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Life table of *Meloidogyne incognita* (Southern root-knot nematode) on tomato

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Abstract
An experiment was conducted at the Department of Entomology, College of Horticulture, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during 2014-15. A life table for *M. incognita* was developed on tomato cultivar 'Solan Lalima' under laboratory conditions. The mortality rates were very high during egg and J2 stages prior to root penetration. The mortality of subsequent life stages was low and virtually constant. Egg laying started on 22 day and egg laying was completely stopped on the 33 day of the pivotal age. The gross reproductive rate and net reproductive rate of the nematode on tomato was 396.16 eggs/ female. The species had the approximate generation time of 26.36 days. The innate capacity for natural increase was 0.20. The values of true intrinsic rate found to be 0.217. The average time taken by the species to complete one generation was 25.08 days. Finite rate of natural increase and doubling time were 1.53, 3.91 days respectively.

Keywords: *Meloidogyne incognita*, life table, gross reproductive rate, net reproductive rate, approximate generation time, population dynamics

1. Introduction
The root-knot nematode (*Meloidogyne incognita*) is an economically important plant parasite with a wide host range and abundant field populations can develop quickly under appropriate conditions [2]. This rapid population growth is mainly due to the completion of several generations during a single growing season, combined with the high female's fecundity. Root-knot nematodes are (RKN) poikilothermic organisms and their development usually depends upon the temperature [18]. A life table is a systematic explication of survival and mortality of a population. It has a profound predictive function and is useful in relating population fluctuations with environmental factors and in identification of key factors responsible for changes in the population size [1] [10]. A life table can be used to determine whether a population is growing, declining, or remaining stable. It can be used to simulate the outcome of management decisions [10]. Development of a life table for an organism requires knowledge of the rate of development of the organism, age specific mortality, survival of the original population with time, and age-specific fecundity. These parameters are, nevertheless, difficult to determine in plant-parasitic nematodes primarily because of their obligate parasitism, microscopic size and subterranean habitat [6]. It is not feasible to observe a single age cohort to determine the development stage, natality and fecundity in plant-parasitic nematodes. Because of these constraints life tables have not been developed for plant- parasitic nematodes although some of the life table parameters were measured for *Meloidogyne arenaria* Chitwood on grape [5] [7] [8] [9]. Life tables for plant-parasitic nematodes, therefore, may have to be constructed by repeated sampling of a population in a habitat. To determine whether the host type and the environmental factors (temperature) had any influence on nematode activity and life cycle of *M. incognita*, the present study on "Life table of *Meloidogyne incognita* (Southern root-knot nematode) on tomato" is an attempt to develop a life table for *M. incognita* on tomato as a prelude to further research on host-nematode-environment interactions.

2. Materials and methods
2.1 Root-knot nematode culture
The experiment was conducted at Department of Entomology, College of Horticulture, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during 2014-15. Single egg mass of *M. incognita* was isolated from tomato roots collected from Entomology farm of University (UHF, Nauni-Solan).

This was placed singly in Petri plate containing distilled water. The second stage juveniles hatched from single egg mass were inoculated on root of brinjal (var. Pusa Purple Long) seedling grown under aseptic conditions in 500g soil capacity plastic pot. After 45 days of inoculation, the plant was uprooted and egg masses were isolated from the roots with the help of a forecep. The egg masses were placed in petri plate containing distilled water. The eggs were allow to hatch and the juveniles were again inoculated in the brinjal seedlings grown individually in 500 cc capacity plastic pots (containing autoclaved soil and sand mixture 1:1 w/w) under greenhouse conditions. The seedlings were allowed to grow for 60 days under aseptic conditions. This nematode culture was used further for mass multiplication

For mass multiplication, seedlings of brinjal were raised in thirty plastic pots of three kg capacity and maintained under aseptic conditions. After one week the seedlings were inoculated. The seedlings were allowed to grow till the culture was required for experiment.

2.2 Identification of the nematode species [13]

The character most frequently used for identification of *Meloidogyne* at species level is the morphology of finger print like perineal pattern, specific of the species, observed in the posterior body region of adult females. This area comprises the vulva-anus area (perineum), tail terminus, phasmids, lateral lines and surrounding cuticular striations. Perineal patterns of individual female of *Meloidogyne* were cut and prepared as per the methods given by Karssen [4].

Fresh galled roots were selected from uprooted pot culture plants to isolate mature females. The galled roots were stained with acid fuchsin/cotton blue-lactophenol method given by Bridge *et al.* [2] The roots were preserved in clean lactophenol. The galls were opened by teasing them carefully with fine needle to expose the root-knot nematode female. The female was taken out in a small drop of water on a clean glass slide and its posterior end was cut under a stereoscopic microscope with the help of a sharp blade. Cut portion was trimmed with a nylon bristle to remove the inner tissue. The perineal pattern was transferred to a drop of anhydrous glycerol on a clean glass slide. The perineal pattern was aligned in such a way that it was in a straight line with anus oriented down. The pattern was gently pressed against the glass with hair brush. A cover slip was placed on it gently. Excess glycerine was removed with the help of filter paper and sealed with glyceel.

Composition acid fuchsin/ cotton blue-lactophenol

Glycerol: 20 ml
Lactic acid: 10 ml
Phenol: 10 ml
Distilled water: 10 ml
Acid fuchsin/ cotton blue dye: 0.1 per cent

2.3 Raising of seedlings

Seedlings of tomato (cv. Solan Lalima) were raised in 3 Kg capacity sterilized plastic pots containing autoclaved soil mixture (soil, sand and FYM in the ratio of 2:1:1 respectively). These pots were kept in glasshouse for germination of the seedlings.

2.4 Preparation of soil mixture

The soil mixture (soil, sand and FYM in the ratio of 2:1:1) was autoclaved in gunny bags at a temperature of 121°C (pressure 15 lbs/ sq inch) for 30 minutes. After autoclaving, the mixture was spread over the polythene sheets for 24 hours to make it free from toxic fumes.

2.5 Isolation/ hatching of egg masses

Egg masses were isolated from the roots of brinjal plants (maintained for the nematode culture) and juveniles and an individual egg mass was kept in a cavity block with distilled water. For life table study of each crop 200 cavity blocks were maintained. These were incubated at 25°C for egg hatching. Juveniles emerged in each cavity block were counted and removed daily for up to 7 days of incubation. The percentage of egg hatching was calculated accordingly.

2.6 Inoculation of juveniles (J2s)

Tomato seedlings were uprooted carefully from the nursery pots without damaging the roots and were transplanted in small (50g capacity) plastic @ single seedling per cup. About 200 seedlings of each crop were maintained at ambient temperature (28.8±2.4°C). After 24 hrs of transplanting each seedling was inoculated with freshly hatched juveniles extracted from individual egg mass (@ 400 juveniles).

2.7 Juvenile penetration and development

The penetration study was conducted from June to July under laboratory conditions. Five inoculated seedlings were uprooted after every 24 hours to check the penetration of juveniles entered in the roots. All juveniles that had entered the roots within the same time constituted an inoculum cohort of the same age.

2.8 Staining of roots

To study each development stage and age specific survival, five seedlings were removed daily and washed free of water. The clean roots were stained with 0.1 per cent cotton blue lactophenol solution and stored in clear lactophenol to remove the excess stain. Penetration was checked by compressing the stained roots between two glass plates (15 × 10 cm) under stereo zoom microscope [12].

Number and different stages of nematode (J2, J3, J4 and adult) were identified under the microscope. The J2s were identified by the presence of stylet (infective and feeding stage) whereas J3 and J4 were non-feeding stages having a spike tail stage. The males and female stages were identified by the presence of different genital primordias. The males of root-knot nematode having 'I-shaped' genital primordia where females having 'V-shaped' genital primordial. The sex ratio was determined at J4 stage.

2.9 Age specific survival and fecundity of females

From 22 days onwards number of females, males and eggs per egg mass were calculated individually. Ten egg masses were collected and the number of egg per egg mass was counted daily to estimate number of eggs laid /female/day separately. The females were assumed dead as soon as the egg laying was stopped. However females were checked for egg laying even for one week.

2.10 Construction of life table

The life table of *M. incognita* was studied under laboratory conditions on tomato (cv. Solan Lalima) at room temperature. The fertility tables were constructed as per the method described by Singh and Sharma [14].

2.11 Age specific survival/mortality life table

The age specific survival/mortality life table was constructed as described by Deevey [4]:

x= age of cohort

l_x= number surviving at the beginning of age x age of individual in days (pivotal age).

L_x = proportion of individuals still alive at age x (age specific survival), l_x for females was calculated from l_x for immature and for adult stages.

m_x = mean of female off spring produced per female in the age interval (x). The daily increase in egg number per female was taken as age specific fecundity.

The following fertility parameters were calculated:

Gross reproduction rate (GRR): $\sum m_x$

Net reproductive rate (R_o): $\sum l_x m_x$

Approximate cohort generation time (T_c): $\sum x l_x m_x / R_o$

Innate capacity for natural increase (r_c): $\log_e R_o / T_c$

True intrinsic rate of increase (r_m): The true intrinsic rate of increase was calculated as per the method of Southwood [17] was used of r_m.

True generation time (T): loge Ro / r_m.

2.12 Statistical Analysis: The experiment was conducted under laboratory condition using completely randomized design (CRD) and the data obtained were analysed in MS-Excel 2007 and presented in the form of tables and graphs.

3. Results and discussion

3.1 Survival and life expectancy (ex) of *Meloidogyne incognita* on at room temperature

Mortality rates of *M. incognita* on tomato at room temperature were high between 0 and 1 day of the pivotal age whereas, the life expectancy was found to be very low (Table 1). Mortality kept on decreasing between 2 and 9 day of the pivotal age whereas, life expectancy kept on increasing respectively. Thereafter on 10 and 11 day of the pivotal age, there was slight increase in mortality rate with decrease in life expectancy. After 12 day up to 14 day mortality was decreasing constantly with increase in life expectancy. The mortality rate increased on 15 day with decreased in life expectancy and afterwards kept on decreasing up to 24 day of the pivotal age along with increase in life expectancy. On 25 day the mortality increased along with the decrease in life expectancy. This mortality rate kept on decreasing up to 20 day of the pivotal age with increase in life expectancy. Further the mortality rate increased between 29 and 30 day and life expectancy decreased. After 31 day the mortality rate kept on decreasing and became zero on 34 day whereas, life expectancy was also found to be decreasing and reached its minimum value on 34 day of the age.

Table 1: Survival and life expectancy (ex) of *Meloidogyne incognita* on tomato at room temperature (28.8±2.4°C).

x	l_x	d_x	100qx	L_x	T_x	e_x
0	450	193	42.89	353.5	568	1.26
1	257	85	33.07	214.5	383.5	1.49
2	172	6	3.49	169	332.5	1.93
3	166	5	3.01	163.5	324	1.95
4	161	1	0.62	160.5	319.5	1.98
5	160	2	1.25	159	316	1.98
6	158	2	1.27	157	312.5	1.98
7	156	1	0.64	155.5	310	1.99
8	155	1	0.65	154.5	308	1.99
9	154	1	0.65	153.5	305	1.98
10	153	3	1.96	151.5	300	1.96
11	150	3	2.00	148.5	294.5	1.96
12	147	2	1.36	146	290.5	1.98
13	145	1	0.69	144.5	287.5	1.98
14	144	2	1.39	143	283	1.97
15	142	4	2.82	140	276.5	1.95
16	138	3	2.17	136.5	270	1.96
17	135	3	2.22	133.5	264.5	1.96
18	132	2	1.52	131	259.5	1.97
19	130	3	2.31	128.5	254.5	1.96
20	127	2	1.57	126	250	1.97
21	125	2	1.60	124	246.5	1.97
22	123	1	0.81	122.5	244	1.98
23	122	1	0.82	121.5	242	1.98
24	121	1	0.83	120.5	239	1.98
25	120	3	2.50	118.5	234.5	1.95
26	117	2	1.71	116	230	1.97
27	115	2	1.74	114	226.5	1.97
28	113	1	0.88	112.5	223	1.97
29	112	3	2.68	110.5	217	1.94
30	109	5	4.59	106.5	209	1.92
31	104	3	2.88	102.5	202.5	1.95
32	101	2	1.98	100	199	1.97
33	99	0	0.00	99	148.5	1.50
34	99	99	100.00	49.5	49.5	0.50

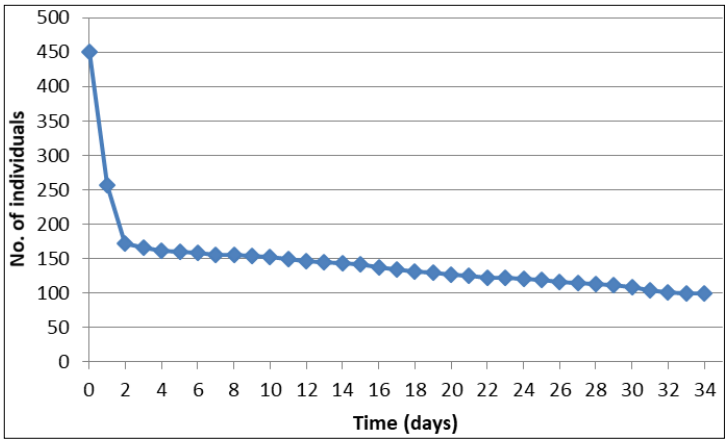

Fig 1: Survival curve for *M. incognita* on tomato at 28.8±2.4°C

3.2 Life Table (For Females) and Age Specific Fecundity

The data on the fertility of *M. incognita* on tomato at room temperature are presented in table 2, perusal of which reveal that survival was 28% at the time of adult emergence on the pivotal age of 22 day which decreased to 27% on 22 day and remained same till 25 day of the pivotal age. On 26 and 27 day of the pivotal age, the survival was 26% whereas, 25% survival was observed during 28 and 29 day of the pivotal age. However, on 30 day, the survival was 24% which decreased to 23% on 31 day of the pivotal age. Further the survival decreased to 22% on 32 and 33 day of the pivotal age. It was observed that all the females died on 34 day of the pivotal age. Egg laying started on 22 day of the pivotal age (81.97eggs/ female). Maximum eggs were laid on the 23 day (149.79), after which the rate of egg laying kept on decreasing reaching a minimum value of 48.87 eggs/ female on the 33 day of the pivotal age. The egg laying was completely stopped on the 32 day of the pivotal age (Table 2).

The data on age-specific survival and age-specific fecundity of *M. incognita*, respectively were utilized to compute fertility parameters of the nematode on tomato (cv. Solan Lalima) and has been presented in Table 3. The gross reproductive rate and net reproductive rate of the nematode on tomato was 902.83 eggs/ female and 230.80 eggs/ female, respectively. The species had the approximate generation time of 26.36. The innate capacity for natural was 0.20 at room temperature. The value of true intrinsic rate found to be 0.217. The first few days of egg laying (22-25 age intervals) contributed more to the value of r_m than other age interval (Table 4). The average time taken by the nematode to complete one generation was 25.08 days. Finite rate of natural increase and doubling time at room temperature had different values i.e. 1.53, 3.91 respectively.

Table 2: Age-specific survival and age-specific fecundity of *M. incognita* on tomato at room temperature (28.8±2.4°C).

Pivotal age in days (x)	Survival at x (l_x)	Number of eggs/female (m_x)	l_xm_x	xl_xm_x	Trial rm $e^{7-rmx}l_xm_x$	
					r_m=0.20	r_m=0.22
0-21	0.28	**Immature stages**				
22	0.27	81.97	22.40	492.90	301.59	194.23
23	0.27	149.79	40.61	934.04	447.65	282.59
24	0.27	96.29	25.89	621.36	233.66	144.58
25	0.27	78.74	21.00	524.95	155.17	94.12
26	0.26	70.71	18.38	477.98	111.19	66.11
27	0.26	78.74	20.12	543.33	99.66	58.07
28	0.25	70.71	17.76	497.15	72.02	41.14
29	0.25	63.55	15.82	458.71	52.52	29.41
30	0.24	57.15	13.84	415.32	37.62	20.65
31	0.23	54.02	12.48	387.01	27.77	14.94
32	0.22	52.28	11.73	375.51	21.37	11.27
33	0.22	48.87	10.75	354.82	16.04	8.29
34	0.00	0.00	0.00	0.00	0.00	0.00
Total	-	902.83	230.80	6083.06	1576.27	965.40

Table 3: Fertility parameters of *Meloidogyne incognita* on tomato at room temperature (28.8±2.4°C).

Fertility parameters	Formulae	Values
Gross reproductive rate (GRR)	$\sum m_x$	902.83
Net reproductive rate (Ro)	$\sum l_x m_x$	230.80
Approximate generation time (Tc)	$\sum x l_x m_x / R_o$	26.36
Innate capacity for natural increase (rc)	logeRo/Tc	0.20
True intrinsic rate of increase (rm)	rm	0.217
True generation time (T)	logeRo/rm	25.08
Finite rate of natural increase (λ)	Antiloge rm	1.53
Doubling time (DT)	Loge2/rm	3.19

Table 4: Contribution of each age group to the value of r_m (r_m = 0.217) in tomato at room temperature (28.8±2.4°C).

Percentage age group (x)	$lXmX\ .e^{7-r}m^x$	Percentage contribution of each age group
22	44.22	18.41
23	37.21	15.50
24	31.26	13.01
25	26.21	10.91
26	21.94	9.14
27	18.34	7.64
28	15.31	6.37
29	12.76	5.31
30	10.63	4.42
31	8.84	3.68
32	7.34	3.06
33	6.10	2.54

4. Discussion

Slobodkin (1962) [16] reported that age specific survival and mortality data of two age intervals during which survivorship (l_x) of *M. incognita* was low; i) during the egg stage and ii) during the second-stage juvenile phase prior to root penetration. Similar results were supported by Deevey (1947) [4] survivorship curve for *M. incognita* resembled that of type IV of or type III of in which mortality of young stages was greatest. Egg mortality, inability of second stage juveniles to penetrate the tomato roots, and (or) juvenile mortality in soil before penetration were presumable factors responsible for low survivorship (l_x) and e_x values. Mortality of pre-parasitic stages in soil was probably higher than that of the subsequent parasitic stages. Many factors such as soil moisture and temperature greatly affect the migration of juveniles in soil and root penetration [15]. Availability of food and suitable environment for nematode development inside the plant roots, and shelter from lesser favourable soil environment might be responsible for lower mortality after root penetration

The intrinsic rate of natural increase (r_m) is a useful parameter for comparing reproductive capacities among different species and under different environmental conditions. Initial egg laying contributed greatly to the value of r_m for *M. incognita* on tomato; which was in accordance with the findings of Singh and Sharma [14] for *Heterodera cajani* and Banu *et al*., [2] for *M. incognita*. Plant host is an important factor which influences the reproduction of plant-parasitic nematodes and it may affect the r_m value, which is likely to change with nematode species, and plant hosts. The r_m value would be an useful parameter for comparing reproductive potential of a nematode species on different plant hosts and between different nematode species. The intrinsic rate of increase (r_m) has been useful as predictive and comparative measure of population growth potential.

5. Conclusion

This study contributes to the understanding of population biology of *M. incognita*. It will be useful in developing life tables for field populations of plant-parasitic nematodes, and eventually it will help in development of nematode pest management models.

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