both salts. In two experiments, reduced infectivity was pronounced on a susceptible cultivar (Hunts) at all salt concentrations, while infectivity was reduced primarily at higher salt concentrations on a moderately resistant host (Beefmaster). Development of entrant juveniles was significantly depressed by both salts on cv. Hunts, but merely delayed on cv. Beefmaster. Impaired infectivity and development resulted in population reduction to 23% and 39% of controls on Beefmaster and Hunts, respectively, six weeks after infection.

RÉSUMÉ

Influence de la concentration en sels sur l'infectivité et le développement de Meloidogyne incognita *sur tomate*

Au cours d'études effectuées en serres, l'infectivité et le développement de *M. incognita* sur tomate ont été d'autant plus affectés que les solutions de NaCl, de $CaCl_2$ ou de ces deux sels dans le sol étaient plus concentrées (ECe 1,5 ; 2,5 ; 3,5 ; 5,0 mmhos/cm). Au cours des deux expériences, l'infectivité a été réduite à toutes les concentrations sur un cultivar sensible (Hunts) alors que la réduction de l'infectivité n'a débuté qu'aux concentrations élevées sur un cultivar modérément résistant (Beefmaster). Le développement des juvéniles ayant pénétré a été réduit significativement par les deux sels sur le cv. Hunts mais seulement retardé sur le cv. Beefmaster. Six semaines après l'infection, les altérations causées à l'infectivité et au développement ont amené une réduction de la population par rapport aux témoins de 23% sur Beefmaster et 39% sur Hunts.

Root-knot nematodes (*Meloidogyne* spp.) are worldwide in distribution and are important parasites of a wide range of economically important plants. Most species of *Meloidogyne* are serious plant pathogens in arid and semi-arid regions. These regions are characterized by high evapotranspiration rates, due to high temperature and low relative humidity, which may result in tremendous accumulation of salts in the root zone. The chemical composition of the soil solution directly affects plants (Bernstein, 1964) and nematodes (Bird, 1977). Saline soils contain variable amounts of cations and anions at different concentrations and complexities. The common dominant anions are chlorides (Cl^-), sulfates (SO_3^{2-}), bicarbonates (HCO_3^-), and sometimes nitrate (NO_3^-). The cations are calcium (Ca^{2+}), sodium (Na^+), magnesium (Mg^{2+}), and sometimes potassium (K^+). These elements can exist individually or in combination with others to form complex compounds.

The influence of such compounds on nematode infectivity is unclear. Na^+, Cl^-, and boron at excessive amounts might have specific toxic effects on plants (Bernstein, 1964 ; Mengel & Kirby, 1978). Egg hatching of *M. javanica* decreased as the concentration of the electrolytes (NaCl, $CaCl_2$, KCl) increased in the solution media (Dropkin, Martin & Johnson, 1958). Wallace (1966) studied egg hatch, survival and

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development under different moisture regimes in which various electrolyte concentrations were obtained.

Heterodera rostochiensis egg hatching (Ellenby & Gilbert, 1958), and reproduction of free-living nematodes (Everard, 1960), were decreased when exposed to saline media. Prot (1978*a*, *b* ; 1979*a*, *b*) observed effects of moisture and salt gradients on movement and behavior of *M. javanica* and four other plant-parasitic nematodes. A low soil moisture content, combined with a higher temperature in the presence of root-knot nematode, increased the number of galls in proportion to the size of the root system (Jones, 1932).

In the absence of a host plant, many plant nematodes, including *Meloidogyne* spp., can remain alive for several months or even years (Bergeson, 1959). Survival of citrus nematode in saline soil has been studied (Kirkpatrick & Van Gundy, 1966), and under field conditions more citrus nematodes were recovered from citrus roots subjected to high salinity (6.5 mmhos/cm) than from citrus growing at lower salinity levels (2.5 mmhos/cm). There is no information on how long *Meloidogyne* spp. juveniles survive and remain infective in saline soils.

The influence of salinity on development of *Meloidogyne* species has not been investigated. Development of *Meloidogyne* was studied by Godfrey and Oliveira (1932) on cowpea and pineapple. The length of time elapsing from initial inoculation to the first egg development was nineteen days in cowpea and 35 days in pineapple. Bird (1959) studied development of root-knot (*M. javanica* and *M. hapla*) on tomato. Studies of environmental influence on development include effects of temperature (Daulton & Nusbaum, 1966 ; Godfrey & Oliveira, 1932 ; Jones, 1975 ; Jones, 1932 ; Tyler, 1933*b*), moisture (Jones, 1975 ; Wallace, 1966) and nutrients (Oteifa, 1953). The present experiments were undertaken to study the influence of increasing concentrations of various salts on infectivity and development of *Meloidogyne incognita* on resistant and susceptible tomato cultivars.

Materials and Methods

Thirty-five-day-old tomato seedlings (*Lycopersicon esculentum* Mill) of cultivars : Hunts 2580 (Wesson Food Co.), Beefmaster (Burpee Seed Co.), and Ronita (Peto Seed Co.) were sown in vermiculite and grown in a greenhouse. After 35 days, the seedlings were transplanted to insulated styrofoam cups filled with blow sand (78% sand, 14% silt, and 8% clay). Each cup was lined with a polyethylene bag to prevent salt leakage. Plants were then treated according to the objectives of the study.

Infectivity studies

Ten days after transplanting, a solution of either sodium chloride (NaCl), calcium chloride ($CaCl_2$) or a combination of a 1 : 1 ratio was added to the cups at different concentrations to obtain desired salinity levels. The range of levels did not exceed those which occur in agricultural soils. Three days after salinization, 400 freshly-hatched second-stage *M. incognita* juveniles from greenhouse populations were either inoculated directly or gradually preconditioned to salt-treated or untreated soil.

Direct inoculation

Freshly hatched juveniles were inoculated directly to salt-treated soil at EC_e levels of 0.0, 1.5, 2.5, 3.5, and 5.0 mmhos/cm. In these experiments either NaCl, $CaCl_2$, or combinations of the two salts were used as salinizers, and Hunts 2580 and Beefmaster tomato seedlings were used as hosts. Each salt and the combination treatment were tested in separate experiments when Beefmaster was host, and were combined in a single experiment when Hunts was the host. Treatments were replicated fifteen times. Five plants were destructively sampled 24 hr, 48 hr, and 1 wk after inoculation. Roots were washed with water and stained in boiling acid/fuschin-lactophenol for 2 min. The stained roots were washed under running water for 2-3 min to remove the excess stain, and transferred to lactophenol for destaining for at least two days. The destained roots were cut free of the root stub and spread over glass plates, in which 3-4 drops of glycerin were added, and pressed firmly to another glass plate. The plates were viewed under a dissecting microscope to determine the total number of juveniles

that had penetrated into the root system. The infectivity percentage was determined by dividing the number of juveniles in the roots by the total nematodes inoculated.

Precondition treatment

Since sudden exposure to saline soil solutions might constitute an unnatural shock to nematodes, a population of *M. incognita* juveniles calibrated at 400 per cup were gradually preconditioned in a salt gradient (i.e., 1.5, 2.5, 3.5, and 5.0 mmhos/cm) for 4 hr at every salinity level before inoculation to either treated (5.0 mmhos/cm) or untreated sandy soil. At the same time, an equal number of juveniles was exposed to distilled water for the same period and inoculated to treated and untreated soils. In these tests, three tomato cultivars were used as indicators of nematode survival : Beefmaster, Hunts 2580, and Ronita. Each treatment was replicated five times and harvested 1 week after inoculation. Roots were processed and infectivity determined as above.

DEVELOPMENTAL STUDY

Ten days after transplanting, NaCl or $CaCl_2$ were added to the soil in solution to establish an EC_e of 0, 3.5 and 5.0 mmhos/cm at equilibrium. Nematodes were inoculated as before. Soil and air temperatures were recorded for the duration of the experiments. Plants were washed from five pots every week for six weeks from each treatment and cultivar. Roots were prepared and examined as before and developmental stages of the nematodes were recorded. Time and temperature relations were expressed in heat units (hu) : each centigrade degree above 10.5 for 1 hour contributing 1 effective unit (Tyler, 1933*b*). Summation of these units provide a measure of the heat unit requirements for a given stage of life history exposed to any salt type and concentration. The stages recorded were : (*i*) second-stage juvenile (J_2) ; (*ii*) third- and fourth-stage (J_3J_4) combined because of the difficulties in differentiating them ; (*iii*) mature females (♀) ; and (*iv*) number of egg masses (E.M.) produced.

Results

INFECTIVITY

Direct inoculation

Nematode infectivity was lower on non-salt treatments of Beefmaster than those of Hunts (Tab. 1 & 2). Under present conditions, however, Beefmaster did not show strong resistance to nematode penetration. The trend of all salt treatments on Beefmaster was for lower infectivity as EC_e increased. This trend was statistically significant only in the $CaCl_2$ and combination treatments. $CaCl_2$ treatments generally showed significant reductions only at the higher salt concentrations, but combination of the two salts had a synergistic effect on penetration during the first day. Infectivity on subsequent days showed little difference between effects of $CaCl_2$ and combination salt treatments.

When the more susceptible cultivar, Hunts, was used as an indicator host, (Tab. 2), increased EC_e again tended to reduce infectivity. Salt effects were generally more pronounced early in the experiment and each type of salt as well as the combination treatment significantly reduced infectivity. Although the combination treatments gave somewhat lower infectivity after 24 hours than the other treatments, the differences were not as striking as those obtained on Beefmaster.

Precondition treatment

Penetration by unpreconditioned larvae was not suppressed in salt treated soils to levels as low as in the previous experiment. Nematodes which were gradually preconditioned to high salt levels before inoculation penetrated root systems of both varieties as well in salt-treated soils as in untreated soils. Unpreconditioned larvae penetrated roots of plants in all treatments at a significantly higher rate than did preconditioned larvae. In several treatments, unpreconditioned larvae had higher rates of penetration in soils of EC_e 5.0 mmhos/cm than in untreated soils in contrast to the previous experiment.

Table 1
Effect of different salinizers on *M. incognita* second stage juveniles infectivity to Beefmaster tomato seedlings.

	NaCl			*$CaCl_2$*			*$NaCl + CaCl_2$*		
	Day			*Day*			*Day*		
mmhos/cm	*1*	*2*	*7*	*1*	*2*	*7*	*1*	*2*	*7*
0.0	10.8a	22.4a	26.4a	12.5b	15.9b	15.8b	21.1b	22.4c	26.2c
1.5	15.2a	22.5a	24.8a	11.3b	14.8b	15.6b	0.0a	16.4b	18.8b
2.5	14.4a	21.4a	21.5a	10.3b	15.8b	18.8b	0.0a	13.5b	14.8a
3.5	12.8a	22.7a	21.0a	8.8b	7.9a	17.8b	0.0a	12.1b	11.8a
5.0	12.8a	20.5a	21.5a	4.3a	9.1a	10.7a	0.0a	3.6a	11.2a

Table 2
Effect of different salinizers on *M. incognita* second stage juveniles infectivity to Hunts 2580 tomato seedlings

	NaCl			*$CaCl_2$*			*$NaCl + CaCl_2$*		
	Day			*Day*			*Day*		
EC_e *mmhos/cm*	*1*	*2*	*7*	*1*	*2*	*7*	*1*	*2*	*7*
0.0	19.8c	26.5c	37.0b	19.8b	26.5b	37.0b	19.8c	26.5b	37.0b
1.5	13.2b	16.3b	23.1a	12.1a	18.3a	24.3a	7.5b	15.6a	21.3a
2.5	12.6b	15.6b	19.5a	9.9a	15.1a	23.3a	8.9b	17.2a	17.9a
3.5	9.8b	14.5b	21.3a	10.4a	14.1a	20.2a	6.2b	14.2a	20.2a
5.0	2.0a	9.8a	18.8a	8.3a	13.3a	19.1a	0.3a	10.3a	19.7a

Table 3
Effect of NaCl and $CaCl_2$ as salinizers on development of root-knot nematode *(Meloidogyne incognita)* on *Lycopersicon esculentum* cv. Beefmaster

Physiological time of exposure (hu > 10.5 °C)	*EC_e (mmhos/cm)*	*Stage of Development* [A] *J2*	*J3/J4*	♀	*E.M.*	*Stage of Development* [B] *J2*	*J3/J4*	♀	*E.M.*
	0	47b	—	—	—	48b	—	—	—
2 092	3.5	49b	—	—	—	67b	—	—	—
	5.0	14a	—	—	—	20a	—	—	—
	0	4a	198b	—	—	—	199b	—	—
4 000	3.5	1a	118a	—	—	4	113a		
	5.0	3a	121a	—	—	2	101a	—	—
	0	—	60a	137b	—	—	15a	60a	—
6 380	3.5	—	40a	67a	—	—	49b	56a	—
	5.0	—	85b	55a	—	—	42b	68a	—
	0	—	—	194b	26a	—	—	202c	29a
8 060	3.5	—	—	145a	50a	—	2	144b	35a
	5.0	—	11	138a	38a	—		112a	11a
10 268	0	3	4a	200a	142c	10a	1	182b	104b
	3.5	—	7a	182a	60b	2a	—	186b	41a
	5.0	—	8a	175a	39a	2a	5	137a	37a
13 054	0	309b	21	192a	156c	381	16	183a	173b
	3.5	42a	—	172a	60b	144	8	184a	60a
	5.0	22a	11	165a	39a	—	—	150a	56a

[A] = NaCl as salinizer
[B] = $CaCl_2$ as salinizer

Table 4
Effect of NaCl and $CaCl_2$ as salinizers on development of root-knot nematode *(M. incognita)* on *Lycopersicon esculentum* cv. Hunts 2580

Physiological time of exposure (hu > 10.5 °C)	EC_e *(mmhos/cm)*	*Stage of Development* (1) *J2*	*J3/J4*	♀	*E.M.*	*Stage of Development* (2) *J2*	*J3/J4*	♀	*E.M.*
2 092	0.0	105b	—	—	—	106b	—	—	—
	3.5	75ab	—	—	—	64a	—	—	—
	5.0	70a	—	—	—	55a	—	—	—
4 000	0.0	5ab	240c	—	—	1a	244c	—	—
	3.5	10a	202b	—	—	11b	77b	—	—
	5.0	16b	110a	—	—	10b	45a	—	—
6 380	0.0	—	27a	96b	—	—	29a	110b	—
	3.5	—	118b	102b	—	—	10a	73a	—
	5.0	3	140b	25a	—	—	50b	74a	—
	0.0	—	—	209c	122c	—	—	149b	78b
8 060	3.5	—	—	134b	63b	—	1	88a	14a
	5.0	—	—	89a	10a	—	21	85a	8a
10 268	0.0	6	—	250c	49b	8	—	216b	50b
	3.5	—	4	194b	62b	—	—	98a	20a
	5.0	—	—	153a	20a	—	—	86a	10a
13 054	0.0	690c	52	250c	110b	1077b	69b	226b	115b
	3.5	250b	—	201b	80ab	237a	8a	109a	22a
	5.0	153a	—	155a	69a	218a	1a	104a	40a

(1) = NaCl as salinizer
(2) = $CaCl_2$ as salinizer

DEVELOPMENT

Larval penetration of roots of both cultivars showed the same trends of suppression at high salt concentrations as in the direct penetration experiment (Tab. 3 & 4). Development up to and including the fourth stage (J_4) required at least 4 000 hu. Total numbers of J_3/J_4 were significantly depressed by the two salt types and concentrations on Hunts, while on Beefmaster, development was merely delayed. A similar trend was noted with development of mature females. At 6 380 hu most salt treated plants had significantly fewer mature females than control plants. This trend remained throughout the experiment on Hunts (Tab. 4), but differences between treatments gradually became non-significant on Beefmaster (Tab. 1). Production of egg masses was depressed by increased salinity on both cultivars, although the differences were not significant on Beefmaster until 10 268 hu. Reflecting this trend, initial penetration by second generation larvae (13 054 hu) was significantly depressed in salt treated plants.

Discussion

The characteristics of the nematode which increase its chances of invasion are important because of the limited time available for infection. Wallace (1966*b*) suggested that newly hatched juveniles remain active for a limited time of about 4-8 days. We found in all cultivars tested, that infectivity was maximum by the 7th day after inoculation. Wallace (1966*a*, *b*) considered that the probability that juveniles will invade a root depends on many factors : (*i*) the presence of a host root ; (*ii*) the proximity of the root ; (*iii*) the speed of the nematode ; (*iv*) the tortuosity of the track the nematode takes in reaching the root ; and (*v*) the speed of penetration. The presence of highly susceptible hosts increases the chance of the nematode to invade the roots. This was true when Hunts 2 580 was used as an indicator. The soil environment around a host root is also very important in determining the fate of nematodes. Juvenile penetration was affected by the concentration and perhaps the type of salt in the soil solutions. Prot (1978*a*, *b* ; 1979*a*) observed

that juvenile root-knot nematodes migrate toward lower salt concentrations regardless of the presence of a host. Present findings do not indicate whether high salt concentrations interfere with nematode sensory perception or reduce its ability to penetrate by other mechanisms. Depletion of body contents in juveniles occurred when exposed to gradual increases in salts, possibly accounting for lower infectivity due to reduced metabolic activity and movement in the soil, which could inhibit the search for infection sites.

At higher salt concentration, calcium translocation in the xylem is reduced, which in turn, may depress the growth of meristematic tissues (Mengel & Kirkby, 1978). This could lead to fewer roots available for infection. Mengel and Kirkby (1978) also reported that NaCl salinity affects the incorporation of inorganic N and thus depresses protein synthesis in young plants. This leads to growth inhibition in young root meristematic tissues, which was observed in Hunts, but not Beefmaster. However, since preconditioned juveniles displayed markedly reduced peuetration of roots in untreated soil, it is unlikely that direct alteration of plants or plant attractants by high salt levels is a sole cause of reduced penetration.

It is unknown whether salt-adapted populations evolve in saline field situations, however, simple preconditioning treatments had the effect of reducing and even reversing penetration trends noted in direct inoculation experiments. Such results may indicate that sudden osmotic shock caused reduced penetration in the direct inoculation series. These data suggest that reduced penetration may not occur in response to salinity in naturally conditioned field populations. However, reduced penetration in response to salinity was noted in the direct inoculation studies and the development studies, while in the preconditioning series even non-preconditioned larvae exhibited greatly reduced penetration in all treatments. For this reason, it is unclear whether unidentified variables confouuded the results of preconditioning on penetration.

Alteration in plant growth due to excess salinity such as those cited above are more likely to influence development of established parasites. Tyler (1933*a*) and others have suggested that a healthy condition of the host plant is important for the development of its parasites. Greater numbers of J_3, J_4 and adult parasites were observed than infective J_2 stages. This is due to difficulty in observing the slender J_2 stage and perhaps suggests that infectivity tests can be delayed until entrant larvae undergo further development and become more easily visible. The influences of salts on nematode development appeared more pronounced on Hunts than Beefmaster. Development to mature females in Beefmaster was delayed by salt treatments, but by 13 054 hu there were no significant differences between treatments. Numbers of egg masses at 13 054 hu remained significantly different from control plants, although newly mature females in salt treated plants may have been capable of further egg production. Whether development is merely delayed or permanently impaired on these cultivars, plants will be subjected to fewer total nematodes and populations at the end of the season should be reduced. Such findings have implications in terms of development of plant damage functions and nematode population models for plants grown under high salinity conditions. Areas of related interest are plant growth responses to salinity which may interact with nematode pathogenesis, and the ability of nematode eggs and larvae to survive in the absence of hosts in saline soils.

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