

## Influence of Temperature on Multiplication and Egg Hatching of *Longidorus africanus*<sup>1</sup>

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**Abstract:** *Longidorus africanus* multiplication on tomato was highest at 29 °C. Few nematodes were recovered after 6 weeks at soil temperatures of 35 °C or below 23 °C. The time to egg hatching was shortest and the percentage of eggs hatching was highest at 29 °C. The minimum temperature and the heat sum above this temperature required for egg development were calculated to be 14.3 °C and 94.08 degree-days, respectively. The thermal times required for egg development by *L. africanus* and *L. elongatus* were nearly identical. For both species the product of the base temperature and the heat sum was near constant, and at a temperature of 22.3 °C the rates of egg development were equal.

**Key words:** egg development, *Longidorus africanus*, *Longidorus elongatus*, nematode, thermal time relationships.

*Longidorus africanus* Merny, damages several vegetable crops in the Imperial Valley of southern California (Kolodge et al., 1987; Radewald et al., 1969). Recently, Ploeg (1998) reported from field studies that fluctuations in population levels of *L. africanus* were strongly correlated with soil temperature and that numbers were highest in the hot summer months when soil temperatures at the 15-cm depth averaged around 30 °C. Also, Lamberti (1969) and Kolodge et al. (1986) suggested that high soil temperatures were favorable for *L. africanus*. From greenhouse studies it was estimated that *L. africanus* can complete its life cycle in 7 to 9 weeks, although it was suggested that this period might be shorter under favorable field conditions (Kolodge et al., 1986). After testing four temperatures (18, 24, 30, and 35 °C), Lamberti (1969) found that *L. africanus* multiplied only at the two higher temperatures, with very few or no nematodes found after 3 months at 18 or 24 °C. Reproduction was highest at 30 °C.

Rates of egg development and hatching are important factors in the population dynamics of nematodes. However, for ectoparasitic nematodes, data on temperature effects on egg development and hatching

are available only for *Xiphinema diversicaudatum* and *L. elongatus*, with optimum temperatures of 25 °C and 20 to 25 °C, respectively (Boag, 1985; Flegg, 1968, 1969). To predict population development and assess risks on plant damage, it is essential that relationships between nematode population dynamics and temperature be understood. The objectives of this study were to determine the effects of temperature on development and hatching of *L. africanus* eggs and compare these effects with the known effects of temperature on the population dynamics of *L. africanus* in southern California.

### MATERIALS AND METHODS

A culture of *L. africanus*, originally obtained from field soil in the Imperial Valley, was maintained on tomato (*Lycopersicon esculentum* Mill. cv. Pixie) in 1-liter pots in a greenhouse at a constant soil temperature of 26 °C in steam-sterilized coarse sand. Subculturing took place every 2 to 3 months. Nematodes used in the experiments were extracted from the cultures with a modified sieving and decanting technique (Brown and Boag, 1988). Final separation was by migration of the nematodes through a 100-µm-pore nylon sieve for 12 hours into a plastic saucer filled with enough water to touch the bottom of the sieve.

**Nematode multiplication:** Four-week-old tomato cv. Pixie seedlings were transferred to steam-sterilized coarse sand in 1.5-liter plastic containers. The containers were placed in waterbaths at 11, 14, 17, 20, 23, 26, 29, 32,

Received for publication 20 July 1998.

<sup>1</sup> This study was funded in part by the California Fresh Carrot Advisory Board.

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The author thanks M. Arciniega for technical help.

This paper was edited by A. F. Robinson.

and 35 °C ( $\pm 1$  °C), five containers per temperature. One week later a shallow hole was made close to the stem of each tomato plant, and 5 ml of a nematode suspension containing  $80 \pm 5$  *L. africanus* of all stages was inoculated into each hole. Six weeks after inoculation, nematodes were extracted from each pot and counted. The experiment was repeated once.

**Egg development and hatching:** Nematode suspensions were examined at  $\times 40$ , and gravid *L. africanus* females were handpicked into a small sterilized watchglass containing 100  $\mu$ l sterile water. For surface decontamination, 60 to 70 nematodes were transferred to the center of a plastic petri dish containing 1% water agar (60 to 70 nematodes/dish) (Huang and Becker, 1997). The dishes were placed in an incubator at 26 °C and examined 3 hours later. Nematodes that had moved through the agar away from the center of the dish were collected from the agar with a thin metal needle and aseptically transferred to a petri dish, 30 nematodes/dish, with 2-week-old excised tomato roots on White's medium (Sigma, St. Louis, MO). Earlier observations on feeding of *L. africanus* had shown that nematodes fed, deposited eggs, and survived longest on this substrate-root combination. The dishes were kept at 26 °C and examined after 8 hours. The location of eggs (28.3 eggs/dish on average) deposited in the agar was marked on the bottom of the dish. After 8 hours the dishes were placed in the dark in incubators at 17, 20, 23, 26, 29, 32, or 35 °C ( $\pm 0.5$  °C). Temperatures in the incubators had previously been calibrated with HOBO digital thermo-readers (Spectrum Technologies Inc., Plainfield, IL). Three petri dishes were incubated at each temperature. Every 12 hours each dish was examined, and hatching was recorded up to 40 days after the start of the experiment. Plates in which fungal or bacterial contamination developed were discarded. The experiment was repeated once.

Analysis of variance was done with SAS statistical software (SAS Institute, Cary, NC), and means were separated with Duncan's multiple-range test.

## RESULTS

**Nematode multiplication:** The *L. africanus* populations observed after 6 weeks at the tested temperatures were not significantly different between the two experiments (factor "experiment"  $P = 0.84$ ; factor "experiment  $\times$  temperature"  $P = 0.99$ ). The temperature effect on the final numbers of *L. africanus* was, however, highly significant ( $P = 0.001$ ). Significantly more *L. africanus* were recovered from the 29 °C treatment than from any of the other temperatures. Numbers of *L. africanus* were low and not significantly different from each other after temperatures of 11, 14, 17, 20, and 35 °C (Fig. 1).

**Egg development and hatching:** The average number of eggs per plate (28.3) was not significantly different between the different temperatures or between the two experiments ( $P = 0.1$ ). During the 40-day observation period, bacterial or fungal colonies developed in 11 plates, which were discarded and not included in the results. The time to first egg-hatching was shortest and the total percentage of hatched eggs was highest at 29 °C. During the 40-day period no eggs hatched at 35 °C, and only one egg hatched after 30 days at 17 °C (Table 1). Regression of the reciprocal of the time to hatching ( $\text{day}^{-1}$ ) against temperatures from 17 to 29 °C accounted for 98% of the variation. The time to egg-hatching at 32 °C clearly did not fit on the regression line and was not in-

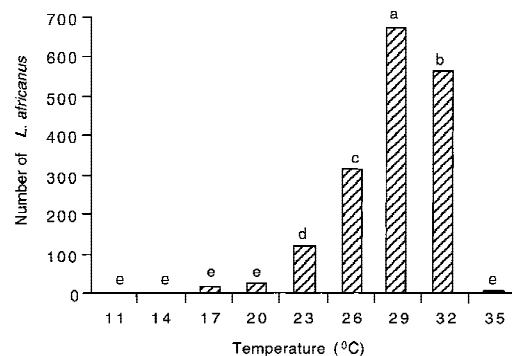


FIG. 1. Effect of soil temperature on the population level of *Longidorus africanus* on tomato, 6 weeks after inoculation with 80 *L. africanus* of mixed stages.

TABLE 1. Influence of temperature on time to hatching and total hatch of *L. africanus* eggs<sup>a</sup>.

		Temperature (°C)						
		17	20	23	26	29	32	35
Experiment 1	Eggs deposited	31.7	29.7	31.7	27.7	29.7	28.0	24.7
	Eggs hatched	0.0 <sup>b</sup>	22.0 <sup>b</sup>	29.5 <sup>b</sup>	19.5 <sup>b</sup>	27.0 <sup>b</sup>	16.7	0.0 <sup>b</sup>
	Days to first hatch	—	18.5	10.0	9.0	6.5	6.5	—
Experiment 2	Eggs deposited	31.3	29.0	25.3	30.0	28.3	26.3	27.0
	Eggs hatched	0.3 <sup>b</sup>	21.0	21.0	28.5 <sup>b</sup>	22.5 <sup>b</sup>	19.5 <sup>b</sup>	0 <sup>b</sup>
	Days to first hatch	30.0	19.5	10.0	8.0	6.0	7.5	—
Average	Eggs deposited	31.0	29.3	28.5	28.8	29.0	27.2	25.8
	% eggs hatched <sup>c</sup>	1	69	88	86	93	65	0
	Days to first hatch	30.0	19.0	10.0	8.5	6.3	7.0	—

<sup>a</sup> Each experiment consisted of three replicates.

<sup>b</sup> One replicate was lost due to contamination.

<sup>c</sup> Contaminated plates were not included.

cluded in the regression. Extrapolation of the regression line to the x-axis (days<sup>-1</sup> = 0) gave a base temperature of 14.3 °C (Fig. 2). The thermal time required above this base temperature ranged from 81 to 108 degree-days (average 94.08 degree-days).

DISCUSSION

Few data are available on the biology and life cycle of *Longidorus* species. The reproduction of *L. elongatus* observed by Yassin (1969) was continuous but slow at 20 °C,

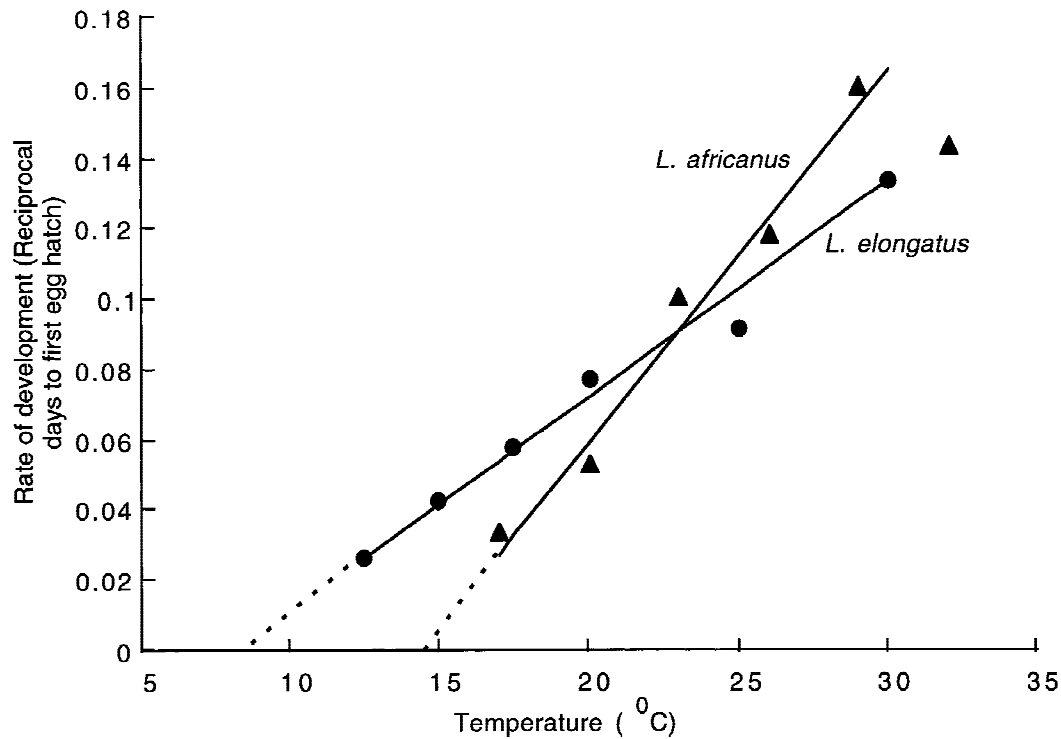


FIG. 2. Relationship between temperature and the rate of egg development of *Longidorus elongatus* (●) and *L. africanus* (▲) (*L. elongatus* data according to Boag [1985]).

with populations increasing only 8 to 10-fold after 16 weeks on *Fragaria vesca* and with an estimated life cycle of ca. 19 weeks. At 30 °C the life cycle of *L. elongatus* was completed in 9 weeks (Wyss, 1970). The current results show that within 6 weeks *L. africanus* populations can increase 8.5-fold, if temperatures are favorable, but increase more slowly or even decrease as soil temperatures deviate from 29 °C. Numbers of *L. africanus* increased only at temperatures within the 23 to 32 °C range, and very few nematodes were found after a 6-week period of 35, 11, or 14 °C. These results correspond with those from field observations on the population dynamics of *L. africanus* in the Imperial Valley, where fluctuations in population levels were highly correlated with soil temperatures. The highest populations observed previously (Ploeg, 1998) occurred during the summer when soil temperatures approached 30 °C, and the lowest population levels occurred during the cooler winter months (Ploeg, 1998). The results also agree with those of Lamberti (1969), who found the highest multiplication at 30 °C. However, at 23 °C some multiplication occurred in this experiment, whereas in Lamberti's results (1969) very few nematodes survived 3 months at 24 °C. Furthermore, Lamberti (1969) observed multiplication at 35 °C, whereas in this study this temperature reduced population levels to almost zero. Cohn and Mordechai (1969) concluded from experiments on the multiplication of Israeli *L. africanus* at 20 to 23 °C that the life cycle was completed within 4 months. Thus, the population in this study appeared to be adapted to cooler soil temperatures than that of Lamberti (1969), and to higher temperatures than that of Cohn and Mordechai (1969). The preference of *L. africanus* for subtropical and tropical conditions is also apparent from its geographical distribution, which includes Egypt, South Africa, Sudan, Portugal, and Zimbabwe (Aboul-Eid, 1970; Bravo and Roca, 1995; Jacobs and Heyns, 1987; Merny, 1966; Zeidan and Coomans, 1992). In California, *L. africanus* occurs mainly in the most southern part of the state (Siddiqui et al., 1973).

Boag (1985) studied the time to hatching of *L. elongatus* in relation to the environment temperature ( $T_e$ ) and reported a minimum temperature for egg development ( $T_b$ ) of 8.3 °C and a required heat sum ( $S$ ) above this temperature of 162.7 degree-days. Tyler (1933) initially showed that the rate of development  $R$ , expressed as the reciprocal time required for development of a *Meloidogyne* sp., was linearly related to  $T_e$ . Data on thermal time relationships of several plant-parasitic nematode species were reviewed by Trudgill (1995) and Trudgill and Perry (1994). They confirmed Tyler's (1933) observation and concluded that different developmental processes (life-cycle duration, egg development) in several plant-parasitic nematode species can be described by the relationship  $R = (T_e - T_b)/S$  for  $T_b < T_e$ .

Using Boag's data (1985), the rate of egg development for *L. elongatus* is given by  $R = 0.0061 \times T_e - 0.051$  (Fig. 2). Our data indicate that for *L. africanus* the relationship between egg development and temperature is also linear over most of the temperature range, with an estimated minimum temperature  $T_b = 14.3$  °C and a required heat sum ( $S$ ) of 94.1 degree-days. Trudgill and Perry (1994) and Trudgill (1995) compared the thermal time relationships for development of the tropical and temperate root-knot nematodes *M. javanica* and *M. hapla*, respectively. They concluded that the lower  $T_b$  and higher  $S$  of *M. hapla* (8.25 °C and 554 degree-days) were matched by a correspondingly higher  $T_b$  and lower  $S$  (12.9 °C and 350 degree-days) in *M. javanica*. They further concluded that, for the biologically similar species *M. hapla* and *M. javanica*, the product  $T_b \times S$  is constant, and hypothesized that this may also be true for other organisms with similar biology and development. The needle nematodes *L. africanus* and *L. elongatus* fulfill this requirement. Both species are ectoparasitic, feeding on the zone just behind the root tips, and causing characteristic swelling or galling (Hooper, 1973; Cohn, 1970; Cohn and Orion, 1970). In both species four juvenile stages are found in the soil, indicating that first-stage juveniles hatch from the eggs (Hooper, 1961; Bravo

and Roca, 1995). The body length of the first-stage juveniles is close to 1.1 mm for both species (Hooper, 1961; Bravo and Roca, 1995). As with the development of *M. hapla* and *M. javanica* (Trudgill, 1995; Trudgill and Perry, 1994), the egg development of the temperate species *L. elongatus* is characterized by a lower  $T_b$  and a higher  $S$ , as compared to the tropical species *L. africanus*. Furthermore, the thermal constants ( $\alpha = T_b \times S$ ) are very similar for the two species (1,342 for *L. africanus*, 1,347 for *L. elongatus*), which supports the hypothesis by Trudgill and Perry (1994) that for biologically similar species  $T_b \times S$  is constant. The temperature ( $T_j$ ) at which the rate of egg-development is equal for both *Longidorus* spp. can be calculated as 22.3 °C (See Fig. 2). It can be shown (Trudgill and Perry, 1994) that, when  $\alpha$  is constant,  $T_i = T_b$  (*africanus*) +  $T_b$  (*elongatus*) = 22.54, which approximates the calculated value for  $T_i$ , and that for both species the optimum  $T_b$  for minimum developmental duration equals  $T_i/2$ .

It is unknown whether the thermal time relationship for egg development of *L. africanus* reflects the temperature requirements for completion of the life cycle. Few data are available, but studies on cyst and root-knot nematodes (Koshy and Evans, 1986; Langeslag et al., 1982; Mugniéry, 1978; Trudgill, 1995) suggest that embryogenesis and completion of the life cycle share a common  $T_b$ . Our results also point to similarities between temperature requirements for *L. africanus* multiplication and egg development, as both processes have temperature optima close to 29 °C and appear to cease at temperatures approaching 35 °C. Reproduction in pots was limited at 20 °C and 17 °C, but in petri dishes eggs still hatched at these temperatures. Assuming similar temperature requirements for embryogenesis and life-cycle completion, it can be hypothesized that (i) only *L. elongatus* can develop at temperatures between ca. 8 and 15 °C, (ii) *L. elongatus* will out-compete *L. africanus* at temperatures between 15 and 22 °C, and (iii) *L. africanus* will out-compete *L. elongatus* at temperatures above 22 °C.

The information presented here provides an important basis for modeling population dynamics, geographical distribution patterns, and yield losses. Further work needs to be done to determine the generation times of *L. africanus* and the importance of other factors (e.g. soil type, host, moisture) affecting its population dynamics.

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