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EFFECT OF SALINITY AND TEMPERATURE ON REPRODUCTION AND EGG HATCHING OF *MELOIDOGYNE INCOGNITA* IN TOMATO

by

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Temperature is an important factor in determining the geographical distribution of root-knot nematodes (*Meloidogyne* spp.), perhaps more so than plants or soil (De Guiran and Ritter, 1979; Ferris and Van Gundy, 1979). Salinity is a problem in some marginal agricultural land but has not been investigated on plant parasitic nematode reproduction, egg hatching and invasion of roots. This work examines the effect of salinity (salt type and concentration) on rate of reproduction of *Meloidogyne incognita* (Kofoid *et* White) Chitw. under different temperatures; and the effect of salinity on egg viability, egg hatch and infectivity of juveniles.

Materials and Methods

A tomato cultivar, Hunts 2580, susceptible to root-knot nematode (*M. incognita*) was used in these studies. Thirty-five-day-old tomato seedlings were transplanted into 2 litres plastic containers filled with blow sand (78% sand, 14% silt and 8% clay). The containers were immersed in water in temperature tanks up to the soil line, and maintained at 25, 30 or 35 °C (Ferris *et al.*, 1955). The seedlings were grown for two weeks before the following salts and concentrations were added: (i) sodium chloride (NaCl) solution at conductivity of 0, 3.5 and 5.0 mmhos/cm; (ii) calcium chloride ($CaCl_2$) solution at conductivity of 0, 3.5 and 5.0 mmhos/cm; (iii) combination of so-

dium chloride and calcium chloride at conductivity of 0, 3.5 and 5.0 mmhos/cm. Ten days after salinization, 800 freshly-hatched juveniles of *M. incognita* were added to each container by inoculating them around the root zone deep in the pot. Each treatment was replicated 16 times for every salt concentration and temperature.

A sample of four containers was taken from every salt type, concentration and temperature, 7, 14, 21 and 28 days after inoculation. Roots taken after seven days were processed (Edongali and Ferris, 1980). The number of mature egg-laying females in the stained roots were counted. The number of eggs per female was determined by dividing the total eggs obtained by the number of females.

To study the effect of salts on egg hatching, egg masses were hand-picked from plant roots grown in CaCl-treated and untreated soil at different temperature regimes. The effect of salt types and concentration on egg hatching was studied artificially using miniature sieves constructed from Nepon tubes (2 cm diameter, cut to 0.8 cm lengths) and 100 μm copper mesh glued between the two sections. The treatments were $CaCl_2$ and NaCl at conductivities of 0, 3.5 and 5.0 mmhos/cm. These were repeated for each temperature (25 °C, 30 °C and 35 °C). Each treatment was replicated four times, with each replicate containing five egg masses. Each sieve was placed in a 3.5 cm diameter plastic petri plate and salt solutions and water control were pipetted into the plates until the solution was slightly above the level of the mesh, and completely immersed the egg masses. The petri plates were covered and kept at 25 ± 2 °C. Egg hatch was observed at 7-day intervals for 21 days. The sieves and egg masses were transferred into fresh salt solutions or distilled water every week. The hatched juveniles were inoculated to Hunts 2580 to test for infectivity.

Results

Reproduction of *M. incognita* was affected by temperature variation and salinity. At the lowest temperature (25 °C), no egg production was observed in either NaCl, $CaCl_2$ or combinations (Table I), two weeks (4,827 heat units) after inoculation. Few females had matured at any salt concentrations. Three weeks (7,308 heat units) later, more females had developed and egg production had started.

Table I - *Effect of salinity and temperature (25 °C) on reproduction of* M. incognita *in Hunts 2580 tomato.*

Treatment (EC_e mmhos cm)	Sampling time (weeks)								
	2 weeks			3 weeks			4 weeks		
	Total No.			Total No.			Total No.		
	♀	Eggs	Eggs ♀	♀	Eggs	Eggs ♀	♀	Eggs	Eggs ♀
NaCl	(1)								
Control	25 a	—	—	270 b	20,336 b	75 a	288 b	370,700 b	1,287 a
3.5	18 a	—	—	191 a	17,600 a	92 a	190 a	321,000 a	1,689 a
5.0	— *	—	—	—	—	—	—	—	—
$CaCl_2$									
Control	45 c	—		404 b	19,505 b	48 b	439 b	300,310 b	684 b
3.5	23 b	—		166 a	2,690 a	16 a	207 a	19,531 a	94 a
5.0	12 a	—		118 a	1,380 a	11 a	185 a	12,680 a	68 a
Combinations									
Control	41 b	—		349 c	20,280 c	58 b	387 b	281,840 c	728 b
3.5	10 a	—		230 b	9,600 b	41 ab	236 a	41,610 b	176 a
5.0	9 a	—		216 a	5,680 a	26 a	224 a	28,681 a	128 a

* Plants died.

(1) In each column within a treatment, averages sharing the same letter do not differ significantly (P = 0.05).

There was no significant difference between the numbers of females in the treatments and control when NaCl was used as the salinizer. The $CaCl_2$ treatments had significantly lower numbers of females than the untreated control as did the combination treatments. Maximum egg production was obtained after 9,744 heat units had accumulated.

At the intermediate temperature (30 °C), females developed in salt treatments after accumulation of 6,552 heat units, but there were no significant differences between the treatments (Table II). No egg production was observed at that period (6,552 heat units). Production of eggs started at all salt treatments after accumulation of 9,828 heat units. Numbers of eggs per female were significantly higher in the control treatments than in treated soils. After 13,104 heat units (4 weeks), egg production per female was significantly higher in the control treatment than in salt treatments.

Two weeks (8,232 heat units) after inoculation at 35 °C, many females were observed but there were few eggs (Table III). Egg production increased with time and decreased at higher salt concentrations regardless of the type of salt. The highest egg production was after 16,464 heat units (4 weeks), when a new generation of juveniles was observed penetrating the root system.

Hatching of egg masses obtained from plants grown at different temperatures (25, 30 and 35 °C) decreased with time and increased as the salt concentration decreased (Tables IV, V and VI).

Hatching of egg masses was higher on plants grown at 30 °C (Table V) than at the other temperatures tested. The lowest hatch was at an EC_e of 0.5 mmhos/cm with both salts at all temperatures tested. Infectivity of juveniles from tomato seedlings decreased with time. A higher hatch, and lower infectivity was obtained in the control. Presence of salt slowed hatching and resulted in higher infectivity. Maximum infectivity was obtained by NaCl treated juveniles from all egg sources i.e. eggs produced in plants grown at the three temperatures.

Conclusions

Godfrey and Oliveira (1932) found that the time from initial inoculation to the first egg development was 19 days in cowpea at 27 °C. Few females were found producing eggs after two weeks at

Table II - *Effect of salinity and temperature (30 °C) on reproduction of* M. incognita *in Hunts 2580 tomato.*

Treatment (EC_e mmhos·cm)	Sampling time (weeks)								
	2 weeks			3 weeks			4 weeks		
	Total No.			Total No.			Total No.		
	♀	Eggs	Eggs ♀	♀	Eggs	Eggs ♀	♀	Eggs	Eggs ♀
NaCl	(1)								
Control	57 a	—	—	189 a	73,930 a	391 a	175 a	230,720 a	1,318 a
3.5	40 a	—	—	230 b	103,800 b	473 b	170 a	349,720 b	2,057 b
5.0	—*	—	—	—	—	—	—	—	—
$CaCl_2$									
Control	50 a	—	—	179 a	40,890 c	228 c	277 b	284,900 b	1,067 b
3.5	40 a	—	—	192 a	29,810 b	155 b	221 ab	145,800 a	660 a
5.0	28 a	—	—	193 a	11,700 a	60 a	182 a	130,200 a	715 a
Combinations									
Control	50 a	—	—	291 b	29,800 b	102 a	206 b	273,500 b	861 b
3.5	47 a	—	—	180 a	14,700 a	81 a	176 a	133,860 a	761 ab
5.0	31 a	—	—	150 a	11,900 a	79 a	191 a	123,790 a	648 a

* Plants died.

(1) In each column within a treatment, averages sharing the same letter do not differ significantly (P = 0.05).

Table III - *Effect of salinity and temperature (35 °C) on reproduction of* M. incognita *in Hunts 2580 tomato.*

Treatment (EC_e mmhos/cm)	Sampling time (weeks)								
	2 weeks			3 weeks			4 weeks		
	Total No.			Total No.			Total No.		
	♀	Eggs	Eggs/♀	♀	Eggs	Eggs/♀	♀	Eggs	Eggs/♀
NaCl	(1)								
Control	85 b	48 a	0.6 a	220 a	50,400 a	229 a	438 b	135,000 b	308 b
3.5	48 a	43 a	0.9 a	210 a	59,520 b	283 a	230 a	34,000 a	148 a
5.0	— *	—	—	—	—	—	—	—	—
$CaCl_2$									
Control	92 a	10 a	0.1 a	208 a	49,100 a	236 b	267 a	100,960 c	378 b
3.5	70 a	9 a	0.1 a	327 b	53,800 a	164 a	363 b	90,000 b	248 a
5.0	81 a	8 a	0.1 a	195 a	76,800 b	137 a	240 a	58,940 a	246 a
Combinations									
Control	92 ab	8 a	0.3 a	208 b	38,400 b	185 b	492 b	83,000 b	169 a
3.5	72 a	5 a	0.1 a	152 a	18,900 a	124 a	240 a	39,000 a	163 a
5.0	110 b	8 a	0.1 a	132 a	20,600 a	150 ab	296 a	40,400 a	137 a

* Plants died.

(1) In each column within a treatment, averages sharing the same letter do not differ significantly (P = 0.05).

Table IV - *Effect of salt treatments on egg hatching and juvenile infectivity to Hunts 2580 tomato seedlings with egg masses obtained from tomato plants grown in NaCl treated soil at 25 °C.*

Treatment (mmhos/cm)	No. juveniles hatched (weeks)			% infectivity (weeks)		
	1	2	3	1	2	3
NaCl	(1)					
3.5	400 b	643 d	164 c	25 b	7 ab	12 c
5.0	561 c	259 b	115 b	24 b	10 b	9 bc
$CaCl_2$						
3.5	705 d	475 c	154 c	25 b	9 bc	5 b
5.0	122 a	55 a	123 b	16 a	5 ab	7 b
Control	1422 e	1395 e	43 a	15 a	3 a	1.2 a

(1) In each column averages sharing the same letter, do not differ significantly (P = 0.05).

Table V - *Effect of salt treatments on egg hatching and juvenile infectivity of Hunts 2580 tomato seedlings with egg masses obtained from tomato plants grown in NaCl treated soil at 30 °C.*

Treatment (mmhos/cm)	No. juveniles hatched (weeks)			% infectivity (weeks)		
	1	2	3	1	2	3
NaCl	(1)					
3.5	202 b	606 d	130 c	35 c	10 a	19 b
5.0	116 a	106 b	92 b	13 a	8 a	17 b
$CaCl_2$						
3.5	282 b	385 c	58 a	26 b	33 b	7 a
5.0	126 a	61 a	63 a	13 a	5 a	13 ab
Control	1063 c	856 d	66 a	22 b	7.6 a	2.5 a

(1) In each column averages sharing the same letter, do not differ significantly (P = 0.05).

Table VI - *Effect of salinity on egg hatching and infectivity of* M. incognita *to Hunts 2580 tomato seedlings, with egg masses obtained from tomato plants in NaCl treated soil at 35 °C.*

Treatment (mmhos/cm)	No. juveniles hatched (weeks)			% infectivity (weeks)		
	1	2	3	1	2	3
NaCl	(1)					
3.5	120 b	510 c	90 b	40 b	25 b	0.3 a
5.0	150 b	100 a	92 b	10 a	8 a	0.03 a
$CaCl_2$						
3.5	66 a	130 a	40 a	12 a	10 a	0.0 a
5.0	50 a	210 b	36 a	6 a	8 a	0.1 a
Control	500 c	560 c	80 b	37 b	20 b	4.0 b

(1) In each column averages sharing the same letter, do not differ significantly ($P = 0.05$).

a higher temperature (35 °C). Tyler (1933) stated that the minimum time required for the life cycle of the root-knot nematode from juvenile to juveniles in tomato was 25 days at 27 °C. In the studies reported (Tables I, II and III) at least 21 days were required before any egg production was observed. The effect of salinity and temperature induced stress in the plant growth, resulting in less root growth. At 35 °C roots were small and confined to the top soil region of the pots.

The optimum temperature for plant growth is also optimum for the parasite. The plants grew better at 30 °C than 35 °C or 25 °C, which is in agreement with Van Gundy (1976). The overall total egg production was higher at 30 °C than any other temperature tested. Salts restricted plant growth at a higher temperature (35 °C).

Juveniles can exist longer than eggs in soils without a host at a lower moisture (Wallace, 1966). Dropkin *et al.* (1958) found that an increase in osmotic pressure of soil solution results in reduction of egg hatch. In these studies, egg hatch decreased as salinity increased, and declined with time. Infectivity of the hatched juveniles showed the same trend, indicating that the survival of hatched larvae when exposed to salt is very limited even in the presence of a host. The source of eggs is very important. Eggs produced in plants grown

at high temperatures might be less viable due to lower hatch. At 35 °C few eggs were hatched, and infectivity declined.

In conclusion, the optimum temperature for *Meloidogyne incognita* reproduction is about 30 °C. Presence of salinity in the soil slows development and reduces the number of eggs produced per female. Eggs produced by females in plants grown at 30 °C produced more juveniles that penetrated plants better than from eggs produced at 25 or 35 °C. A more detailed study on the rate of reproduction in salty marginal lands is necessary for better understanding of the impact of these conditions on survival of the nematodes under field conditions.

SUMMARY

Seedlings of the tomato cultivar Hunts 2580, susceptible to root-knot nematode (*Meloidogyne incognita*), were transplanted into 2 l plastic containers filled with blow sand which then were immersed in water in temperature tanks maintained at 25, 30 or 35 °C. Salt solutions of either NaCl, $CaCl_2$ or combination, were added two weeks after transplanting to produce a conductivity of 0, 3.5 and 5.0 mmhos/cm. Eight-hundred juveniles were added ten days after transplanting to each pot. A sample of four containers were taken 7, 14, 21, and 28 days after inoculation. The roots were stained to determine the number of females and egg production. Egg hatching was studied using miniature sieves constructed from Nepon tubes. The egg masses were placed in the sieves and immersed in NaCl, or $CaCl_2$ solutions at conductivity of 3.5 and 5.0 mmhos/cm. Tomatoes grew better at 30 °C than any other temperature tested and this was also the optimum temperature for the parasite. Salts restricted plant growth at higher temperature (35 °C), where roots are small, and confined to the top soil region of the pots. Egg hatching decreased as salinity increased and declined with time. Infectivity of the hatched juveniles showed the same trend, indicating that survival of hatched juveniles when exposed to salt is very limited even in the presence of a host. Eggs produced in plants grown at high temperatures might be less viable due to lower hatch; at the highest temperature (35 °C), less eggs were hatched, and infectivity declined.

LITERATURE CITED

De Guiran G. and Ritter M., 1979. Life cycle of *Meloidogyne* species and factors influencing their development. *In* Root-knot Nematodes (*Meloidogyne* species) Systematics, Biology and Control. F. Lamberti and C. E. Taylor Eds. Academic Press, London, New York, San Francisco. pp. 173-191.

Dropkin V. H., Martin G. C. and Johnson R. W., 1958. Effect of osmotic concentration on hatching of some plant parasitic nematodes. *Nematologica*, *3*: 115-126.

Edongali E. A. and Ferris H., 1980. Interaction of salinity and root-knot nematode (*Meloidogyne incognita*) on tomato (*Lycopersicon esculentum* Mill.). Ph. D. Thesis. UCR. 124 pp.

FERRIS J. M., LEAR B., DIMOCK W. and MAI W. F., 1955. A description of Cornell temperature tanks. *Plant. Dis. Reptr., 39*: 875-878.

FERRIS H. and VAN GUNDY S. D., 1979. *Meloidogyne* ecology and host interrelationships. *In* Root-knot Nematodes (*Meloidogyne* species) Systematics, Biology and Control. F. Lamberti and C. E. Taylor Eds. Academic Press, London, New York, San Francisco. pp. 205-230.

GODFREY G. H. and OLIVEIRA J., 1932. The development of the root-knot nematode in relation to root tissues of pineapple and cowpea. *Phytopathology, 22*: 325-348.

TYLER J., 1933. Development of root-knot nematodes as affected by temperature. *Hilgardia, 7*: 392-415.

VAN GUNDY S. D., 1976. Ecology of root-knot nematodes. Proc. Research Planning Conference on Root-knot Nematodes, *Meloidogyne* spp. IMP, Raleigh, N. Carolina, 12-16 Jan. 1976; pp. 64-73, Univ. N. Carolina.

WALLACE H. R., 1966. Influence of moisture stress on the development, hatch and survival of eggs of *Meloidogyne javanica*. *Nematologica, 12*: 57-69.

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