Supplement to the Journal of Nematology 29(4S):635–639. 1997.

Minimal Growth Temperature of *Pasteuria penetrans*[1]

Z. X. CHEN[2] AND D. W. DICKSON[3]

Abstract: *Pasteuria penetrans* is an obligate, mycelial, and endospore-forming bacterial parasite of *Meloidogyne* spp. with promise for the management of root-knot nematodes. Our objective was to use regression analysis of developmental time (days) to various temperatures to determine the minimal temperature for growth and development of *P. penetrans* in *Meloidogyne* spp. The data set for regression originated from a previously published report. The data fit well to hyperbolic equations. For various developmental stages of *P. penetrans*, the minimal growth temperature ranged from 16.7 °C to 17.8 °C and averaged 17.2 °C.

Key words: bacterium, biological control, degree-day, developmental threshold, endospore, *Meloidogyne* spp., minimal growth temperature, *Pasteuria penetrans*, root-knot nematode, temperature.

Pasteuria penetrans (Thorne) Sayre & Starr is an obligate, mycelial, and endospore-forming bacterial parasite of *Meloidogyne* spp. that has shown potential as a biological control agent against root-knot nematodes (Chen et al., 1996; Dickson et al., 1994). Future successful use of the organism depends on our ability to manipulate its development. *Pasteuria penetrans* has not been grown on artificial media (Bishop and Ellar, 1991). Currently, the parasite must be reared on *Meloidogyne* spp., which, in turn, must be reared on susceptible plants (Sharma and Stirling, 1991) or in a nematode-root explant system (Verdejo and Jaffee, 1988). The former method, using potted tomato plants in a greenhouse or growth chamber, is widely used for production of endospores of *P. penetrans*. Several factors have been shown to affect the development of *P. penetrans* in such culture. Ammonium nitrate is capable of slowing the development of *P. penetrans* (Z. X. Chen and D. W. Dickson, unpubl.), and some chemicals, such as chloropicrin and methyl bromide, prevent development but do not prevent attachment (Freitas, 1997). However, the most important factor affecting development may be temperature (Hatz and Dickson, 1992; Serracin et al., 1997). Bacteria have a characteristic optimal growth temperature at which they exhibit their highest growth and reproduction rates, a minimal growth temperature below which they are metabolically inactive and do not demonstrate growth, and an upper temperature limit beyond which they fail to grow (Atlas and Bartha, 1993). Both the optimal growth temperature and the ranges of temperatures that microorganisms can tolerate determine their distribution and ecological significance (Atlas and Bartha, 1993).

The production of endospores increases with temperatures above 20 °C, and an optimum temperature for growth has been reported as 35 °C (Hatz and Dickson, 1992). The objective of this study was to provide further information on the temperature requirements of a Florida isolate of *P. penetrans* by determining the minimal growth temperature for its development.

Materials and Methods

Data source: All data were derived from a publication by Hatz and Dickson (1992). The development of *P. penetrans* in the nematode pseudocoelom was categorized into nine stages as follows: A) early vegetative stage, B) later stage, C) mycelial branches, D) fragmentation, E) sporangial development, F) sporangium size increasing, G) endospore development, H) sporangia separation, and I) mature sporangium. The developmental time (days) for each stage at various temperatures was reported. Stages A, C, D, F, H, and I were selected for this study because they are the most definitive growth stages.

Received for publication 3 February 1997.
[1] Florida Agricultural Experiment Station Journal Series No. R-05582. Supported in part by Southern Region IPM Grant No. 93-34103-8396.
[2] Graduate Research Associate, and [3] Professor, Entomology and Nematology Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL, 32611-0620.
E-mail: dwd@gnv.ifas.ufl.edu

Data analysis: For each selected developmental stage of *P. penetrans,* a hyperbolic equation was used to describe the relationship between time (days) and temperatures. The minimal temperature was derived from the hyperbolic equation (Sentilles, 1989):

$$y = a + \frac{c}{x - b} \quad (1)$$

where y is the mean time (days) for development of each selected stage of *P. penetrans;* x is temperature (°C); c is the reciprocal of the slope parameter for the linear regression of x against $1/(y - a)$ (see Equation 3 in results and discussion section), approximate to the thermal constant (K); a is the horizontal asymptote, approximate to the minimal growth period at most favorable conditions; and b is the vertical asymptote, approximate to the minimal growth temperature requirement at which temperature y approaches plus infinity. Here, b is defined as the minimal growth temperature. A diagram of Equation 1 is shown in Fig. 1. All calculations were accomplished by using a Curve Fit module in SigmaPlot (SPSS, Chicago, IL). The parameter a in Equation 1, however, was empirically determined as 3, 5, 6, 8, 12, and 15 days for stages A, C, D, F, H, and I, respectively, before computing the data.

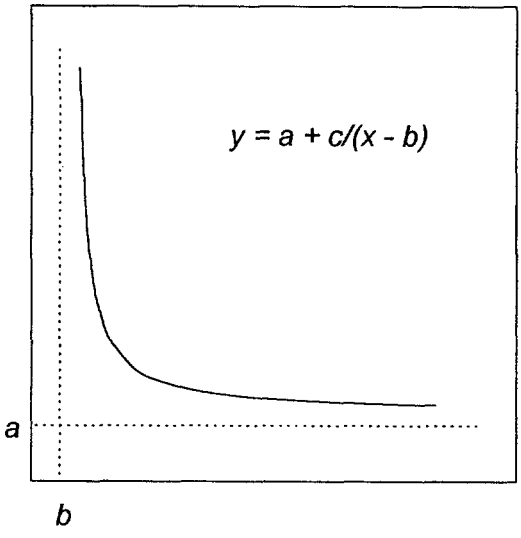

FIG. 1. Diagram of hyperbolic equation of $y = a + c/(x - b)$. The variables and parameters of the equation were defined as follows: y is the mean developmental time (days) of each stage of *Pasteuria penetrans;* x is temperature (°C); c is the thermal constant (degree days); a is the horizontal asymptote, approximate to the minimal growth time of *P. penetrans* growing under most favorable conditions; and b is the vertical asymptote, approximate to the minimal growth temperature (threshold of development) of *P. penetrans* at which temperature y approaches plus infinity.

RESULTS AND DISCUSSION

Rate of development rather than time of development has generally been used in estimating minimal growth temperature for insects and nematodes (Bastian and Hart, 1991; Ferris et al., 1978; Jackson and Elliott, 1988), and the subject was recently reviewed by Trudgill (1995). Rearranging and taking the reciprocal of Equation 1, then,

$$\frac{1}{y - a} = \frac{x - b}{c} \quad (2)$$

let $y' = y - a$, $b' = 1/c$, $a' = b/c$, rewrite Equation 2, then,

$$\frac{1}{y'} = -a' + b'x \quad (3)$$

where $1/y'$ is the rate of development of *P. penetrans,* and a' and b' are the intercept and slope parameters of the linear regression of the rate of development to temperature.

Apparently it is easier to use developmental rate (1/time) for linear regression; however, several complications arise when using rate (Equation 3) instead of time (Equation 1) for regression analyses (Kramer et al., 1991). In least-square estimations, one tries to minimize the function $f(Y - y) = \Sigma(Y - y)^2$, where Y is the predicted response value and y is the observed response value. Minimizing the squared error for rate is not equivalent to minimizing the squared error for time, and the parameter estimation using time was superior to that of rate (Kramer et al., 1991). Thus, we regressed time using Equation 1.

The relationships between the growth time and temperature fit well using Equation 1 (Fig. 2). Coefficients of determination (r^2), ranging from 0.969 to 0.997, were significant in all regressions ($P \leq 0.05$). The minimal growth temperature for the various stages of *P. penetrans* (parameter b, Eq. 1)

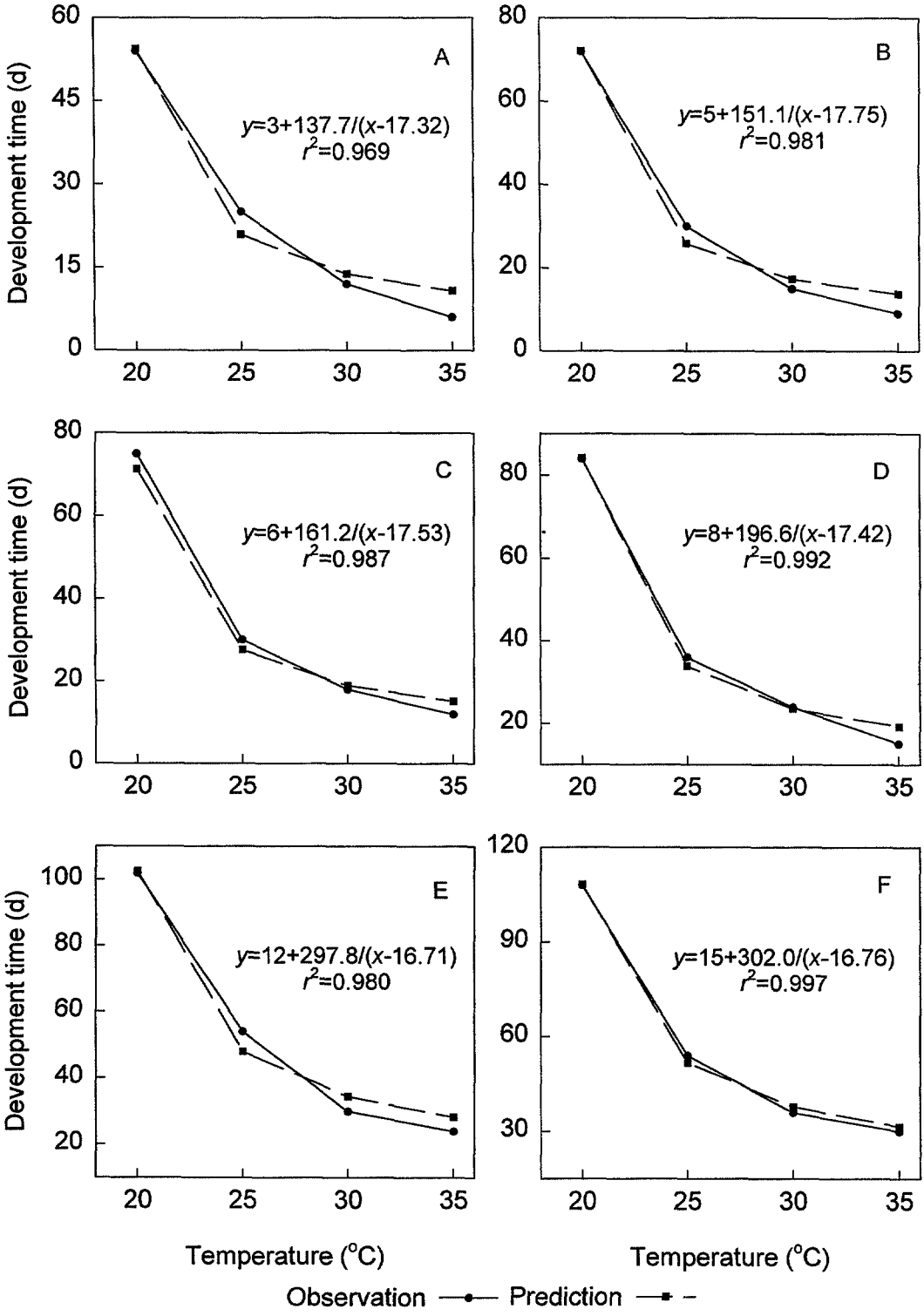

FIG. 2. Regression of the developmental time (days) of various stages of *Pasteuria penetrans* to temperatures. Definitions of the developmental stages were as reported by Hatz and Dickson (1992). Six of the nine developmental stages reported by Hatz and Dickson (1992) were selected for regression analyses: A) Early vegetative stage. B) Mycelial branches. C) Fragmentation. D) Sporangium size increasing. E) Sporangia separation. F) Mature sporangium.

was determined to range from 16.7 °C to 17.8 °C. The narrow temperature range suggests that *P. penetrans* might have the same minimal growth temperature requirement for all developmental stages. The mean minimal growth temperature for all life stages of *P. penetrans* was 17.2 °C. The thermal constants (parameter *c*, Eq. 1) for stages A, C, D, F, H, and I were approximately 138, 151, 161, 197, 298, and 302 degree-days, respectively (Fig. 2A–F).

It is known that temperature-growth time curves fit hyperbolae only for a certain range of temperatures. The curve departs from the hyperbola at either end of the favorable range, resulting from both high- and low-temperature inhibition. Consequently, some authors have suggested that only the data at the favorable range may be used to estimate the parameters of regressions (Jackson and Elliott, 1988; Trudgill, 1995). The coefficients of determination in our regressions were close to 1, indicating that the data fit well to the hyperbola and that the tested temperatures were in the favorable range for the development of *P. penetrans*.

The horizontal asymptote *a* is frequently set to zero to simplify computation (Equation 1) (Bastian and Hart, 1991; Ferris et al., 1978; Jackson and Elliott, 1988). Theoretically, *a* is the minimal growth time of *P. penetrans* at most favorable conditions. We estimated these *a* values to be 3, 5, 6, 8, 12, and 15 days for stages A, C, D, F, H, and I, respectively. The regressions of growth time to temperature, however, were significant for all developmental stages when the *a* values were set to zero ($P \leq 0.05$). Consequently, in the case where *a* is set to zero, the calculated thermal constants for stages A, C, D, F, H, and I were 179, 216, 239, 313, 495, and 560 degree-days, and minimal growth temperatures were 16.7, 17.0, 16.8, 16.3, 15.2, and 14.8 °C, respectively. The minimal growth temperature for each developmental growth stage was more variable in this case than when we used different *a* values for each. When *a* values were set to zero, the mean minimal growth temperature for all developmental stages of *P. penetrans* was 16.1 °C.

Degree-days have been used to predict the development of *P. penetrans* (Bird, 1986; Serracin et al., 1997; Sharma and Stirling, 1991; Stirling, 1981). Since we had different minimal growth temperatures based on the values used for determining *a*, the calculation of degree-days was different. For calculating degree-days (DD) using minimal growth temperature (*b*) of 16.1 °C, the equation is $DD = D(T - b)$, where T is the temperature (°C) and D is the growth time (days). Otherwise, using minimal growth temperature of 17.2 °C, the equation is $DD = (D - a)(T - b)$ (Fig. 2A–F).

Pasteuria penetrans is considered a mesophilic bacterium (Hatz and Dickson, 1992). Thus, this endospore-forming parasite of nematodes may have a high minimal growth temperature. An accurate determination of the minimal growth temperature is currently impossible without in vitro cultivation. However, using regression analyses, we determined the minimal growth temperature to be approximately 17 °C, which is considerably higher than the 10 °C minimal growth temperature required for *M. arenaria* (Ferris et al., 1978). Minimal growth temperature is an important parameter for understanding the biology of *P. penetrans*. The life cycle of *P. penetrans* was considered to be synchronized with the development of root-knot nematodes (Bird, 1986; Mankau, 1981). However, this concept was challenged by some recent observations. Various developmental stages of *P. penetrans* were observed simultaneously in the pseudocoelom of root-knot nematodes (Chen et al., 1997). *Pasteuria penetrans* also develops in second-stage juveniles (D. W. Dickson, unpubl.; Giblin-Davis et al., 1990) and males (Dickson et al., 1994; Page and Bridge, 1985) of *Meloidogyne* spp. The different minimal growth temperature requirements of *P. penetrans* and *M. arenaria* further verify that the development of both organisms is not synchronized. At low temperature, the two organisms may undergo two completely different life cycles, with *M. arenaria* completing its life cycle and the development of *P. penetrans* being inhibited. Thus, understanding minimal growth temperature may facilitate our ability to amplify the bacterium in field

situations, as well as improve our ability to cultivate the bacterium in vivo.

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