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Degree-day Models for Predicting Egg Hatch and Population Increase of *Criconemella xenoplax*[1]

S. W. Westcott III[2] and P. M. Burrows[3]

Abstract: A degree-day model was derived to predict egg hatch for *Criconemella xenoplax.* Eggs collected from gravid females were incubated in distilled water at constant temperatures of 10–35 C. Sixty-six percent of all eggs hatched between 13 and 32 C, and 42% hatched at 10 C. All eggs aborted above 32.5 C. Between 25 and 32 C, 8.5 ± 0.5 days were required for egg hatch. Degree-day requirement for egg hatch at 10–30 C was estimated to be 154 ± 5 with a base of 9.03 ± 0.04 C. This base of 9 C was adopted in studies of the relationship between degree-days and nematode population increase on *Prunus* seedlings grown 9–11 weeks in a greenhouse. Degree-day accumulations were based upon daily averages from maximum and minimum air temperatures. Ratios of final to initial population densities exhibited an exponential pattern in relation to degree-day accumulations with proportionate doubling increment of 0.100 ± 0.049 every 139 ± 8 degree-days. These results provide a means of predicting nematode population increase under greenhouse conditions and a basis for choosing sampling intervals when evaluating nematode multiplication.

Key words: *Criconemella xenoplax,* degree-day, egg hatch, *Mesocriconema xenoplax,* modelling, nematode, peach, *Prunus,* population increase, ring nematode, temperature.

As with other poikilothermic organisms, nematode development and reproduction are influenced by temperature. Simulation models of nematode population dynamics have been based upon temperature and moisture (6). There is little information describing responses of *Criconemella xenoplax* (Raski) Luc & Raski to temperature. Highest population densities of this nematode in the field have been found during winter months (4,7). Lownsbery (4) suggested that lower densities during summer months were due to high temperatures or seasonal drought in Merced County, California. Too little is known about the biology of this nematode to determine the importance of either factor in limiting its reproductive potential.

Two reports describe the effects of temperature on population increase of *C. xenoplax*: 1) Lownsbery (5) determined change in nematode population densities on grape and peach cultivars grown under greenhouse conditions for 4 months at specific temperatures between 13 and 28 C. Highest nematode population densities, recorded on grape at 26 C, were a 30-fold increase over the initial population of 2,000 nematodes/plant. Final nematode population densities at 21 and 28 C were similar and about half those observed at 26 C, whereas populations at 13 C were unchanged after 4 months. Final population densities on peach were nearly the same as the initial population densities at all temperatures. 2) Seshadri (8) found no differences in nematode population increase on grape grown for 3 months at three temperatures between 22 and 26 C. In that period the nematode population densities increased 74 times the initial population of 300 nematodes/plant.

The objectives of our research were to evaluate the influence of temperature on *C. xenoplax* egg hatch in water and population increase on *Prunus* in a greenhouse by investigating the relationships between the nematode behavior and degree-day accumulations.

Materials and Methods

Egg hatch: Criconemella xenoplax from collections made at the Sandhills Research and Education Center near Elgin, South Carolina, were grown on Nemaguard peach in a greenhouse. Nematodes were extract-

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[2] Visiting Assistant Professor, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

[3] Professor, Department of Experimental Statistics, Clemson University, Clemson, SC 29634-0367.

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ed from soil by elutriation (1) combined with centrifugal-flotation (3). From 10 to 30 gravid females of *C. xenoplax* were transferred by hand to distilled water in 1.5-ml glass dishes and allowed to deposit eggs (ca. 2.5 eggs/female each day) at 25 C for 24 hours. Females were removed and the dishes were transferred to incubators maintained in the dark at constant temperatures of ca. 10, 15, 20, 25, 30, 32, and 35 C (± 2 C). Evaporation from dishes was limited by enclosure in a petri dish. A 25-C treatment was included as a standard for comparison with all other temperatures in each experiment. Three to five replications, each consisting of a single dish containing 30–90 eggs, were incubated at each temperature. Experiments were repeated one to three times.

Egg survival was determined by comparing the number of eggs that hatched with the initial number in a dish. After 2 weeks at temperatures above 30 C, unhatched eggs were transferred to 25 C and were considered aborted if not hatched within another 2-week period.

The time required for hatching was determined by counting the number of second-stage juveniles each day. Counting was discontinued when the remaining eggs showed no signs of development. The average number of days (D) for hatching of all eggs in a single dish was computed and considered a single replicate:

$$D = 0.5 + \sum_{t=1}^{k} t(n_t - n_{t-1})/n_k$$

(equation 1)

where n_t is the number of second-stage juveniles counted on day t, there being k days in a hatching period. An additional 0.5 day was included in D to account for the average age of eggs at the beginning of the incubation period; egg deposition by gravid females was distributed over the entire 24-hour period.

Estimation of degree-day accumulation required for egg hatch was accomplished by adopting a model for expected values, D(T), of the observed average days to egg hatch at incubation temperature T:

$$D(T) = (\delta + 0.5[T - T_o])/(T - \tau)$$

(equation 2)

where τ is the base temperature above which degree-days are accumulated and δ is the degree-day requirement for hatch. This model assumes an accumulation of $(D - 1)(T - \tau)$ degree-days augmented with $0.5(T - \tau)$ and $0.5(T_o - \tau)$ degree-days for the first day of incubation, T_o (= 25 C) being the temperature prior to transfer to incubation temperature. This model was fitted to observed days to egg hatch by weighted nonlinear least squares, weighting by the n_k count used in equation 1.

In subsequent trials to evaluate performance of this model and reliability of estimates for τ and δ, eggs were incubated under a regime of alternating temperatures. Eggs, prepared as before, were placed in a growth chamber that cycled between 11 C (11 hours) and 21 C (13 hours) daily. Average days to egg hatch was determined as before. That experiment was repeated three times.

Nematode population increase: Seeds from 254 accessions representing 16 species of *Prunus* and including 42 interspecific hybrids were selected from collections at Clemson University and the Sandhills Research and Education Center near Elgin, South Carolina. Stratified seeds were planted in steamed sand (60–70 C for 30 minutes) in 150-cm^3 plastic cone pots (38 mm × 20 cm) in a greenhouse with an annual range of air temperatures of 15–37 C. A different mixture of seeds from several accessions was included in each trial. Plants were fertilized weekly with ca. 5 ml of half-strength Hoagland's solution with K_2NO_3 and $CaNO_3$ as the sources of nitrogen (2). Approximately 100 adult and juvenile *Criconemella xenoplax* were suspended in water and added to a depression around the crown of the root of each plant 1 week after planting.

Daily average air temperature in the greenhouse fluctuated widely, especially during sunny periods, and was higher in summer than in winter in spite of efforts to maintain a uniform temperature. Air temperatures exceeded 33 C for several hours during many summer days, but the 24-hour average remained below 30 C. Based on a few observations during the course of these studies, soil temperatures could be 3–6 C below air temperatures during the hottest part of a day and subsequently be near air temperatures the following night. During winter when the greenhouse heater was active, air temperatures fluctuated rapidly (ca. 30–60 minute cycle) in a 4–7-C range.

Duration of these experiments ranged from 61 to 96 days (av. 69 days). After incubation, nematodes were separated from the roots and soil by elutriation for 6.0–6.25 minutes at a flow rate of ca. 60 ml/second (1) combined with centrifugal-flotation (3). An aliquot of the nematode suspension was examined to determine the number of nematodes extractable from each plant (Cf). The estimate for extraction efficiency was 53% when nematodes were added to soil immediately prior to recovery. Assuming extraction efficiency did not vary across experiments, the estimate for the final population (Pf) of nematodes on a plant was derived as:

$$\mathrm{Pf} = \mathrm{Cf}/0.53 \qquad \text{(equation 3)}$$

Maximum and minimum air temperatures were recorded daily at ca. 9:00 a.m. A hygrothermograph also was operated continuously to record duration of air temperature fluctuations during the day. Occasionally, soil and air temperatures were measured and plotted on an hourly basis for up to 3 days.

Accumulated degree-days for each experiment were computed from the average of daily maximum and minimum air temperatures. Base temperature for this calculation was determined from results for egg hatch (9 C) or included as an additional parameter to be estimated when the model was fitted. The relationship between degree-days and expected nematode population increase (Pf/Pi) was formulated as:

$$(\mathrm{Pf}/\mathrm{Pi}) = 1 + \alpha(2^{d/\beta} - 1) \qquad \text{(equation 4)}$$

where Pi is the initial nematode population, d is the degree-day accumulation over the period during which the population increases from Pi to Pf, parameter β is the doubling constant in degree-day units, and parameter α is the proportionate doubling increment. Thus, for example,

when $d = \beta$,
$(\mathrm{Pf}/\mathrm{Pi}) = 1 + \alpha$;
when $d = 2\beta$,
$(\mathrm{Pf}/\mathrm{Pi}) = 1 + \alpha + 2\alpha$;
when $d = 3\beta$,
$(\mathrm{Pf}/\mathrm{Pi}) = 1 + \alpha + 2\alpha + 4\alpha$.

An average of 47 plants was grown in each of 25 trials. Plants supporting final populations of nematodes lower than initial populations were excluded from the results because they are believed to represent failures in establishment of nematode populations. The model (equation 4) was fitted by weighted nonlinear least squares, weighting by the number of plants used to calculate average Pf/Pi values.

An additional set of seven trials was completed to evaluate performance of this model and reliability of estimates for α and β. One of these trials contained 20 Nemaguard and 20 Lovell seedlings, two cultivars commonly used in the peach industry as rootstocks.

RESULTS

Egg hatch: All eggs aborted at constant temperatures above 32.5 C, and 42% ± 16% of eggs hatched at a constant 10 C (Fig. 1). The percentage of egg hatch at nonlethal temperatures varied enormously among replicates. A few eggs were unaccounted for by the end of the observation period in some dishes; presumably the eggs burst at some point during incubation. Aborted eggs were characteristically filled with a dark disorganized material unlike the material in healthy eggs.

At constant 25 C, eggs hatched in ca. 9.4 days; whereas at constant 10 C, eggs hatched in ca. 152 days (Fig. 1). Within a single replicate dish of 30–90 eggs, the range from first to last egg hatched was typically 3 days at 25 C (most eggs hatched

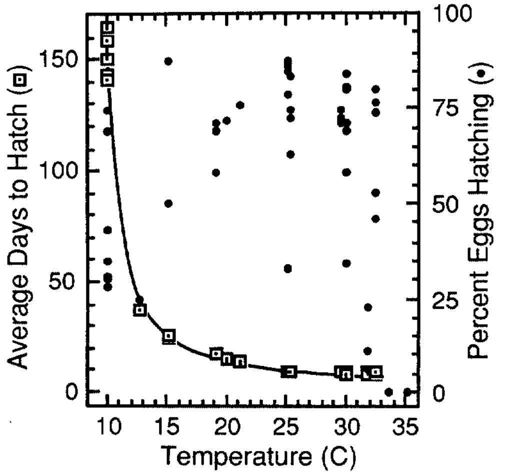

FIG. 1. Relation of temperature to the average time required for eggs of *Criconemella xenoplax* to hatch (⊡) and the percentage of egg hatch at various temperatures (●) when incubated in water. A degree-day model that allows 154 degree-days (base of 9 C) for hatch appears as a solid line.

on day 9), but the range was up to 50 days at 10 C, with egg hatch distributed evenly over this period. From the degree-day model (equation 2) we estimate δ as 154 ± 5 degree-days required for egg hatch, with a base (τ) of 9.03 ± 0.04 C. In the trials with alternating temperatures, eggs hatched after an average of 19.3 days, only 0.7 ± 1.4 days sooner than predicted by the degree-day model with these parameter estimates.

Nematode population increase: Final nematode population counts (Cf) indicated that 94% of inoculated plants supported population increase by the nematode and were retained for use in model parameter estimations. The initial population (Pi) varied from 73 to 159 nematodes/plant; average coefficient of variation about the mean of each Pi was 10%. Across all trials, the average final fresh weight was 3.6 g/plant. The Cf values ranged from 346 to 32,132 nematodes/plant, and the average coefficient of variation was 60%. Degree-day accumulations ranged from 903 to 1,702 degree-days, with a base of 9 C (av. 1,128 degree-days).

With the base of 9 C for calculation of degree-days, the estimate for the proportionate doubling increment (α) was 0.100 ± 0.049 every 139 ± 8 degree-days (Fig. 2A). The results follow the model closely except at the observation made for 1,426 degree-days which was above the predicted value. When the base for degree-day calculation was included as an unknown parameter, rather than 9.0 C, its least squares estimate was 8.9 ± 6.5 C. There is no evidence that 9 C should be rejected as the appropriate estimate for base temperatures.

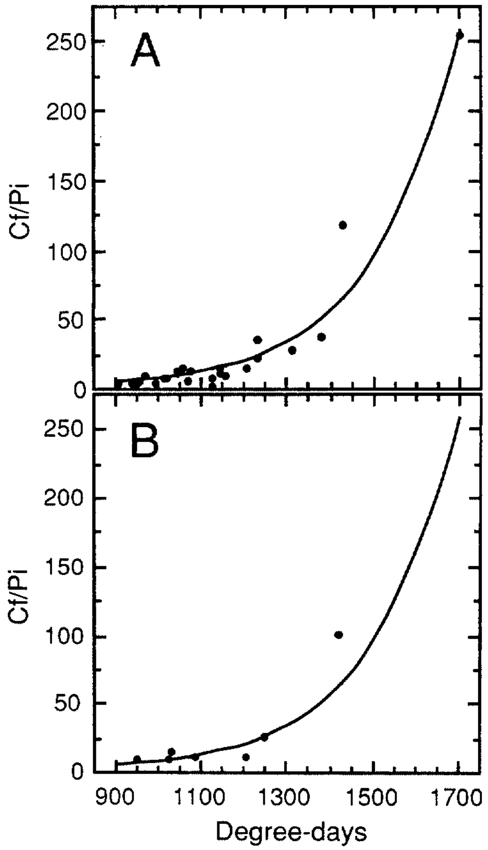

FIG. 2. Observed population increase (Cf/Pi) of *Criconemella xenoplax* grown on *Prunus* seedlings after accumulation of a measured number of degree-days (base 9 C) in greenhouse studies. A) Original set of 25 trials; a degree-day model was fitted to the results and is shown as a solid line. B) Additional set of seven trials. The predicted response appears as a solid line and is based upon the degree-day model using parameter estimates from the original results illustrated in Figure 2A.

No differences in parameter values were detectable when the second set of seven trials was compared with the original trials;

these results and predictions from the original model are shown in Figure 2B. The first observation on the graph (Cf/Pi = 8.5; d = 948) was from a trial composed of seedlings from Lovell and Nemaguard peaches. The average Pf for each cultivar was the same in this experiment. If it is assumed that $\alpha = 0.1$ for these two cultivars, the corresponding doubling constant (β) is 131 degree-days according to the model (equation 4).

DISCUSSION

The response of eggs to constant temperatures between 10 and 35 C provided sufficient information to model egg hatch and determine upper lethal limits. The degree-day model predicts that about 154 degree-days are required for egg hatch. The apparent high precision for the estimation of the base (9.03 ± 0.04) for computation of degree-days does not reflect the variations in actual temperatures that occurred during incubation of eggs (ca. ± 2 C). However, it does indicate that observations over a wide range of temperatures consistently support the use of 9 C as the base for computation of degree-days.

Thomas (9) reported that *C. xenoplax* eggs require about 15 days for egg hatch at 20 C. The model predicts that eggs should hatch in 14 days at this temperature. If the time required for hatch is recalculated based only upon results from 20 C, there is a closer agreement with Thomas' results. An average of 14.9 days for hatch at 20 C was observed after collecting eggs at 25 C for 1 day. If 8 degree-days accumulated during the average of 0.5 day at 25 C, then the duration for egg hatch at a constant 20 C from the time of deposition should be 15.1. If accurate predictions are critical, they should be based upon a model fitted to observations in the temperature range of interest.

Studies with eggs might estimate reasonably some parameters in models that predict population dynamics. This conclusion is supported by the similar estimates for base temperature that were determined from study of egg hatch (9.03 C) and population increase by the nematode in the greenhouse (8.9 C). However, effects of temperature on juveniles and adults may vary from those with eggs and could be critical to accurate modelling of population dynamics. Effects of fluctuating temperatures on eggs, juveniles, and adults must be studied as well to understand effective lethal temperatures and to be able to relate those to conditions in field soil.

Our model for nematode population increase under greenhouse conditions predicts the results well except at the observations made at ca. 1,400 degree-days (Fig. 2). This outcome may indicate that at 1,700 degree-days the nematode population may have reached the carrying capacity of some plants in that trial. Population increase may have slowed or stopped and the average rate of change declined. For accurate evaluation of the maximum rates of nematode population increase, this situation should be avoided.

Lownsbery (5) reported that the highest final nematode populations on grape after 4 months at soil temperatures ranging from 13 to 28 C occurred at 26 C. Although the model for nematode population increase is based upon air temperatures, some useful observations may be made by predicting final populations (Table 1) for the conditions established by Lownsbery (5). Predictions based on the model may underestimate the potential final populations in experiments by Lownsbery because soil temperatures lagged behind air temperatures during high temperature periods of the day in our study. The predictions do not include consideration for extraction efficiency in their system, but if extraction efficiency was ca. 50%, the predictions for 13 C would be similar.

Final populations in experiments by Lownsbery (5) probably were limited by carrying capacity of the plants rather than the rate of population increase at the higher temperatures, assuming that the rate of nematode population increase on grape is as high as that observed on peach in our experiments. On grape, Lownsbery found a positive correlation between final nema-

TABLE 1. Comparison of published results for population increase by *Criconemella xenoplax* on grape with predictions from the degree-day model developed for peaches.

Initial population	Duration (days)	Temperature (C)	Final population	
			Observed	Predicted†
		Lownsbery (5)		
2,000	120	13	2,000	3,991
		18	10,000	45,448
		21	26,500	264,593
		26	60,000	5.24×10^6
		28	24,000	1.73×10^7
		Seshadri (8)		
300	90	22	25,887	10,526
		24	20,742	25,435
		26	19,743	62,017

† Predicted from $Pi(1 + 0.1[2^{d/139} - 1])$; where Pi = initial nematode population density and d = accumulated degree-days at a base of 9 C.

tode populations and root weights, which might be expected if the carrying capacities of plants were reached. His observations that the final populations on peach were not much higher than the initial populations of 2,000 nematodes/plant also could be an expression of carrying capacity for these plants.

In an attempt to refine Lownsbery's determination of the effects of temperature, Seshadri (8) evaluated nematode population increase at soil temperatures of 22, 24, and 26 C. By monitoring nematode populations after 3 months and using initial populations of 300 nematodes/plant, Seshadri established conditions substantially different from those used by Lownsbery. Seshadri did not detect differences in nematode population increase at these temperatures. Under these conditions our model may underestimate the Pf (Table 1). If, however, average soil temperatures in our study were as little as 3 degrees below average air temperatures and extraction efficiency for Seshadri's method was 50%, Pf could range from 21,000 to 126,000 nematodes/plant under Seshadri's conditions. This estimate is near the observed Cf for 22 C and indicates again that at higher temperatures the average carrying capacity of the plants might have been a factor. The possibility of misrepresenting the effects of temperature on nematode population increase are evident again.

The model for estimating nematode population increase on peach seedlings in a greenhouse may be useful to predict the appropriate duration for other experiments employing similar conditions. Its use should be limited to situations where Pi is low and the carrying capacity of the host plants will not be exceeded. Efforts to establish estimates for the carrying capacity for root systems of various sizes and ages of host plants will improve the current understanding of factors that control population dynamics. We also recommend that several intermediate observations be made to assure that the nematode population remains in the exponential phase of growth for the duration of the experiment.

The model for nematode population increase is based on daily maximum and minimum air temperatures. Recording temperatures for these estimates is easy and inexpensive. For many experiments this provides an adequate measure for prediction of the appropriate times for termination. Under some circumstances a model based on soil temperatures may be preferred, and evaluation of temperature variation within the experimental area may be important. These changes would be required to model population dynamics in the field and determine the causes for seasonal fluctuations of field populations that have been observed (4,7).

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