Nematol. medit. (1998), 26: 189-194

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, Andhra Pradesh, India

COMPARISON OF TWO POPULATIONS OF *MELOIDOGYNE JAVANICA* BASED ON MORPHOLOGY, BIOLOGY AND PATHOGENICITY TO GROUNDNUT [1]

by

M. SREE LATHA, S. B. SHARMA, T. B. GOUR and D. D. R. REDDY

Summary. Two populations of *Meloidogyne javanica* race 3 were isolated from groundnut plants collected from Pallipalem (Prakasan district) and Tirupati (Chittoor district) areas of Andhra Pradesh, India. The populations were characterized on the basis of morphology and morphometrics of females, males, and second-stage juveniles, morphology, life cycle, egg-hatch, and pathogenicity on two groundnut cultivars, JL 24 and Robut 33-1. Differences in morphology and morphometrics between the two populations were minor and it was not easy to differentiate them. Both populations had three esterase bands typical of *M. javanica*. The life cycles of both the populations were completed in 24 days at an average ambient temperature of 26.5 °C. Thermal optimum for egg-hatch was 25-27 °C for the Pallipalem population, and 25-30 °C for the Tirupati population. The Pallipalem population reduced the dry shoot mass and shoot length of JL 24 at initial population densities of 1 and 10 eggs/cm^3 soil, respectively. The Tirupati population reduced the dry shoot mass of JL 24 only at an initial population density of 10 eggs/cm^3 soil and it did not cause any reduction in shoot length. The Pallipalem population produced greater number of galls and eggsacs on both the cultivars when compared with the Tirupati population and it was more virulent than the Tirupati population particularly on JL 24.

The root-knot nematode *Meloidogyne javanica* (Treub) Chitw. is an important pest of agricultural crops in the tropics. It is very widespread and polyphagous. Three races of *M. javanica* have been designated based on pathogenicity to pepper and groundnut (Sharma *et al.*, 1995). Race 1 is widespread and it does not attack pepper and groundnut, race 2 is present in Italy and Morocco and it is pathogenic to pepper but not to groundnut, race 3 is reported from Brazil, Zimbabwe, Egypt, India, and USA and it is pathogenic to groundnut but not to pepper. Populations of *M. javanica* that can parasitize both pepper and groundnut (race 4?) have not been reported (Sharma *et al.*, 1995). The frequency of occurrence of *M. javanica* on groundnut is gradually increasing (Prasad *et al.*, 1964; Ibrahim and El-Saedy, 1976; Sakhuja and Sethi, 1985; Patel *et al.*, 1988; Tomaszeweski *et al.*, 1994). Our preliminary studies have shown that variability among field populations of race 3 occurs in India. The differences in pathogenic variability may alter the nematode management strategy and highly virulent populations would require a different management protocol to that for the avirulent population. The objective of this investigation was to study and characterize two populations of *M. javanica* race 3 (Sharma

[1] Approved as ICRISAT JA No. 2245.

et al., 1995) based on morphology, morphometrics, esterase phenotypes, biology and pathogenicity on two groundnut cultivars.

Materials and methods

The two populations were collected from infected groundnut plants in the Pallipalem (district Prakasam) and Tirupati (district Chittoor) areas of Andhra Pradesh, India. The nematode populations were reared on groundnut (*Arachis hypogaea* L.) cv. Robut 33-1 in 25-cm diam plastic pots containing an autoclaved mixture of sand and black cotton soil (3:1) in a glasshouse at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) Center, Patancheru, India. Populations of different life stages (second-stage juveniles, males, females) for different experiments were obtained from these plants.

Juveniles and males were extracted from 100 cm^3 soil samples by suspending them in water, passing them through nested sieves 20 mesh (850 µm-pore), 80 mesh (180 µm-pore) and 400 mesh (38 µm-pore), and placing the residue from the 38 µm-pore sieve on a modified Baermann funnel. Females were obtained from the infected roots of Robut 33-1. Morphology (female and male stylet morphologies; perineal pattern shape; head morphology of males; head, stylet and tail morphology of juveniles) and morphometrics (body length, stylet length and opening of the dorsal gland duct of females, males and juveniles; body width, vulva-anus distance and vulval slit length of females; tail length of juveniles) of 20 specimens each of females, second-stage juveniles and males of both the populations were studied under high resolution BH 2 Olympus light microscope. The females were mounted on glass slides and perineal patterns were cut and mounted in lactophenol. Males and juveniles were fixed in 2% formalin and then transferred to hot lactophenol. After 5 min, the specimens were mounted in glycerine on glass slides and studied under the microscope.

Twenty fully developed females of each population were picked, ten in each Eppendorf, and macerated in extraction buffer (pH 7.4) with 20% glycerol, 2% triton X-100, and 0.1% bromophenol blue dye. Electrophoresis of macerates of females was accomplished with an automated apparatus (Pharmacia LKB. Multiphor II) on 10% polyacrylamide gels. Esterase phenotypes were determined by staining polyacrylamide gels for esterase activity.

Two seeds of groundnut cv. JL 24 were planted in each of 60 (10-cm diam) plastic pots filled with sand plus black soil (3:1). Five-day-old plants in 30 pots were inoculated with the Pallipalem population and another 30 with the Tirupati population. Each pot was inoculated with 1000 second-stage juveniles in shallow depressions around the roots. Two plants for each population were harvested after every 24 h for one week and later after every 48 h. The roots were stained and spread on glass plates (22 cm x 9.5 cm) to observe nematode invasion and development. The nematodes inside the roots were separated out of the root tissues and observed with a high resolution microscope at x 1000 magnification to confirm the stage of nematode development.

Fresh and fully formed eggsacs of either the Pallipalem or the Tirupati population were collected from the roots of groundnut cv. Robut 33-1. Petri dishes each containing five eggsacs in water were incubated at 15, 20, 25 and 30 °C in incubators and at room temperature (24-30 °C) with six replications. The hatched juveniles were counted at 1-day intervals for eight days and then at 2-day intervals until there was no further juvenile emergence. The number of unhatched eggs in each Petri dish was counted at the end of the experiment and cumulative percentage juvenile hatch was calculated. Data were arcsin transformed for statistical analysis.

Pathogenicity was studied on two cultivars, JL 24 and Robut 33-1. Seeds of each groundnut

cultivar were sown in 1000 cm^3 steam sterilized sand soil mixture (3:1) in 15-cm diam plastic pots. For inoculum preparation, eggs of both populations were collected from infected groundnut roots using 0.25% solution of sodium hypochlorite. Each cultivar was inoculated with five inoculum levels (0, 1000, 5000, 10,000, 20,000 eggs/pot). Each inoculum level was replicated ten-times for the Pallipalem population and six-times for the Tirupati population. All the pots were placed on glasshouse benches in a randomized block design. The nematode inoculum was added to the soil together with the seed. Pots were irrigated every alternate day and nutrient solution was added once a week. Data on shoot length, dry shoot mass, and nematode-caused gall number, gall size, % root galling, and eggsacs were assessed 60 days after inoculation. Roots were treated with 0.25% trypan blue to stain the eggsacs using the technique of Sharma and Mohiuddin (1993). Roots were rated on a 1-9 scale for gall index (GI), gall size (GS), percent galled area (% GA) and eggsac index (EI). A damage index (DI) was calculated by dividing the sum of GI, GS and %GA by three (Sharma *et al.*, 1993). Significant differences in nematode and plant parameters were determined using the analysis of variance technique.

Results and conclusions

No major morphological and morphometric differences were found between the Pallipalem and Tirupati populations. Morphometrics of females, males, and second-stage juveniles were within the range as previously reported for *M. javanica* (Eisenback *et al.*, 1981). The females of the Pallipalem population were slightly bigger than the females of the Tirupati population (Table I). Females of both the populations had a conus of the stylet that was longer than the shaft, that was pointed and tapered gradually towards the tip and broadening at the junction with the shaft. The coefficient of variation for characters studied was generally much greater in females of the Tirupati population than in females of the Pallipalem population. Intrapopulation variation in males of both populations for body length and the dorsal oesophageal gland opening was greater than that for stylet length. All morphometric characters of second-stage juveniles of both populations showed low variability, with coefficient of variation less than 10 (Table I). Both populations had three esterase bands and were phenotypically identical to *M. javanica*.

The two populations were identical in the time taken to complete their life cycles and had similar growth and developmental patterns in the roots. The second-stage juveniles of both populations started invading the roots of JL 24 within 24 h of inoculation and maximum numbers within the root were observed two days after inoculation. The juveniles gradually started increasing in width and swollen nematodes were observed six days after inoculation. Third and fourth stage juveniles and young females were observed 9, 10 and 12 days after inoculation, respectively. Mature females were observed after 16 days and eggsacs with eggs were observed after 19 days. The next generation of second-stage juveniles was observed 23 days after inoculation. The life cycles of both populations were completed in 24 days at an ambient average temperature of 26.5 °C.

The egg-hatch of the Pallipalem population was quicker and greater at 25 °C than at other temperatures, except the room temperature (27 °C); egg-hatch was lowest at 15 °C. About 98% of the total egg-hatch occurred in 12 days at 25, 30, and room temperature. The cumulative percentage hatch (arcsin transformed) in 28 days was 62% at 15 °C, 73% at 20 °C, 80% at 25 °C, 69% at 30 °C and 74% at room temperature. With the Tirupati population it was quicker at 30 °C; however, cumulative percentage hatch at 30 °C was not different ($P=0.05$) from that at 25 °C and at room temperature (Table II). Initially,

TABLE I - *Morphometric comparison of two populations of* Meloidogyne javanica.

Character	Pallipalem population Mean ± SE (Range)	CV	Tirupati population Mean ± SE (Range)	CV
Female (n=20)				
Body length	757.7±12.20 (678-849.7)	7.2	713.6±23.30 (544.9-910.5)	14.6
Body width	566.8±14.10 (470.5-678.4)	11.1	518.0±26.67 (338.7-729.8)	23.0
Stylet length	15.9±0.20 (14.1-17.2)	5.6	15.8±0.20 (14.5-17.5)	5.7
DGO	3.1±0.14 (2.0-4.1)	20.2	3.0±0.15 (1.9-4.1)	22.4
Vulva-anus distance	16.2±0.56 (12.0-19.9)	15.4	16.3±0.56 (12.2-20.0)	15.4
Vulval slit length	23.3±0.70 (18.7-31.2)	13.4	24.4±0.84 (18.1-31.2)	15.4
Male (n=20)				
Body length	1917.9±50.73 (1420.6-2295.0)	11.8	1831.7±43.84 (1429.4-2190.0)	10.7
Stylet length	19.6±0.27 (17.0-21.0)	6.2	19.7±0.23 (18.0-21.0)	5.2
DGO	2.9±0.09 (2.2-3.6)	13.9	2.9±0.09 (2.2-3.6)	13.9
Second-stage juvenile (n=20)				
Body length	458.4±6.80 (402.0-515.0)	6.6	451.5±5.64 (420.0-477.5)	5.6
Stylet length	15.0±0.11 (14.0-16.0)	3.3	15.0±0.14 (14.1-16.0)	4.2
DGO	3.3±0.06 (3.0-4.0)	8.1	3.5±0.06 (3.1-4.0)	7.7
Tail length	55.3±0.83 (50.5-61.5)	6.7	54.1±0.64 (51.2-61.2)	5.3

All measurements are in µm. DGO = Dorsal oesophageal gland opening.

more than 50% eggs hatched within six days at 30 °C and room temperature whereas egg-hatch at 25 °C accelerated at nine days after incubation. Egg-hatch at 15 °C was slow and gradual and continued until the end of the experiment. The cumulative percentage hatch (arcsin transformed) in 28 days was 75% at 15 °C, 63% at 20 °C, 84% at 25 °C, 86% at 30 °C, and 83% at room temperature. The Tirupati population was apparently more flexible in thermal limits for egg-hatch than the Pallipalem population. It seems that the Tirupati population prefers higher temperatures than the Pallipalem population.

The Pallipalem population reduced the dry

shoot mass and shoot length of JL 24 at inoculum levels of 1 and 10 eggs/cm^3 soil, respectively (Table III). The Tirupati population reduced the dry shoot mass of JL 24 at the inoculum level of 10 eggs/cm^3 soil; there was no adverse effect on shoot length (Table III). Plant growth of Robut 33-1 was not affected by either of the populations even at an inoculum level of 20 eggs/cm^3 soil. The number of galls produced by both populations increased with increase in inoculum levels on both cultivars. However, the number of galls produced by the Tirupati population at an inoculum level of 20 eggs/cm^3 soil was even less than that produced by the Pallipalem population at an inoculum level of 5 eggs/cm^3 soil. The Tirupati population induced very small galls on both cultivars, regardless of inoculum levels. The Pallipalem population induced medium to large galls at inoculum levels greater than 5 eggs/cm^3 soil. Reproduction (based on eggsac numbers) of the Tirupati population was also low on both cultivars in comparison with that of the Pallipalem population. The Pallipalem population was more virulent than the Tirupati population on groundnut.

Natural diversity in pathogenicity to groundnut, and thermal requirement for egg-hatch were evident in the two morphologically-similar populations of *M. javanica* race 3. The differences in pathogenicity were marked and the Tirupati population was less-virulent than the Pallipalem population. A groundnut cultivar (Robut 33-1) was tolerant to both the populations. Sakhuja and Sethi (1985) found that the *M. javanica* population in parts of Punjab in northern India was

TABLE II - *Egg batch of two* M. javanica *populations at five incubation temperatures.*

Population	Temperature °C					LSD (P=0.05)
	15	20	25	30	RT	
Pallipalem	62.2 (77.4)	72.6 (90.9)	79.6 (96.6)	68.5 (85.6)	73.8 (92.1)	6.05
Tirupati	75.1 (92.7)	62.5 (77.8)	84.4 (98.9)	86.0 (99.5)	82.9 (98.4)	5.60

Figures in parenthesis are cumulative percent hatch and out with the parentheses are arcsin transformed values. The average room temperature (RT) was 27 °C (24-30 °C).

TABLE III - *Pathogenicity of Pallipalem and Tirupati populations of* M. javanica *on cv. JL 24.*

M. javanica eggs/cm soil	Shoot length (cm)	Dry shoot mass (g)	Gall index	Gall size	% Galled area	Eggsac index	Damage index
Pallipalem							
0	7.9	2.6	0	0	0	0	0
20	5.2*	1.1*	9	7	9	9	8
Tirupati							
0	7.7	1.9	0	0	0	0	0
20	7.3	1.4*	8	3	7	9	6

* Significant at 5% level. Cultivar Robut 33-1 was tolerant to both the populations and no reduction in shoot length and shoot mass was observed in nematode-infested soils; the Eggsac and Damage indices were between 8 and 9.

highly pathogenic to groundnut. It is important that variability in virulence and reproduction in field populations of *M. javanica* (race 3) is recognized when making nematode management decisions.

Literature cited

EISENBACK J. D., HIRSCHMANN H., SASSER J. N. and TRIANTAPHYLLOU A. C., 1981. *A guide to the four most common species of root-knot nematodes* (Meloidogyne *species), with a pictorial key.* Raleigh: North Carolina State University Graphics, 48 pp.

IBRAHIM I. K. A. and EL-SAEDY M. A., 1976. Development and pathogenesis of *Meloidogyne javanica* in peanut roots. *Nematol. medit., 4*: 231-234.

PATEL D. J., PATEL B. A., CHAVDA D. C. and PATEL H. V., 1988. Record of *Meloidogyne javanica* on groundnut in Gujarat, India. *Internat. Arachis Newsl., 3*: 16-17.

PRASAD S. K., DASGUPTA D. R. and MUKHOPADHYAY M. C., 1964. Nematodes associated with commercial crops in North India and host range of *Meloidogyne javanica. Indian J. Entomol., 26*: 438-446.

SAKHUJA P. K. and SETHI C. L., 1985. Growth of groundnut as influenced by different inocula of *Meloidogyne javanica. Indian J. Nematol., 15*: 135-137.

SHARMA S. B. and MOHIUDDIN M., 1993. Trypan blue stains egg sacs of the root-knot nematodes, *Meloidogyne* spp. *Internat. Pigeonpea Newsl., 6*: 54-55.

SHARMA S. B., REMANANDAN P. and MCDONALD D., 1993. Resistance to *Meloidogyne javanica* and *Rotylenchulus reniformis* in wild relatives of pigeonpea. *J. Nematol., 25*: 824-829.

SHARMA S. B., SMITH D. H. and MCDONALD D., 1995. Host races of *Meloidogyne javanica* with preliminary evidence that the "groundnut race" is widely distributed in India. *Internat. Arachis Newsl., 15*: 43-45.

TOMASZEWSKI E. K., KHALIL M. A. M., EL-DEEB, POWERS T. O. and STARR J. L., 1994. *Meloidogyne javanica* parasitic on peanut. *J. Nematol., 26*: 436-441.

Accepted for publication on 15 June 1998.