

# Temperature-based Prediction of Egg-Mass Production by *Meloidogyne incognita*

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**Abstract:** A maturation-rate relationship for *Meloidogyne incognita* on *Lycopersicon esculentum* 'Rutgers' was derived and used to estimate harvest dates for maximum egg hatch from laboratory cultures at ambient temperatures. Daily maturation increments were totaled (nematode maturation total, NMT) and correlated with hatch from isolated white, yellow, and amber egg masses. Hatch per mass fluctuated periodically from ca. 1.0 NMT, when egg masses were first visible, to 2.5 NMT by which time plants showed stress. Maximum yields from white and yellow masses occurred, with a shorter than expected periodicity, at 1.5–1.8 and 2.1–2.2 NMT. White masses decreased from 90% of the total masses at 1.0 NMT to 5% at 2.5 NMT, as the proportion of yellow and amber masses increased concomitantly. Harvested masses per gram of root varied from 97 to 276; hatch per gram of root, 11,000 to 86,000. **Key Words:** Root-knot nematode, *Lycopersicon esculentum*, maturation.

The effect of temperature on maturation rate is well established for endoparasitic *Meloidogyne* in tomato plants in the laboratory (2, 6), and accumulated degree-days above a characteristic threshold have been correlated with part of this nematode's life cycle in at least one field crop (5). However, accurate prediction of egg-mass production or of hatch per host plant remain practical problems requiring balancing of conflicting chronologies, i.e., pest development and host decline.

As long as the host provides adequate nutrition, nematode development should be predictable from temperature relationships already known for portions of a single *Meloidogyne* life cycle. Available data on egg incubation time (4) and time from hatch to egg-laying (2) indicate that at 27 C one life cycle occurs in 24 days, which is consistent with Tyler's estimate of 25 days at that temperature (6). We used these correlations for simplifying routine maintenance of cultures and for following *Meloidogyne* population development beyond the first nematode generation in Rutgers tomatoes. The experiments described here relate egg-mass production to anticipated life cycle and reveal an unexpected variability in hatch per egg mass as the nematode population increases to the point of irreversible impact on the host.

## MATERIALS AND METHODS

The *M. incognita* Kofoid and White (Chitwood) culture was isolated from *Glycine max* L. and maintained in *Lycopersicon esculentum* Mill. 'Rutgers'.

Rutgers tomato plants intended for Study A were grown in an environmental chamber at 25 C day and 23 C night temperatures with 14 h days (54,000 lm/m<sup>2</sup>). Plants intended for Study B were grown at 20 C day and 18 C night temperatures with 8 h days. All plants were 47–49 days old with 3–5 leaves prior to transplanting and inoculation.

Study A was initiated by pouring a suspension of 10 egg masses on the roots of each plant during transplanting. In Study B, each plant was inoculated with a suspension of ca. 3,000 larvae directly onto the roots. To correct for the inoculum difference, the 2 days required for eggs to hatch were added to Study B when comparing culture development throughout the two studies. Inoculated plants were grown under Gro-Lux lights (Sylvania, Danvers, Massachusetts) with a 16.5 h photoperiod in 17-cm-diam pots filled with sandy loam: sand (1:2), dry-heat-sterilized for 3 h at 102 C. Temperatures for Study A were 27–33.5 C; Study B, 26–31 C. Average room temperatures for individual plants fell within 29.0–29.5 C for Study A and 28.5–29.0 C for Study B.

Single plants were harvested at specified times. Each was rinsed, blotted dry, weighed and all external egg masses were removed, counted, and sorted into three categories: (i) white with no yellow color; (ii) yellow with no amber tinge; and (iii) amber. This

Received for publication 3 December 1977.

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color change has been attributed (1) to polyphenol oxidase activity. Each group of egg masses was put on a double Kimwipe (Kimberly-Clark, Neenah, Wisconsin) layer over cheesecloth in contact with tap water for 1 week at daily average temperatures of 27–31 C in Study A or 26–30.5 C in Study B. Larvae in the tissue were then rinsed through the tissue and the resulting suspension was diluted to a known volume. Larvae in 3 or more 1-ml aliquots were counted and counts were converted to hatch per egg mass.

## RESULTS AND DISCUSSION

Working with *M. incognita* in Rutgers tomato roots, Davide and Triantaphyllou (2) found that newly hatched larvae develop to egg-laying females in 60, 30, and 13 days at 15, 20, and 30 C, respectively. The rate of this maturation is, for practical purposes, a linear function of temperature (Fig. 1, solid line). We have defined daily increment as the reciprocal of the number of days required for development through the period being discussed. At 27 C, hatch to egg-laying requires 16 days ( $1 \div$  daily increment). At the same temperature, egg incubation *in vitro* requires 8 days (4); i.e. one-third of the total life cycle at 27 C. This proportion should apply at all temperatures in this 15–30 C range, since Tyler's data (6) suggest that temperature has a similar effect on both egg development and nematode maturation. These observations allowed us to construct the dashed line in Fig. 1 correlating temperature with maturation rate for the entire life cycle. Interestingly, the threshold temperature and life cycle times predicted from this plot are not markedly different from those obtained by Tyler (6), which demonstrates a consistency among data on seemingly disparate nematode cultures.

We predicted culture development, after inoculation with egg masses, by totaling daily nematode maturation increments determined using an average temperature for each day. We used a table derived from  $y = 0.00269x - 0.0301$ , which represents Fig. 1 (dashed line) for 15–31 C. Life cycles for the majority of the population in each culture were approximated conveniently by nematode maturation totals (NMT's) of 1.0, 2.0, and so on. For 3 years, this compilation suc-

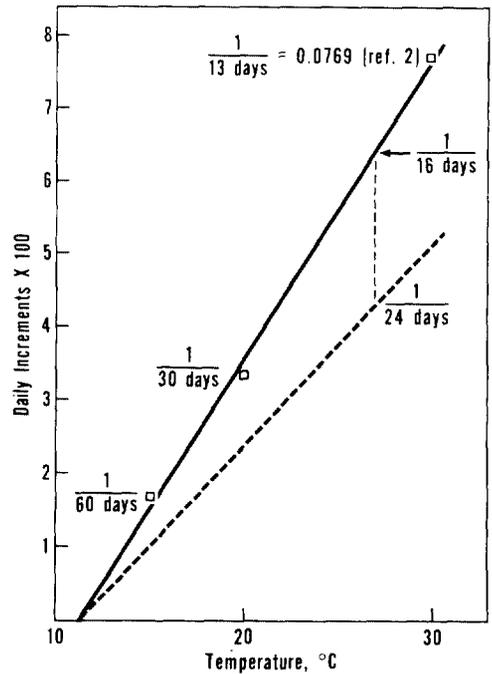


FIG. 1. Hatch to egg laying (2) [At 27 C,  $1 \div$  daily increment (arrow) =  $1 \div 0.0639 = 16$  days for hatch to egg laying.] — — — = life cycle calculated from hatch-to-egg-laying plot. [At 27 C, 16 days for hatch to egg laying + 8 days (4) for egg laying to hatch = 24-day life cycle.  $16 \div 24 = 67\%$ . Therefore, daily life cycle increment =  $67\% \times$  daily increment from hatch to egg laying.]

cessfully predicted when white egg masses were prevalent on roots even though daily average temperatures varied as much as 12 C. Egg masses were always small and sparse on roots through at least 1.0 NMT, and the best harvests were realized after slightly longer incubation periods. At constant temperatures, the desired NMT value divided by the daily increment provided a quick estimate of the time at which a host plant should be harvested. At a constant temperature of 30 C, e.g., only 20 days are required to reach 1.0, while at 21 C, 38 days are needed. Laboratory culture of *M. incognita* is simplified appreciably by such estimates.

Females maturing from an inoculum (first generation) should lay eggs from 0.7 through at least 1.7 NMT since *Meloidogyne* females remain alive (4) and lay eggs (6, 7) through at least one additional generation. Accordingly, a plot of hatch per mass against NMT should reflect an increase in average egg mass size on the

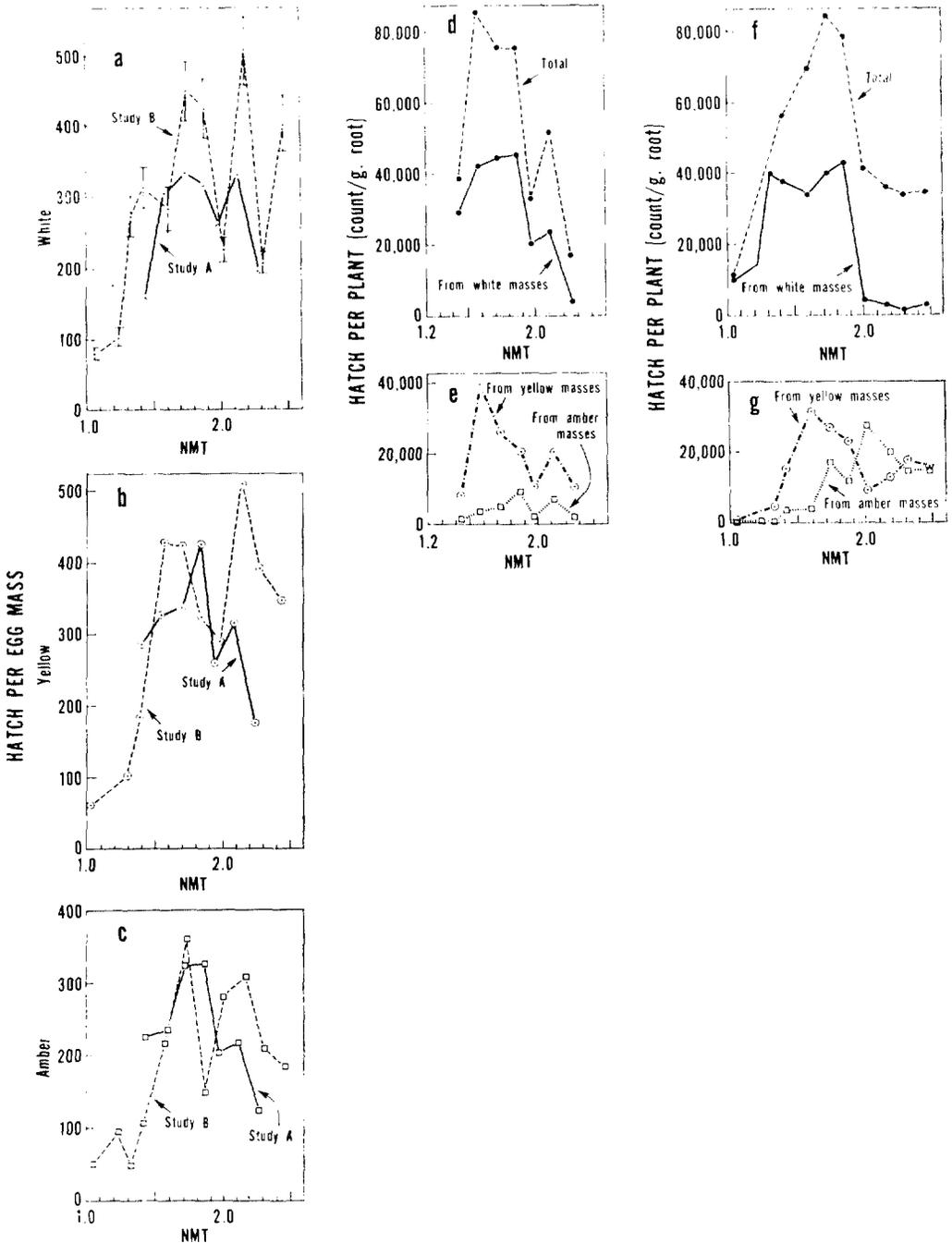


FIG. 2. Hatch from (a) white, (b) yellow, and (c) amber egg masses at various stages of development. Brackets indicate 95% confidence limits based on precision of counts on 1-ml aliquots. Hatch per plant (larva/g root) in (d, e) study A and (f, g) study B. NMT = Nematode maturation total.

root exterior until initiation of egg laying by the succeeding generation at or near 1.7 NMT. Thereafter, the plot would depend on mortality and fecundity of all females, rates of egg laying, and egg development. Different development rates among the

population might logically result in one smooth increase and decline in this plot. But Figs. 2a-c, which tend to confirm predictions about egg laying, reveal multiple maxima in larval populations and a similar periodicity regardless of egg mass color. In

Fig. 2a, these maxima, separated by 0.4 NMT (ca. 8 days) rather than 1.0 NMT as expected, are reproduced in both studies. Points were considered significantly different when 95% confidence limits for precision of larval counts, converted to hatch per egg mass, showed no overlap. Thus the principal maxima in Fig. 2a-c are significant. However, they do not correlate with number of egg masses per sample (smaller samples could have given greater error) or with temperatures during either periods between harvests (2-4 days) or the 7-day hatch. There were no additional stresses on the plants throughout the two studies. It also seems unlikely that these peaks represent the normal rise and fall in egg-laying rate for each generation (3) because maxima occur more often than the 0.7 NMT required for second-stage maturation to egg laying.

Based on Fig. 2 and our other observations, the largest egg masses and the highest hatch from white or yellow egg masses should be obtained from roots at NMT's of 1.5-1.8 or 2.1-2.2; for amber, 1.6-1.8 or 2.1. The average hatch per egg mass for all colors combined was over 200 larvae at NMT = 1.5 to 2.2 in study A; NMT = 1.3 to 2.5 in B. Harvest before 1.4 NMT should be avoided. Values for total larvae per g of root (Fig. 2d,f) reinforce these recommended ranges. The increase in hatch per mass up to 1.6-1.8 NMT parallels increased egg laying rate following the hatching of

the second generation observed by Tyler (7). White and yellow egg masses, which had the most viable eggs of the three types (Fig. 2d-g), could contribute most of a plant's larval population.

The total number of external egg masses fluctuated differently in the two studies, but egg masses per g of root varied over the same range; 99-276, study A; 97-254, study B. In each study (Table 1), white egg masses constituted about 85% of the total near 1.2 NMT and decreased to less than 40% near 2.0 NMT.

A notable increase in amber egg masses occurred (ca. 1.9 NMT, Table 1) at about the same time as irreversible wilt and/or yellowing in some plants. These plants were not harvested (three plants in study A between 1.85 and 2.05 NMT; four plants in study B between 2.1 and 2.3 NMT). A 0.3 decrease in these wilt NMT's (to correct for 7-day hatch and allow direct comparison with NMT's at wilting) shifts them under the maxima for total larvae in Fig. 2d and f. Therefore, irreversible wilt of infected plants seems to be an accumulated effect of numerous second-stage larvae hatching and feeding. The initiation of female maturation [ca. 2.4 NMT predicted from (2)] would create additional stress on surviving plants.

Chemical reasons for the spacing of maxima reported here remain obscure, but pest-host interaction is apparent. In both studies, the number of fresh (white) egg

TABLE 1. Hatch from and numbers of *Meloidogyne incognita* egg masses removed from ' Rutgers' tomato roots having various nematode maturation totals (NMT).

NMT	Root wt. <sup>a</sup>	Total No. masses	% of total masses			Hatch per mass		
			W <sup>b</sup>	Y	A	W	Y	A
1.1 <sup>c</sup>	1.27	188	88	10	2	78	61	4
1.2	2.62	456	83	14	3	101	—	93
1.3	2.43	479	75	22	3	273	102	48
1.4	0.93	220	51	35	15	314	184	105
1.6	2.89	611	57	35	8	283	429	234
1.8	1.94	390	44	32	24	451	423	359
1.9	1.87	475	40	28	32	425	324	147
2.0	2.37	358	13	22	66	226	289	278
2.2	5.42	525	6	26	68	508	511	306
2.3	3.39	417	6	37	58	206	391	206
2.5	1.87	258	5	34	61	402	347	181

<sup>a</sup>Weight of fresh root in g.

<sup>b</sup>W = white, Y = yellow, A = amber.

<sup>c</sup>Study B.

masses per g of root declined rapidly at or after 1.9 NMT as if the taxed plants no longer supported larval maturation and egg production. The viability (hatch/mass) of egg masses harvested after 1.9 NMT remained unaffected and continued to fluctuate as it had earlier. As decline in egg production appears to be related to plant decline, so also might periodicity in egg viability reflect periodic changes in the host plant. The exact cause is still to be elucidated.

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