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Effect of Temperature on the Embryogenesis of Geographic Populations of *Rotylenchulus reniformis*

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Abstract: The effect of temperature on the embryonic development of three populations of reniform nematode (*Rotylenchulus reniformis*) from the southeastern United States was studied. The development of eggs from single-cell stage to eclosion of second-stage juvenile was monitored at 20, 25, 30, and 35°C. All populations completed embryogenesis in 7 days at 25°C. The greatest differences among populations in time to completion of embryogenesis were observed at 20 and 35°C. Results at the intermediate temperatures (25 and 30°C) were similar for the three populations. The optimal temperature for embryogenesis was calculated to be 31.4°C for the population from Alabama, 28.4°C for the one from Mississippi, and 37.5°C for the one from South Carolina.

Key words: embryogenesis, *Rotylenchulus reniformis*, reniform nematode, temperature.

Rotylenchulus reniformis is a broadly distributed species in tropical, subtropical, and some temperate regions of the world. It affects a large number of cultivated plants (Robinson et al., 1997; Gaur and Perry, 1991) as an obligate, sedentary, semi-endoparasite of roots. The life cycle of *R. reniformis* was initially described by Linford and Oliveira (1940). Mature females lay one-celled eggs into a gelatinous matrix, where the embryo develops into a first-stage juvenile (J1). The first cuticle molt occurs while the nematode is still in the egg. The second-stage juvenile (J2) emerges from the egg, and subsequent juvenile stages (J3 and J4) remain in the soil until adulthood is reached (Robinson et al., 1997). Eggs are able to hatch in water without the influence of a host plant and juveniles will develop into males and pre-adult females without feeding.

Cotton crop specialists and nematologists report an increase in the distribution and prevalence of reniform nematode in the United States over the last decade (Robinson, 2007). Its incidence has been documented to be on the rise in the southeastern cotton-growing areas and it is considered to have replaced root-knot nematode (*Meloidogyne incognita*) as the major nematode pest of cotton in Mississippi, Louisiana, and Alabama (Robinson, 2007). It is unknown whether this observed increase in importance is related to the emergence of novel, more aggressive populations, or of populations with increased fitness. Although the life history and some management aspects of this nematode are known, most of its basic biology remains to be investigated.

The effects of temperature on embryonic development may comprise an important factor in the ecology and distribution of reniform nematode. The thermal time requirements for embryogenesis in reniform nematode are not known. Sivakumar and Seshadri (1971) studied the embryonic development of this nematode in detail, reporting that eggs develop into second-stage juveniles in six to seven days, but did not include any temperature information in their study. The time necessary for reniform nematode to complete its life cycle has been shown to vary with temperature and host species (Rebois, 1973; Heald and Inserra, 1988). Differences between populations in their sensitivity to temperature have also been observed (Heald and Inserra, 1988). However, there are no studies on the effect of temperature on the embryogenesis of this organism. The thermal requirement for embryonic development can be very high in plant-parasitic nematodes. For example, in *Meloidogyne javanica*, it constitutes 40% of the energy required for development to maturity (Trudgill, 1995a). This understudied aspect of reniform nematode biology could be an important factor in the overall fitness of the nematode and its ability to extend its distribution range. The objective of this study was to compare the effect of temperature on the embryogenesis of three populations of reniform nematode isolated from cotton fields in the southeastern United States.

Materials and Methods

Reniform nematode populations were obtained from cotton fields in Huxford, Alabama (AL), Glendora, Mississippi (MS), and Saint Matthews, South Carolina (SC). These populations were morphologically indistinguishable (Agudelo et al., 2005). Infested field soil was used to increase the nematodes in the greenhouse on soybean (*Glycine max*) cv. Braxton. Seeds were germinated in a germination mix and transferred to one-gallon plastic containers after the first true leaves emerged. Three to four plants were maintained in each container holding infested field soil mixed with a pasteurized sandy loam. The populations were maintained separately for two months prior to the beginning of this study.

After 60 days, infected roots were carefully washed to remove soil particles. Egg masses were removed from roots and gently crushed to release eggs. Individual freshly-laid eggs, in the one-celled stage, were arbitrarily

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selected and transferred to wells of a 24-well plate (VWR, Polystyrene) containing 1 ml of 0.5% water agar. The plates were sealed with Parafilm and incubated in the dark at 20, 25, 30, and 35°C ± 0.5°C. The eggs in each well were examined twice daily to monitor development of the embryo until eclosion of the second stage juveniles. A 24-hour period was added to all data in order to account for the time elapsed between eggs being laid and the transfer to the plates. A minimum of 20 replicates were used for all data averages presented in this study.

The rate of development for each population was expressed as the reciprocal of the number of days required for completion of embryonic development. The cumulative hatch was calculated by successive addition of second-stage juveniles hatched each day until the last eclosion and expressed as a percentage of the total observed. The data was analyzed with Statistical Analysis Software® (SAS, 2003, v9.1, Cary, NC) using the NLmixed procedure and a random effect for plate.

Results

Average days to eclosion: The time necessary for eclosion varied greatest among populations at the lowest (20°C) and the highest (35°C) incubation temperatures (Table 1). Similar average days necessary for eclosion were observed for all populations at the two intermediate temperatures (25 and 30°C). The three populations had an average of seven days to hatch at 25°C. Both MS and SC had an average of 6 days to hatch at 30°C, while AL had an average of 5 days at this incubation temperature. Both MS and SC had an average of 8 days to hatch at 20°C, while AL averaged 10.7 days to hatch at this temperature. At 35°C, AL and SC were similar with an average of 6.0 and 5.7 days to hatch, respectively. The population from Mississippi averaged 8 days to hatch at 35°C.

Table 1. Average days to eclosion for three geographic populations (Huxford, Alabama (AL), Glendora, Mississippi (MS), and Saint Matthews, South Carolina (SC)) at four incubation temperatures (20, 25, 30, 35°C), with average rates, median, mode, range, standard deviation, and number of replicates at each temperature.

Population	Temp. (°C)	Average[a]	Rate[b] (days^{-1})	Median	Mode	Range	Standard Deviation	Replicates
AL	20	10.7	0.09	11	12	7-14	2.14	23
	25	7.1	0.14	7	7	6-9	0.09	24
	30	5.4	0.18	5	5	5-6	0.50	26
	35	6	0.13	6	6	5-8	1.02	25
MS	20	8.8	0.11	9	9	7-10	1.00	21
	25	7.3	0.14	8	8	6-8	0.76	24
	30	6.1	0.16	6	7	5-7	0.86	29
	35	8.1	0.12	9	8	6-11	1.35	20
SC	20	8.5	0.12	9	8	6-10	1.18	24
	25	7.2	0.14	7	8	6-8	0.82	39
	30	6.1	0.16	6	5	5-8	0.98	26
	35	5.7	0.18	6.5	6	5-7	0.80	20

[a] Average of days from single-cell egg to eclosion of second-stage juveniles.
[b] Average rate of development per day.

Average rate of development: The average rate for egg development was the same for MS and SC at 30°C. At 30°C, AL had the same average rate of development as SC at 35°C. The rate of development was linear between 20 and 30°C for all populations. SC was the only population to have a linear rate of development between 20 and 35°C. The rate of development for AL was slowest at 20°C and fastest at 30°C. SC had the same rate of development at 35°C as AL did at 30°C.

Cumulative hatch: When the cumulative hatch was compared among populations and across temperatures, the largest variation occurred at 20 and 35°C (Figure 1). For AL at 20°C, the majority of eclosions occurred 12 days after egg laying. For MS, the majority of eclosions occurred between nine and ten days. SC had the fastest cumulative hatch with most eclosions occurring between eight and nine days. At 25°C, the majority of eclosions for AL occurred at seven days, which was similar to MS and SC. Both MS and SC populations had the majority of eclosions occur between seven and eight days. The results for 30°C were similar across all populations, with most eclosions occurring between five and six days. At 35°C, AL had a similar percentage hatch to SC, with a peak of eclosion between five and six days. The MS population varied greatly from the AL and SC populations, having an eclosion peak between eight and nine days. The AL population had the longest development time to eclosion at 20°C, and 30°C was the best observed hatching temperature for this population. Hatching at 35°C was faster than at 25°C for this population. For MS, 30°C was also the best observed temperature for embryogenesis, but 25°C was more favorable for development than 35°C. For SC, 35°C was more favorable than 30°C. For all populations, hatching at 25°C and 30°C was similar.

SAS analysis: A quadratic regression was used to model the time to eclosion data as a function of incubation temperature. Hypothesis tests for equality of regression functions across locations were conducted. The regression fits allowed for estimation of the optimal temperature associated with embryonic development. A significance level of 0.05 was used for all hypothesis tests. The predicted optimal temperatures for each population were calculated: AL (31.4°C), MS (28.4°C), and SC (37.5°C) ($P < 0.0001$) (Figure 2). There were significant differences ($P < 0.0005$) among populations in their predicted optimal temperatures (inflection point). There was no significant difference between locations when incubation at 25 and 30°C was compared. The effect of temperature was significant across locations ($P < 0.0001$).

Discussion

We observed distinct effects of temperature on embryonic development of the three reniform nematode

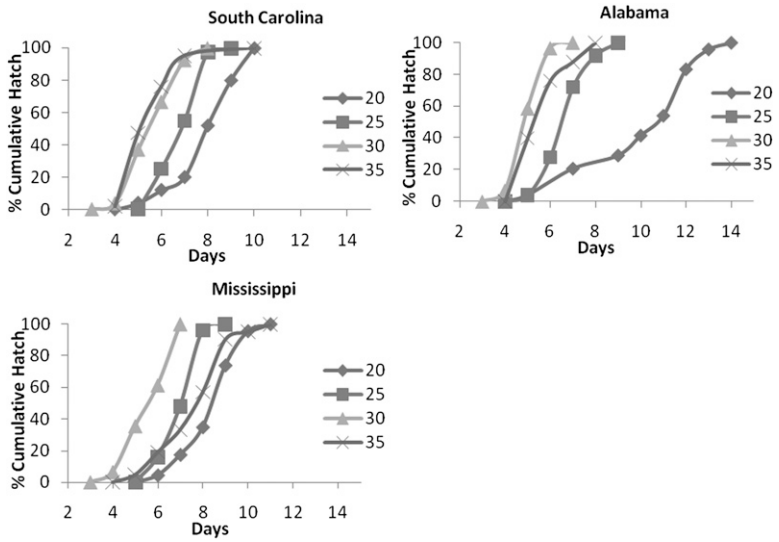

FIG. 1. Cumulative hatch of reniform nematode populations from three geographic locations (Alabama, Mississippi, and South Carolina) at four temperatures (20, 25, 30, and 35°C).

populations included in this study. The population isolated from a cotton field near Huxford, Alabama appears to be the most sensitive to lower temperatures, exhibiting the slowest development at 20°C. This behavior may be explained by the fact that winter temperatures in Huxford are normally 2-4°C warmer than in the other two locations (Table 2). Heald and Inserra (1988) reported a differential sensitivity to low temperatures in a study comparing reproduction of reniform nematode populations from Louisiana, Texas, and Puerto Rico on lettuce. They found that the population from Puerto Rico was unable to reproduce on lettuce at 15°C, and suggested that the populations from Texas and Louisiana were better adapted to cooler temperatures. The fastest rate of development for AL was observed at 30°C and an optimal temperature of 31.4°C was calculated for this population. These temperatures are comparable to those reported by Rebois (1973) in his study of the effect of soil temperature on infection and reproduction of reniform nematode on soybean, where he found that fecundity was highest at 29.5°C. However, in our study we have eliminated the effects of temperature on the plant and all the indirect effects on nematode development through the interactions between the nematode and the plant. This allowed for a more accurate calculation of the optimal temperature for each reniform nematode population.

Of the three locations included in this study, Glendora, Mississippi has the lowest normal high temperatures (Table 2) consistently throughout the year, which may explain why the population from MS had the lowest calculated optimal temperature (28.4°C) of the three. The behavior of the population from South Carolina is more difficult to explain because it exhibited the highest calculated optimal temperature (37.5°C), but this location consistently has the lowest normal low temperatures of the three locations. We can only speculate that this population may have higher plasticity

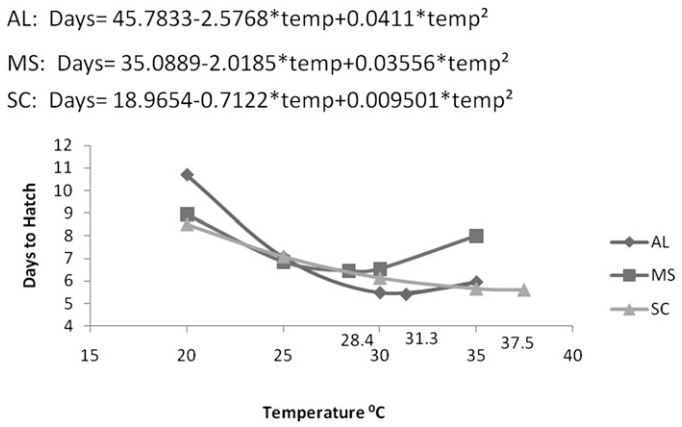

FIG. 2. Quadratic regression fit to number of days to hatch against incubation temperatures for the three populations, Alabama (AL), Mississippi (MS), and South Carolina (SC).

TABLE 2. Normal low and high temperatures for the locations from where the three populations included in this study were isolated. (NOAA National Weather Service http://www.ncdc.noaa.gov/oa/ncdc.html)

	Huxford, Alabama		Glendora, Mississippi		Saint Matthews, South Carolina	
	Normal Low	Normal High	Normal Low	Normal High	Normal Low	Normal High
January	3.3	15.6	1.1	11.7	0.5	13.3
February	5	18.3	3.3	15	1.7	16.1
March	8.9	21.7	7.2	19.4	6.1	20
April	11.7	25.6	11.1	23.9	9.4	24.4
May	16.7	29.4	16.7	28.3	14.4	28.3
June	20.6	32.2	20.6	32.2	18.9	31.7
July	21.7	33.3	22.2	33.9	21.1	33.3
August	21.7	33.3	21.7	33.3	20.6	32.8
September	19.4	31.7	18.3	30.6	17.8	30
October	12.2	27.2	11.7	25.6	10.6	25
November	8.3	21.7	6.7	18.9	6.1	20
December	5	17.2	2.8	13.9	1.7	15

than the other two. Reniform nematode is known to be a highly polymorphic species, for which several phenotypic differences among geographic populations have been reported (Dasgupta et al., 1968; Nakasono, 2004; Agudelo et al., 2005).

Rebois (1973) reported that no nematode reproduction occurred when roots were grown at soil temperatures of 15 and 36°C, and indicated that further research was needed to determine if the higher temperature restricted the host's ability to provide nutrients for the nematode or if there was a direct effect of temperature on the nematode. Our results suggest that embryonic development of reniform nematode is not stopped, or even significantly delayed, at 35°C. Furthermore, the population from South Carolina had the fastest rate of development at this temperature, and a calculated optimal temperature of 37.5°C. Our results indicate that eggs are capable of normal development at 36°C, and that the absence of nematode reproduction observed in other studies at this temperature may be through an effect on the plant and not the nematode.

The optimal temperature for embryogenesis of *Heterodera glycines* is 24°C, with second-stage juveniles failing to emerge from cysts when incubated below 16°C or above 36°C (Alston and Schmitt, 1988). When compared with our results for reniform nematode, these temperatures correlate well with the more temperate distribution of soybean cyst nematode than reniform nematode in the United States. Bird (1972) reported that the optimal temperature for embryogenesis of *Meloidogyne javanica* lies between 25 and 30°C, but he also noted that although embryogenesis is slightly faster at 30°C, more eggs complete development at 25°C. Additionally, he observed that development was twice as fast at 25 and 30°C than it was at 20°C. Our reniform nematode population from Alabama behaved very similarly, developing twice as fast at 30°C (5.4 days on average) than at 20°C (10.7 days on average). We also observed faster development at 25 and 30°C than at 20°C for the other two populations, but the development time was not doubled.

Diez et al. (2003) showed that life-stage development of *M. incognita* was delayed in the presence of equal or higher number of *R. renifomis*, showing that *M. incognita* was more susceptible to antagonism by *R. reniformis* than the reverse. Temperature sensitivity may play a role in the explanation to the perceived displacement of *Meloidogyne incognita* by reniform nematode as the most important nematode pest of cotton in Mississippi, Louisiana, and Alabama (Robinson, 2007). Other authors have discussed ecological advantages of reniform nematode over other nematode species, such as its ability to remain viable in dry soils over long periods of time (Gaur and Perry, 1991; Koenning, 2004; Robinson, 2007). The anhydrobiotic stage may extend the life cycle by up to two years, thus facilitating survival even when a suitable host is absent or allow the nematode to be dispersed long distances in dust storms or when farming equipment is moved from area to area.

In his review of host and plant temperature effects on nematode development rates and nematode ecology, Trudgill (1995b) discusses the ecological significance of linear relations between temperature and development rates. However, he also briefly mentions that several clearly non-linear relations have been demonstrated and cites examples in *Aphelenchus avenae* and *Xiphinema diversicaudatum*, among others. In contrast to the better known cases of these types of studies, i.e. for *Meloidogyne*, *Heterodera*, and *Globodera* species, where linear relations are described, we found a quadratic relationship between temperature and development rate for reniform nematode. This type of relation is less straightforward to use for predictive purposes than a linear relationship.

Reniform nematode populations may have the ability to increase distribution range through variants able to reproduce in a wider temperature range, allowing for increased numbers in new areas. According to a report presented in 2000 by the US Global Change Research Program (USGCRP), average temperatures in the United States have increased between 0.6°C and 2°C for the coastal Northeast, upper Midwest, and the

Southwest over the past 100 years. The largest warming across the nation has occurred in winter. We do not have evidence to support that this increase in temperature has allowed the range of distribution for reniform nematode to expand and/or if it has provided longer seasons for increased reproduction. We do know, however, that reniform nematode populations present in the cotton-growing region of the United States have different abilities to respond to variations in temperature.

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