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THERMAL CONSTRAINTS TO POPULATION GROWTH OF BACTERIAL-FEEDING NEMATODES

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Summary—Bacterial-feeding nematodes are important participants in decomposition pathways and nutrient cycles in soils. The contribution of each species to component processes depends upon the physiology of individuals and the dynamics of populations. Having determined the effects of temperature on metabolic rates of several species of bacterial-feeding nematodes, we now present the effects of temperature on population growth rates and relate those data to observed field dynamics. Of nine species of bacterial-feeding nematodes screened for reproductive performance at 20°C, finite rates of population increase ranged from 4.833 d^{-1} for *Caenorhabditis elegans* Dougherty to 1.160 d^{-1} for *Panagrolaimus detritophagus* Fuchs. Species in the family Rhabditidae generally reproduced more rapidly than those in the Cephalobidae. From the nine species, finite rates of population increase and instantaneous population growth rates were measured at temperatures between 10 and 35°C for *Acrobeloides bodenheimeri* Thorne, *A. buetschlii* Steiner and Buhrer, *Bursilla labiata* Andrássy, *Caenorhabditis elegans*, *Cephalobus persegnis* Bastian, and *Rhabditis cucumeris* Andrássy. *Caenorhabditis elegans* at 20°C had the maximum finite rate of population increase while *R. cucumeris* at 35°C had the minimum rate (4.540×10^{-25} d^{-1}). We utilize the geometric mean of the finite rates of population increase (d^{-1}) as an integral measure of the innate capacity of a species to maintain reproduction across temperatures. The geometric mean varied from 1.03×10^{-4} for *R. cucumeris* to 1.45 for *Cephalobus persegnis*. In the field, temperatures exceeded the upper thermal threshold of *R. cucumeris* for a significant portion of the 1993 growing season. Population dynamics of this nematode closely matched predicted trajectories. Differences in population growth rates may partially explain the amount of N mineralized by each species. © 1997 Elsevier Science Ltd

INTRODUCTION

Microbial-feeding nematodes, along with protozoa, are the primary grazers of bacteria and fungi in the soil. The grazers function in decomposition and nutrient-cycling pathways by stimulating microbial activity and excreting mineral nitrogen. In these pathways, microbial-feeding nematodes may also serve as prey to higher trophic organisms (e.g. predatory nematodes, mites and nematophagous fungi). The trophic group is composed of numerous taxa which differ qualitatively and quantitatively in their functional roles in food webs. Although identification requires skill, they are among the smallest soil-inhabiting organisms for which we can delineate the functions of individual species (Freckman, 1994). The potential contribution of each nematode species to ecosystem processes depends upon the availability and quality of habitat, the metabolic and growth rates of individuals, and the dynamics and size of populations.

Temperature fundamentally affects the physiological processes and population dynamics of most nematodes. Metabolism, embryogenesis, egg-hatch, growth and activity are affected and each process may have different thermal "optima" or constraints (reviewed in Nicholas, 1975; Van Gundy, 1985). For a limited number of bacterial-feeding taxa, the effects of temperature have been measured on respiration (Santmeyer, 1956; Anderson, 1978; Dusenbery *et al.*, 1978; Procter, 1987; Ferris *et al.*, 1995), fecundity (Sohlenius, 1968; Popovici, 1973; Greet, 1978; Grewal, 1991), development (Sohlenius, 1968; Sohlenius, 1973; Yeates, 1970; Schiemer *et al.*, 1980; Ferris *et al.*, 1996a) and activity (Dusenbery *et al.*, 1978). Yet temperatures for optimal (i.e. maximal) physiological rates may not support optimal rates of population growth. For instance, high respiration rates may occur when an organism is physiologically stressed (Atlas and Bartha, 1993).

Measurements of population growth integrate the physiological and behavioral attributes of individuals. The effects of temperature on population development have also been investigated for certain bacterial-feeding nematodes (Nicholas, 1962; Popovici, 1973; Sohlenius, 1973; Anderson and Coleman, 1982; Procter, 1984), though generally at temperatures conducive to growth. Within the lower and upper thermal tolerances of many poikilotherms, both physiological and population-growth

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rates increase linearly or log-linearly with increasing temperatures. This assumption underlies predictions of individual and population development based on heat-unit accumulation, or degree-days, across a range of temperatures (Curry and Feldman, 1987). Above the thermal maximum, however, development rates are either assumed to remain constant, to decline at some unknown rate, or to cease entirely.

Having determined the effects of temperature on metabolic rates for several species of bacterial-feeding nematodes (Ferris *et al.*, 1995), we now report on thermal effects at the population level to better characterize the potential roles of these species in agricultural soils. We hypothesize that the population growth rates of bacterial-feeding nematodes change in a species-specific manner with changes in temperature. Our objectives were: (1) to measure population "growth" rates of nematode species across a range of temperatures; (2) to identify the maximum and minimum temperatures conducive to growth for each species; and (3) to assess the role of physiological tolerances on population dynamics in the field.

MATERIALS AND METHODS

Origin and isolation of bacterial-feeding nematodes

Between 1991 and 1992, *Acrobeloides bodenheimeri* Thorne, *A. buetschlii* Steiner and Buhrer, *Bursilla labiata* Andrássy, *Cephalobus persegnis* Bastian, *Cruznema tripartitum* Zullini, *Panagrolaimus detritophagus* Fuchs, and *Rhabditis cucumeris* Andrássy were isolated from the Sustainable Agriculture Farming Systems (SAFS) project at the University of California at Davis (for a discussion of the SAFS project, see Temple *et al.*, 1994). At weather stations adjacent to the SAFS project, daily maximum soil temperatures vary seasonally from 5 to 34°C at 10 cm depth under bare ground and from 6 to 30°C under grass sod. Soil temperatures during the growing season for tomatoes (the primary economic crop in the project) range from 15 to 30°C at 10 cm depth under grass sod. *Diploscapter coronata* Cobb was isolated from a soil sample taken from Holtville in the Imperial Valley of southern California.

Nematodes were extracted from soil using a semi-automatic elutriator and sugar centrifugation (Barker, 1985). Approximately 1 ml of the bulk nematode suspension was placed onto water agar. After about 1 week, single females of bacterial-feeding types, identified by stoma structure, located near eggs were hand picked and placed onto individual dishes of nematode growth medium (NGM) (Sulston and Hodgkin, 1988). Bacteria associated with the nematodes flourished and provided an adequate food source. A laboratory culture of *Caenorhabditis elegans* Dougherty var. Bristol (wild type strain N2) was used as a comparative standard. Fresh stock cultures of all nematode species were maintained on NGM with associated bacteria at room temperature except for *R. cucumeris*, which was maintained at 17°C due to its inability to consistently reproduce at ambient laboratory temperature.

Prior to experimentation, nematodes were brought into gnotobiotic culture with *Escherichia coli* strain OP50 using a method modified from Grewal (1991). Individuals were rinsed from the surface of a stock culture with 10 ml of deionized water. The rinsate was centrifuged for 2 min at 740 **g**, and the supernatant was discarded. The nematodes were resuspended in 5 ml of sterile deionized water and stored at room temperature overnight to allow the nematodes to digest, or void, bacteria in their intestines. An equal volume of 0.1% "Thimerosal" (Sigma, St Louis, MO, U.S.A.) solution (w/w) was then added to kill any bacteria contaminating the surfaces of nematodes and the mixture was gently agitated for 30 s. An aliquot of the nematode suspension was placed on a NGM plate on the opposite side from an *E. coli* lawn that had grown at 35°C for 24 h. After 12–16 h, nematodes which had migrated to the *E. coli* were transferred to fresh NGM dishes.

Growth rates for all species

Ten to 15 replicates of 0.1 x nutrient agar amended with cholesterol (NAC) (0.05% Bacto-peptone, 0.03% yeast extract, 0.01% NaCl, 1.5% Bacto-agar, and 0.0005% cholesterol [5 mg ml^{-1} ethanol]) in 60 × 15 mm plastic Petri dishes were each inoculated with 10 μl of a turbid suspension of *E. coli* in sterile deionized water. The suspension was placed in the center of the medium and allowed to dry in a laminar flow hood. The dishes were sealed with laboratory film and stored at 35°C for 18–24 h.

When the *E. coli* lawn had formed in each dish, nematodes were rinsed with sterile deionized water from the surface of a gnotobiotic stock culture into an autoclaved glass Petri dish. Using aseptic technique, a single male and/or a fourth-stage juvenile, depending on the reproductive strategy of the species, was placed 1.5 cm from the edge of the *E. coli* lawn. Juveniles, expected to become females, were identified on the basis of size characteristics (Ferris *et al.*, 1995). Juveniles developed into males on fewer than 5% of all dishes containing amphimictic species. Dishes were left with lids ajar in a laminar flow hood to allow any condensate to evaporate. The dishes were sealed with laboratory film and stored at 20 ± 0.5°C. Each replicate (i.e. each Petri dish) was observed daily for the onset of oviposition, after which, the total number of vermiform nematodes was counted daily for at least 1 week or until there were more than 800 nema-

todes. The onset of oviposition was conservatively set at the time of the observation prior to the detection of eggs. Replicates were discarded if they became contaminated, if a parent nematode climbed the edge of the Petri dish, or if the juvenile was male. This procedure was repeated for the nine nematode species.

Calculating r and λ

To ensure that calculations were based on observations made while populations were in an exponential growth phase, the observations from all replicates for one species at one temperature were pooled and fitted to a logistic growth model

$$N_t = \frac{K}{(1 + Be^{-rt})}.$$

In the model, N_t is the number of vermiform nematodes at time t; K is the estimated "carrying capacity" or maximum number of individuals that the available resources can support; B equals $(K - N_0)/N_0$; e is the natural base; r is the instantaneous growth rate; and t is time in units of hours or days after the onset of oviposition. Data were fitted using the Microsoft Excel add-in program Xlmath, which iteratively alters parameters in a function to minimize the sum of the squared deviations between observed and predicted values. After the curve was fitted, observed points beyond $K/2$ or beyond the time to $K/2$ were considered to be outside the exponential growth phase and were excluded from further analyses.

From the equation for exponential growth

$$N_t = N_0 e^{rt}$$

where N_t, e, r, and t are defined as before, and N_0 is the size of the population at the start of an experiment (either one or two individuals), it follows that

$$r = \frac{\ln\left(\frac{N_t}{N_0}\right)}{t}.$$

Therefore, a linear regression of N_t/N_0 vs t provided an estimate for r, the instantaneous growth rate. Because

$$N_t = N_0 \lambda^t$$

it follows that e^r provides an estimate of λ, the finite rate of increase. Both continuous population growth rates and finite rates of increase were calculated on a daily and hourly basis. Since species were observed sequentially, rates were not subjected to analysis of variance. However, 95% confidence intervals were calculated for each rate.

Growth rates across temperatures

Of the original set of nematodes, *A. bodenheimeri*, *A. buetschlii*, *B. labiata*, *Caenorhabditis elegans*, *Cephalobus persegnis*, and *R. cucumeris* were selected for further investigation (for justification, see Results). Replicates for each nematode were prepared as before and were maintained at 15 ± 0.3°C, 25 ± 0.4°C, 30 ± 0.2°C or 35 ± 0.2°C, respectively, in an upright incubator. Replicates were kept at room temperature for less than 15 min each time observations of population development were made. Separate replicates were incubated and observed in a walk-in cooler at 10 ± 1.0°C.

If a temperature proved lethal to a species, a population depletion curve analysis (Silvertown, 1987) was conducted for that species. Six replicates of 0.1xNAC were inoculated with *E. coli*, and nematodes were collected as before. The nematode solution was poured into a sterile test tube and enough of the solution was applied to provide approximately 100 to 150 vermiform nematodes per replicate. Plates were left with lids ajar in a laminar flow hood to allow excess liquid to evaporate. Initial populations were counted and incubated. The number of living nematodes was counted daily for each replicate, except for *Caenorhabditis elegans* and *R. cucumeris* at 35°C which were observed every 2 h. Nematodes were classified as dead if they appeared ruptured or devoid of body contents, or failed to move after mechanical stimulation (Zimmerman and Cranshaw, 1990); no eggs were produced under these conditions. However, inactive nematodes may have simply been in a dauer state, an alternative development stage specialized for long-term survival. After all nematodes became inactive, replicates were moved to room temperature to allow any dauer larvae to continue to develop. Because no dauer larvae were found, λ and r were estimated as described above and will be referred to as growth rates even though, in these environments, the variables describe non-growth situations.

Estimating the thermal growth function

To interpolate between observed data points, we fitted a poikilotherm model (Schoolfield *et al.*, 1981) to the data using the curve-fit function in Sigmaplot (v 5.00, Jandel Corporation). The model has the form

$$\lambda(T) = \frac{T \cdot \exp(a1 - \frac{a2}{T})}{1 + \exp(a3 - \frac{a4}{T}) + \exp(a5 - \frac{a6}{T})},$$

where $\lambda(T)$ is the population growth rate at temperature, T; T is the temperature in °C + 273; and $a1$–$a6$ are curve-fit parameters. The parameters $a1$–$a6$ were iteratively altered until the sum of squares of the residuals was minimized. To estimate the basal temperature, the minimum temperature required for populations of a species to grow, straight lines were fitted to the most linear portion of observed data points (r [h^{-1} and d^{-1}]); data were

not transformed. From each of these lines and from the poikilotherm model, we extrapolated to find the temperature at which population growth stopped. The "optimal" growth temperature, at which the population growth rate was maximal, was identified from the poikilotherm model. We estimated the upper threshold temperature, beyond which temperatures become lethal to a species, from the poikilotherm model and by linear interpolation between the observed population growth rates at the two temperatures which bracketed the transition from a growth- to a lethal-environment.

Field assessment

In 1993, plots of organically-grown tomatoes in the SAFS project were sampled 10 times at approximately 2-week intervals. For each of four plots, a soil sample consisted of 30 cores (2.5 cm dia x 15 cm depth) which were bulked and mixed. Nematodes were extracted from a 350–400 cm^3 subsample, counted, and identified to genus or species (Ferris *et al.*, 1996b). Nematode counts were not corrected for extraction efficiency.

Daily maximum and minimum soil temperatures were collected for each Julian day (JD) of the growing season from two weather stations adjacent to the SAFS project. At one station, soil was bare and non-irrigated. At the other station, soil was covered with sod and irrigated regularly. Days when maximum soil temperatures at either station exceeded laboratory estimates of upper thermal thresholds were tallied, respectively, for the five field isolates. We then simulated temperature-dependent dynamics for each nematode species assuming worst-temperature conditions. If the range of daily soil temperatures across weather stations was conducive to growth, growth occurred at the slowest rate allowable within that range of temperatures. If temperatures exceeded thermal tolerances, death occurred at the maximum rate within that range of temperatures. The predicted dynamics, expressed as a percentage of the maximum simulated population size, were then compared with observed population data, expressed as a percentage of the maximum population encountered in any replicate over time. For each sampling date, differences between observed and predicted values were analyzed for deviation from zero using the Student's *t*-test with a Bonferroni adjustment to maintain a family level of significance at $\alpha = 0.05$.

RESULTS

Population growth rates for bacterial-feeding nematodes at 20°C

All of the nine species originally screened were able to reproduce at 20°C (Fig. 1). Finite rates of population increase ranged from 4.833 to 1.160 d^{-1}. The list of species could be divided into six groups

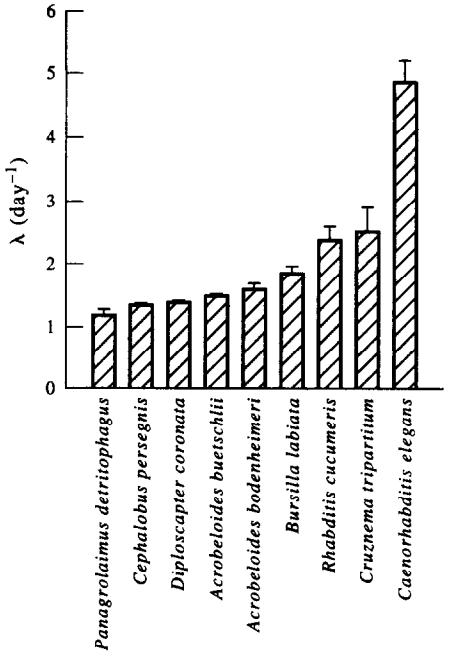

Fig. 1. Finite rates of population increase (d^{-1}) for nine species of bacterial-feeding nematodes at 20°C. Bars indicate 95% confidence intervals.

based on similar degrees of reproduction. *Caenorhabditis elegans* exhibited the greatest population growth rate which was nearly twice as fast as the next closest species, *Cruznema tripartitum*. Of the nematodes isolated from field soil, *Cruznema tripartitum* and *R. cucumeris* demonstrated comparable finite rates of increase of 2.476 and 2.348 d^{-1}, respectively. At 20°C, populations of these species grew faster than all other field isolates. *Bursilla labiata*, with a rate that was 37% of the maximum observed value, was the only species in the third group. *Acrobeloides bodenheimeri* and *A. buetschlii* made the fourth group with intermediate growth rates, which were approximately 1.25-fold greater than the minimum observed rate. The fifth group was composed of *Cephalobus persegnis* and *D. coronata*. *Panagrolaimus detritophagus*, the sole member of the final group, reproduced more slowly than any other species, at a rate 25% of that for *Caenorhabditis elegans* and 47% of that for *Cruznema tripartitum*.

With the exception of the *Acrobeloides*-group and the *Panagrolaimus*-group, one species was selected from each category for determination of growth rates across temperatures. *Panagrolaimus* was excluded from further analysis due to its proclivity to climb the edge of the dish; this behavior created a significant research problem. Both species of *Acrobeloides* were included for further investigation because of their taxonomic similarity.

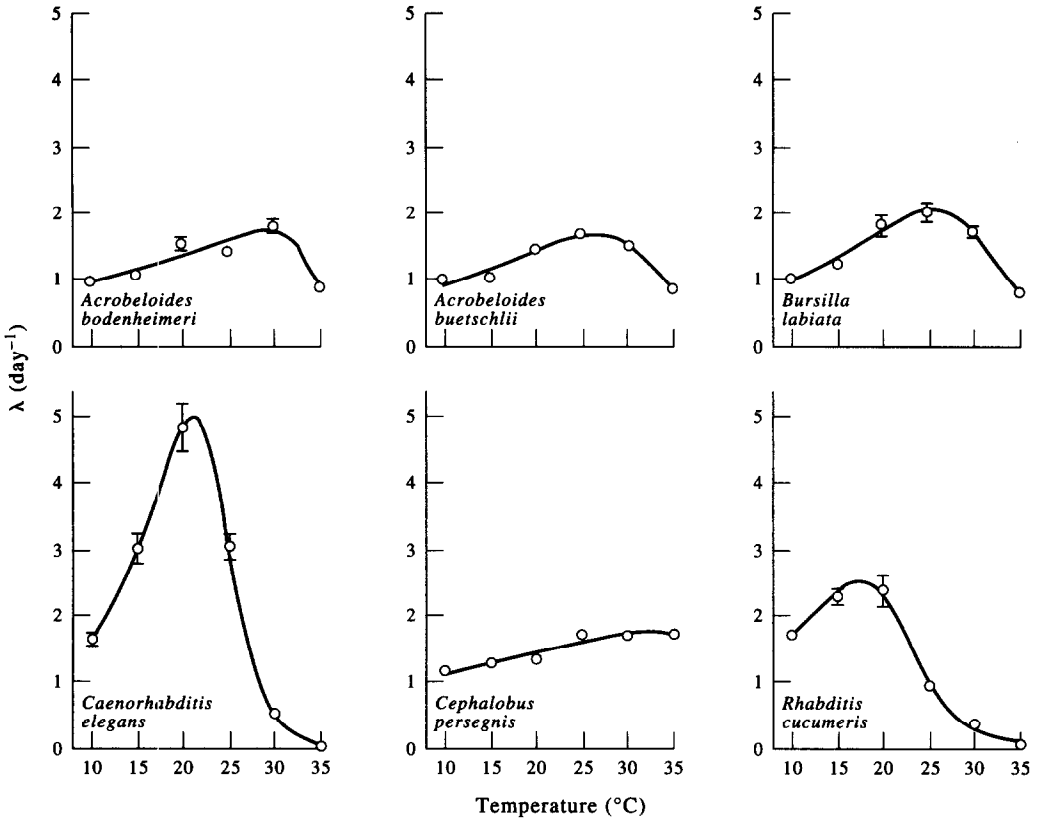

Fig. 2. Relationship between the finite rate of population increase (d^{-1}) and temperature for six species of bacterial-feeding nematodes. Bars indicate 95% confidence intervals. Unseen bars are obscured by symbols. Solid line indicates predicted growth rate from a poikilotherm model.

Finite rates of increase across temperatures

Finite rates of population increase for the six nematode species ranged from 4.833 d^{-1} for *Caenorhabditis elegans* at 20°C to 4.54×10^{-25} d^{-1} for *R. cucumeris* at 35°C (Fig. 2). Across all temperatures, the poikilotherm model accurately predicted population growth rates (Table 1). All species were able to survive extended periods (> 3 weeks) or to successfully reproduce at temperatures between 10 and 20°C, inclusive. Reproduction was considered successful if females deposited eggs and juveniles emerged. At 10°C, in two replicates, *A. bodenheimeri* produced an average of 1.25 eggs female^{-1} d^{-1}, and in six replicates, *A. buetschlii* laid an average of 1.86 eggs female^{-1} d^{-1}. In either case, juveniles were not seen to emerge from eggs, so reproduction was not successful.

As temperatures increased above 20°C, not all species reproduced or survived for extended periods. The upper threshold temperatures estimated by linear interpolation closely matched the threshold temperatures predicted from the poikilotherm model (Table 2). Although linear interpolation between observation points could not predict a lethal temperature for *Cephalobus persegnis*, the poikilotherm model suggested that populations of this nematode would decline at 42.2°C. When temperature sensitive species were exposed to lethal conditions, nematodes did not become active when moved to room temperature, indicating that dauer stages were not present.

Within their respective thermal tolerances, the species also differed in the sensitivity of their population growth rates to temperature. From 10 to 20°C, the change in daily finite growth rates varied from a 3.2-fold increase for *Caenorhabditis elegans* to a 1.2-fold increase for *Cephalobus persegnis*; the growth responses of *A. bodenheimeri*, *A. buetschlii* and *B. labiata* were intermediate. Population growth rates for *R. cucumeris* changed non-linearly over the same temperature range. To avoid assumptions of linearity and to provide a more robust measure of sensitivity to temperature, the coefficient of variation (CV) of the mean growth rates across the six study temperatures was calculated for each species (Table 3). The CV indicates the degree of deviation due to temperature and reflects the degree of deviation from a no-change population growth rate across temperatures (Ferris *et al.*, 1995). If population growth rates for a species remain unaffected by temperature, the CV will equal zero. As the change (either positive or negative) in population growth rates increases due to temperature, the CV correspondingly increases. Of all the species

Table 1. Coefficients of a poikilotherm model[a] (Schoolfield *et al.*, 1981) to describe the relationship between finite rates of population increase (λ, d^{-1}) and temperature (T, °C + 273) for six species of bacterial-feeding nematodes

Parameters	*Acrobeloides bodenheimeri*	*Acrobeloides buetschlii*	*Bursilla labiata*	*Caenorhabditis elegans*	*Cephalobus persegnis*	*Rhabditis cucumeris*
*a*1	−8.4281	0.84887	3.2869	9.4909	0.030613	5.1676
*a*2	−2959.9	−302.67	705.27	155.74	−473.03	1135.1
*a*3	156.86	−5.8276	−8.4493	146.64	−0.61267	−11.99
*a*4	46319	−3814.4	−4226.3	39775	−2221.9	−5173.6
*a*5	−11.852	92.204	88.775	−19.042	81.361	108.27
*a*6	−5531.3	26129	25260	−9371.4	23542	30209

[a] $$\lambda(T) = \frac{T \cdot \exp(a1 - \frac{a2}{T})}{1 + \exp(a3 - \frac{a4}{T}) + \exp(a5 - \frac{a6}{T})}.$$

studied, *Caenorhabditis elegans* showed the greatest variation in finite rates of population increase (d^{-1}) due to temperature. Of the species isolated from soil, *R. cucumeris* was most variable and *Cephalobus persegnis* was least.

Another measure of the relative reproductive performance of each species across the range of experimental temperatures came from the geometric mean of the finite rates of population increase (d^{-1}) (Table 3). Unlike the CV, the geometric-mean growth rate reflects the capability for, and magnitude of, reproduction of a species across the six temperatures. Without accounting for the time a species might spend at a particular temperature under field conditions, *Cephalobus persegnis* had the greatest capability to reproduce, given adequate food and moisture, across the entire range of temperatures. *Rhabditis cucumeris* showed the lowest capability.

Instantaneous population growth rates across temperatures

While the finite rates of population increase (d^{-1}) provided coarse measures, instantaneous rates (h^{-1}) provided more refined measures of population growth (Fig. 3). Instantaneous growth rates ranged from 0.0656 h^{-1} for *Caenorhabditis elegans* at 20°C to −2.3354 h^{-1} for *R. cucumeris* at 35°C. For each species, the fundamental relationships between population growth and temperature did not change whether the relationship was measured using finite rates of population increase or instantaneous rates of population growth. Because instantaneous population growth rates are not bounded by zero, the absolute value of the CV of instantaneous growth rates (h^{-1}) allowed greater distinction between species relative to their sensitivity to temperature (Table 3); the CV for *Caenorhabditis elegans* was nearly twice as great as that for *R. cucumeris*. *Acrobeloides buetschlii* was more sensitive than *A. bodenheimeri* and *B. labiata*.

Because finite rates of population increase expressed on an hourly basis and instantaneous growth rates expressed on a daily basis provided little additional information, these values are not reported.

Population growth thresholds

Based on the linear-fit of the data, the minimum temperatures required for population growth varied for each species and ranged from 14.8°C for *A. buetschlii* to −0.1°C for *Cephalobus persegnis* (Table 2). For the *Acrobeloides* spp. and *B. labiata*, because no population growth was observed at 10°C, growth thresholds were estimated from population growth responses over the range of 15–20°C. The thresholds accurately predicted the lack of growth at 10°C.

Minimum temperatures required for population growth, as estimated from the non-linear poikilotherm model, differed from the requirements predicted from the linear model (Table 2). Each species

Table 2. Critical temperatures (°C) affecting population growth of bacterial-feeding nematodes as estimated from linear regressions and from a poikilotherm model (Schoolfield *et al.*, 1981) fit to observed finite rates of population increase (d^{-1})

Species	Linear regressions		Poikilotherm model		
	Basal	Upper threshold	Basal	Optimum	Upper threshold
Acrobeloides bodenheimeri	13.8	34.4	10.5	29.2	34.7
Acrobeloides buetschlii	15.0	33.9	11.9	26.6	33.9
Bursilla labiata	10.6	33.8	10.7	25.6	33.7
Caenorhabditis elegans	5.0	29.0	5.9	21.1	28.2
Cephalobus persegnis	−0.1	NA	5.8	32.2	42.4
Rhabditis cucumeris	1.4	24.6	4.0	17.7	24.8

NA = not applicable

Table 3. Coefficient of variation (CV, %) of observed finite rates of population increase (d^{-1}) and instantaneous population growth rates (h^{-1}) across a range of temperatures, and geometric mean (GM) of observed finite rates of population increase (d^{-1}).

	Acrobeloides bodenheimeri	*Acrobeloides buetschlii*	*Bursilla labiata*	*Caenorhabditis elegans*	*Cephalobus persegnis*	*Rhabditis cucumeris*
CV (λ, d^{-1})	28.3	26.1	34.7	82.8	17.0	79.6
CV (r, h^{-1})	121.3	142.0	127.1	461.9	47.4	250.2
GM (λ, d^{-1})	1.263	1.209	1.343	0.424	1.45	1.03×10^{-4}

of bacterial-feeding nematode had an approximate basal temperature of 10 or 5°C.

Physiological tolerances and field dynamics

Based on data from weather stations adjacent to the SAFS plots, during the 141-day growing season, *R. cucumeris* could have encountered 86 d when temperatures would have prevented reproduction; *B. labiata*, 3 d; and both *Acrobeloides* spp., 1 d. *Cephalobus persegnis* was unlikely to encounter any days where temperature would preclude reproduction [Fig. 4(A)]. Because *R. cucumeris* was the only nematode likely to experience a substantial restriction in habitat due to temperature, the simulations of temperature-dependent dynamics for the other four species were not reported.

The simulated dynamics of *R. cucumeris* suggested that populations of the nematode would grow relatively slowly through the first 3 weeks of April (JD 95–110). Populations would then increase, with two downturns, to a maximum population size occurring in May (JD 135). After this time, populations would plummet, but might recover for 2 weeks in June (JD 155–167). After JD 167, the simulation suggests that populations would decline precipitously and would remain undetectable through the remainder of the growing season [Fig. 4(B)]. Although the size of the simulated population was substantially greater than the field population, by expressing both observed and predicted values relative to their respective maximum values, the population trajectories for both became comparable.

The relative predicted and observed population trajectories did not significantly differ ($P > 0.05$). Populations were observed to increase from April

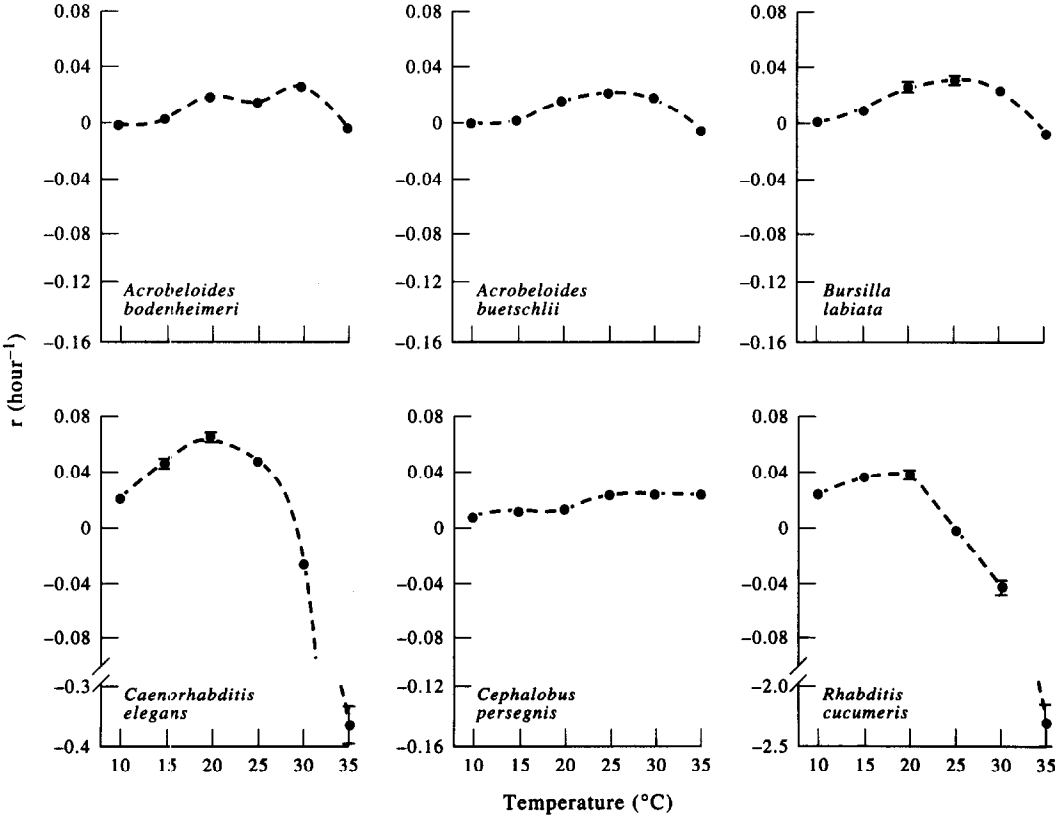

Fig. 3. Relationship between the instantaneous population growth rate (h^{-1}) and temperature for six species of bacterial-feeding nematodes. Bars indicate 95% confidence intervals. Unseen bars are obscured by symbols.

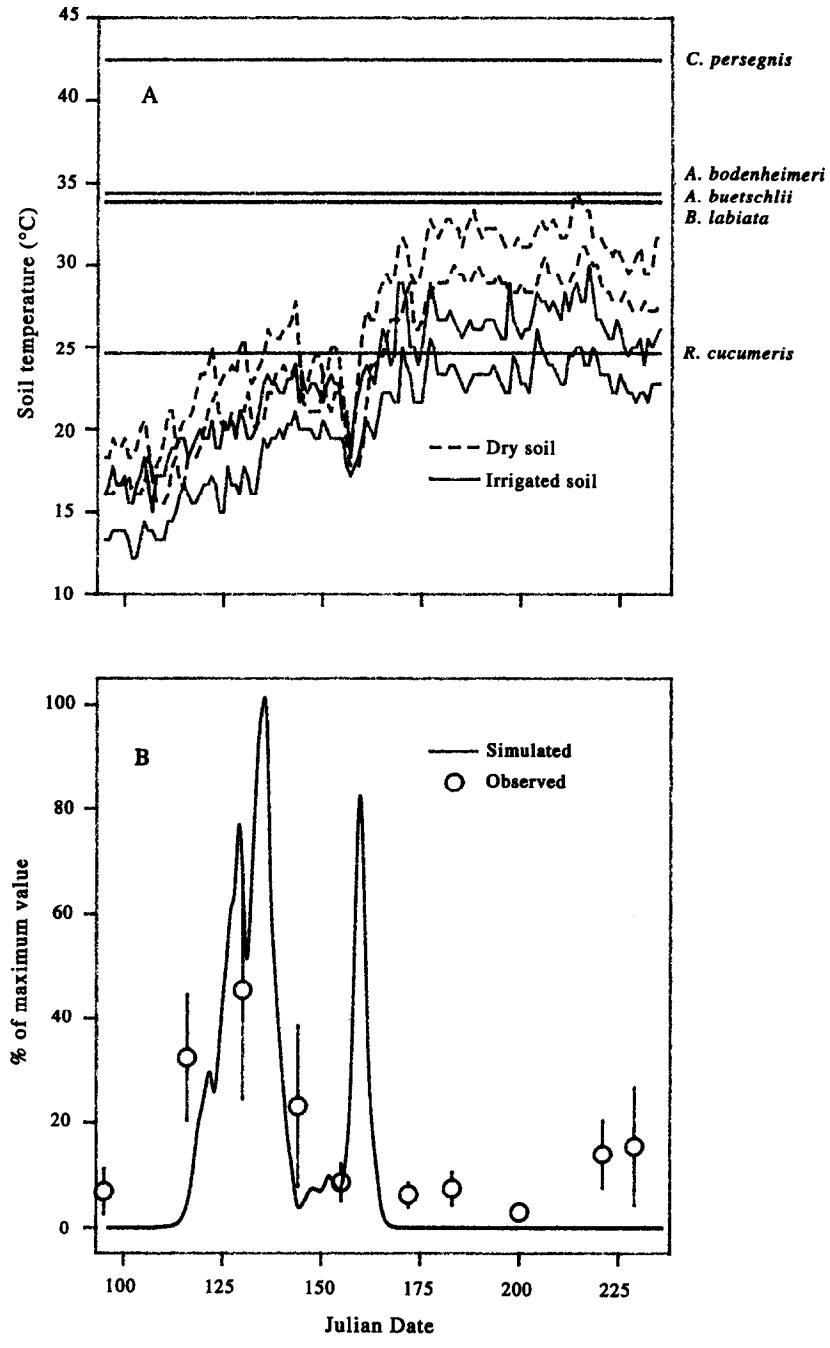

Fig. 4. (A) Maximum and minimum daily soil temperatures (°C) at 10 cm depth in 1993 from two weather stations adjacent to the Sustainable Agriculture Farming Systems project near Davis, CA. Soils at the two stations are maintained either irrigated under grass sod or dry without vegetation. Horizontal lines indicate the upper thermal tolerances for *Acrobeloides bodenheimeri*, *A. buetschlii*, *Bursilla labiata*, *Cephalobus persegnis*, and *Rhabditis cucumeris*. (B) Simulated temperature-dependent dynamics of *Rhabditis cucumeris* in 1993. Symbols indicate the average relative density (% ± SE) of *R. cucumeris* in the organic tomato plots of the SAFS project.

(JD 95) to May (JD 130), then to decline until June (JD 155) ($P < 0.05$). Then, through September (JD 235), populations remained unchanged and statistically no different from zero ($P > 0.05$) [Fig. 4(B)].

DISCUSSION

Selecting nematode species

Bongers (1990) provides a framework to segregate nematode families on the basis of known and

assumed life history characteristics and relative sensitivity to stress. Of the families that are bacterial-feeders, Rhabditidae and Panagrolaimidae are "colonizer" families and are enrichment opportunists. Member populations grow most rapidly when supplies of food increase and are generally the first nematode species to establish populations in newly formed habitats (De Goede *et al.*, 1993). In contrast, Cephalobidae, "persister" bacterial-feeders, are more stress tolerant but are unable to respond as quickly to increases in food availability (T. Bongers, personal communication).

With a few notable exceptions, our initial screening of nine bacterial-feeding species tends to support Bongers' framework. At 20°C, members of Rhabditidae (i.e. *B. labiata*, *Caenorhabditis elegans*, *Cruznema tripartitum*, and *R. cucumeris*) reproduce at comparable rates which exceed the rates for members of Cephalobidae (i.e. *A. bodenheimeri*, *A. buetschlii*, and *Cephalobus. persegnis*). This pattern exactly matches the simulations of Ferris *et al.* (1996a) and generally confirms the notion that species in the Rhabditidae are more capable than those in the Cephalobidae to quickly exploit available food. Additionally, if nematode fauna can be considered stressed at high temperatures, our indices of temperature sensitivity suggest that the Rhabditidae are generally more vulnerable to thermal stress than are the Cephalobidae. However, two of the species originally studied do not seem to fit neatly into the colonizer-persister framework. *Diploscapter coronata*, a member of the Rhabditidae, reproduces as slowly as the members of Cephalobidae. Moreover, of all the species surveyed at 20°C, *P. detritophagus*, a designated enrichment opportunist, reproduced most slowly. So, although characteristics of nematode families may accurately describe the behavior of many member species, exceptions may occur under particular environmental conditions, as has been discussed (Yeates, 1994).

The species selected for further investigation are interesting representatives of nematode diversity because of the similarities and differences in their life histories. Judged by population growth rates at 20°C, the species fit the enrichment-opportunist vs the stress-tolerant split that is identifiable at the family level. However, these similarities mask several key distinctions between species. Firstly, the modes of reproduction vary. *Caenorhabditis elegans*, *A. buetschlii*, *Cephalobus persegnis*, and *R. cucumeris* require only one individual (either a female or a hermaphrodite, depending on the species) to reproduce, while *A. bodenheimeri* and *B. labiata* need both a male and female. Secondly, the weight of adult *R. cucumeris* and *A. bodenheimeri* is at least twice as great as any of the other species. Finally, the metabolic rates across a range of temperatures for *Cephalobus persegnis* and *A. bodenheimeri* are greater than those of other species included in this study (Ferris *et al.*, 1995).

Effects of temperature on population growth rates

With the exception of *Caenorhabditis elegans*, a model organism in developmental biology and genetics, little is known of the effects of temperature on the population growth rates of many bacterial-feeding nematode species. The impact of temperature on population development has been investigated for certain genera (Nicholas, 1962; Sohlenius, 1969; Sohlenius, 1973; Popovici, 1972, 1973; Anderson and Coleman, 1982), but such data are infrequently used to calculate intrinsic rates of increase (Schiemer, 1983; Vranken and Heip, 1983; Procter, 1984). Due to the difficulty of accurately identifying bacterial-feeding nematodes, species names are not reported in many cases.

Elements of this study generally confirm data presented by other authors. Anderson and Coleman (1982) report that the temperature-niche breadth of genera, isolated from a Colorado shortgrass prairie, range from 15 to 30°C for *Rhabditis* sp., from 20 to 30°C for *Caenorhabditis* sp. and from 15 to 35°C for *Acrobeloides* sp. Nicholas (1962) indicates that populations of *A. buetschlii* were able to reproduce from 20 to 32°C and witnessed egg production at 34°C without subsequent hatch. Of course, species isolated from different geographic regions may be adapted to reproduce at a different range of temperatures. This study also further corroborates the finding that *Caenorhabditis elegans* has a maximum reproductive rate near 20°C (Grewal, 1991).

Beyond the optimal temperatures for population growth, the observed changes in growth rate for all species did not conform to a particular pattern. Many degree-day models assume either constant or no growth above the temperature, at which population growth rates are maximal (Curry and Feldman, 1987). However, populations of *A. buetschlii*, *B. labiata* and *Caenorhabditis elegans* continued to grow as temperatures increased past the optimum, but at a steadily decreasing rate (Figs 2 and 3). In contrast, populations of *Cephalobus persegnis* grew at an essentially constant rate above 25°C. Yet populations of *A. bodenheimeri* and *R. cucumeris* effectively ceased growing at temperatures above their respective optima for population growth. To some extent, the patterns are affected by the temperatures selected for observation. Temperatures were chosen to span the range of observed soil temperatures during the growing season in the SAFS project. For soil temperatures above approximately 20°C, the different thermal response patterns complicate the broadcast use of one heat-unit model for all bacterial-feeding nematode species.

Between the basal temperature required for population growth and the temperature where popu-

lation growth is most rapid, the population growth rate appears to increase linearly with increases in temperature. However, this temperature range encompasses only one-third to one-half of the observed data points. Due to the limited amount of data, linear extrapolation from these points to identify the minimum temperature for population growth may be less accurate than the poikilotherm model which uses the entire data set. Although the poikilotherm model has not been used by others to predict nematode development, the basal growth temperatures it identifies are consistent with other basal temperature estimates for plant-parasitic nematodes (Schneider and Ferris, 1987; Trudgill, 1995)

Optimal temperatures for population growth (Table 2) could not be predicted from optimal temperatures for respiration or metabolism. We previously measured maximum respiration–metabolic rates for *A. bodenheimeri* and *A. buetschlii* at 30°C; for *Cephalobus elegans*, *Caenorhabditis persegnis*, and *R. cucumeris* at 25°C; and for *B. labiata* at 20°C (Ferris *et al.*, 1995). Only *A. bodenheimeri* had maximal population-growth and respiration rates at the same temperature. The rapid respiration rate of *R. cucumeris* at 25°C was most likely a stress response because the temperature proved to be mildly lethal to the species.

Physiological tolerances and field dynamics

Although nematodes were observed on agar media at constant temperatures, admittedly quite different from field soils, the reported population growth rates reflect innate characteristics of the species. Numerous additional factors, including moisture, food availability, food type, as well as predation and parasitism rates, may ultimately interact with temperature to constrain population growth in the field. Yet our measurements provide some of the requisite knowledge to determine when and where temperature itself may restrict population growth.

For the five species originally isolated from the SAFS project, soil temperatures in 1993 were unlikely to exceed thermal tolerances for *A. bodenheimeri*, *A. buetschlii*, *B. labiata* or *Cephalobus persegnis* for any significant amount of time. Populations of these nematodes could conceivably grow exponentially through most of the growing season. Limited food availability was likely to preclude that result. In contrast, populations of *R. cucumeris* declined during a period when ample food was available (Ferris *et al.*, 1996b). The concurrence of predicted fluctuations with observed changes in population size heavily implicates temperature as the sole factor responsible for the mid-season collapse of the population.

The laboratory measures of population growth rate across temperatures imply that different nematode species innately have different capacities for withstanding thermal variation. The geometric mean of population growth rates captures the essence of the temperature response curve and provides some indication of this capacity. If the geometric mean exceeds 1, a species demonstrates its capability to reproduce despite temperature variation. Species with the greatest geometric mean have the greatest ability to reproduce across environments, but may not be the most capable in particular environments. For example, *Cephalobus persegnis* has the greatest geometric mean of the six species investigated, but only grows faster than any other species at 35°C. In contrast, *R. cucumeris* grows faster than any other field isolate from 10 to 20°C, but has the lowest geometric mean (Table 3).

In the field, differences in innate thermal tolerances may allow multiple species to coexist (Anderson and Coleman, 1982) and may also affect each species' contribution to ecosystem processes, especially nutrient mineralization. Since the amount of nitrogen mineralized by a species is presumably density-dependent, the relative contribution of each population through time should vary in accordance with the geometric mean of population growth rates. If the geometric mean is calculated based on the expected population growth rate for each daily maximum soil temperature in the SAFS project, the weighted geometric mean for *A. bodenheimeri* becomes 1.56; *A. buetschlii*, 1.43; *B. labiata*, 1.62; *Cephalobus persegnis*, 1.61; and *R. cucumeris*, 0.46. Without accounting for differences in size or respiration, the net population growth rates, based solely on the effects of temperature, suggest that *B. labiata* could have mineralized the most N during the 1993 growing season. The relative contributions of each species, with the exception of *Cephalobus persegnis*, closely match the predictions of Ferris *et al.* (1997).

Heterogeneity in the field complicates the exact prediction of the effects of temperature on nematode dynamics and function. The complexity of the soil profile through space and time may provide numerous microhabitats varying in suitability for reproduction. During crop production periods, soil temperatures fluctuate diurnally and increase closer to the surface. As temperatures approach lethality, nematodes could conceivably migrate to more favorable locations, beneath the zones where climatic parameters were measured. We arbitrarily set the bounds of nematode habitat for this study at the border of experimental plots and at 15 cm depth. The top 15 cm of soil is an area of interest as that is where organic matter is incorporated, which provides a substrate for bacteria to flourish. In fact, numerous microhabitats exist within that zone. Our assessment of nematode population dynamics and habitat quality in the field relies on aggregate representations of those habitats. The effort required to measure populations or con-

ditions in each microhabitat through time seems prohibitive. However, when temperatures exceed thermal tolerances at a given depth, it is highly probable that microhabitats at shallower depths will be warmer and at least equally unsuitable.

As Shelford (1913) indicates, when sufficient measures of a habitat are available, the geographic distribution of a species may be predicted based on the known change in population growth rate as environmental parameters vary. When conditions limit or preclude reproduction, a species should be excluded from that area. Temperatures above 24.6°C for *R. cucumeris*, 33.8°C for *B. labiata*, 33.9°C for *A. buetschlii*, 34.4°C for *A. bodenheimeri* and, potentially, 42.2°C for *Cephalobus persegnis* exceed the thermal tolerances for those species. While microbial dynamics, soil moisture and activity of predators may also affect population growth rates for bacterial-feeding nematodes, we can only account for the impact of temperature at this time.

Measurements of physiological constraints to population growth can be used to predict the geographic distributions of species and their possible contribution to ecosystem processes. Obviously, when species are excluded, they cannot participate in ecosystem functions. The geometric mean of population growth rates across temperatures measures the innate thermal tolerances of a species, and when integrated with climate data for a region, yields a gross assessment of the relative contribution of a species to nutrient mineralization. The assessments for the investigated species corroborate other predictions from detailed carbon and nitrogen budgets (Ferris *et al.*, 1997). Further, when more detailed measures of climate are available, changes in population size through time and space can be predicted. Quantifying the response of bacterial-feeding nematode populations to changes in environmental parameters will greatly enhance our understanding of the potential contributions of taxa to soil and ecosystem processes.

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