Pergamon

PII: S0038-0717(97)00176-4

Soil Biol. Biochem. Vol. 30, No. 7, pp. 949–960, 1998

Printed in Great Britain
0038-0717/98 $19.00 + 0.00

INFLUENCE OF BACTERIAL TYPE AND DENSITY ON POPULATION GROWTH OF BACTERIAL-FEEDING NEMATODES

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(Accepted 18 June 1997)

Summary—The contribution of bacterial-feeding nematodes to litter decomposition and nutrient mineralization depends, in part, on the abundance of particular nematode species. Population dynamics will be constrained by edaphic factors, food availability and food quality. We report the population growth rates for six nematode species as affected by different bacterial isolates and by changes in food supply. Populations of *Caenorhabditis elegans* grew faster than any other nematode-bacterium combination when *Bacillus polymyxa* was supplied as food ($\lambda = 12.26\ d^{-1}$). *Caenorhabditis elegans* also exhibited the greatest variation in population growth rate across the set of bacteria investigated. *Acrobeloides bodenheimeri*, *A. buetschlii*, *Bursilla labiata*, *C. elegans*, *Cephalobus persegnis*, and *Rhabditis cucumeris* did not develop or reproduce when fed *Streptomyces halstedii scabies*. Within the range of food concentrations considered, the six nematode species approached their respective maximal population growth rate between 10^4 and 10^5 colony-forming-units (CFUs) per nematode. Populations stopped growing when food concentrations declined to 10^3–10^4 CFUs per nematode. Between 10^3 and 10^6 CFUs per nematode, variation in population growth rate due to changes in food supply was greatest for *C. elegans* and was least for *A. buetschlii*.

INTRODUCTION

Bacterial-feeding nematodes are among the primary grazers of bacteria in soils. The nematodes enhance rates of nitrogen mineralization (Ingham *et al.*, 1985), increase microbial activity (Yeates and Coleman, 1982), reduce pathogen load (Chantanao and Jensen, 1969), and redistribute saprophytic bacteria (Freckman, 1988) and plant symbionts (Cayrol *et al.*, 1987). As described by Bongers (1990), the nematode trophic group contains numerous taxa classified from *r*-selected to *K*-selected species. The effects of a particular species on nutrient-mineralization rates and microbial-community structure will vary as a function of nematode morphology, physical activity, metabolic rate, developmental rate, nutritive requirements and population size (Ferris *et al.*, 1995, 1996a,b, 1997; Venette and Ferris, 1997).

The population dynamics of all organisms are affected by requirements for food and energy. The requirements of many soil-dwelling organisms in general, and bacterial-feeding nematodes in particular, are poorly understood. Knowledge is limited, in part, because it is not yet possible to observe prey selection and ingestion in soil. In fact, for nematodes, the designation "bacterial-feeder" is often inferred from the structure of mouth parts (Freckman, 1982). Laboratory observations on agar-based media typically confirm this designation but occasionally provide incongruent results (Yeates *et al.*, 1993). Without knowing the dietary requirements or preferences of a nematode, accurate characterization of the quantity and quality of its food resources in a soil is difficult.

Bacterial-feeding nematodes are often associated with sites of high microbial activity. Shifts in the number of bacteria affect the abundance and activity of grazers. Even when edaphic factors do not constrain growth, numbers of bacterial-feeding nematodes lag slightly behind changes in food availability. Little information is available to quantitatively account for changes in population growth rates of bacterial-feeding nematodes as a function of food supply (Schiemer, 1982a,b).

Changes in the types of bacteria available to nematodes as prey may also affect the dynamics of the grazers. The soil contains numerous bacterial genera, typically dominated by Gram-positive organisms (Atlas and Bartha, 1993). Although the relative abundance of taxa varies considerably across soils, *Arthrobacter* spp., *Bacillus* spp. and *Pseudomonas* spp. are frequently the most abundant genera (Alexander, 1977). The relative effects of different bacteria on nematode reproduction has been investigated for a limited number of nematode taxa (e.g. Nicholas, 1962; Sohlenius, 1968;

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Chantanao and Jensen, 1969; Grewal, 1991). In such investigations, prey were selected arbitrarily, based on the availability of isolates, or relative to the presence of isolates in soils from which the nematodes were extracted.

To account for observed differences in the population dynamics of five, naturally cohabiting species of bacterial-feeding nematodes (Ferris *et al.*, 1996b), we quantified the thermal constraints to population growth for those species (Ferris *et al.*, 1995, 1996a; Venette and Ferris, 1997). We now report on the role of food as a determinant of population dynamics. We hypothesize that not all bacteria support the same rate of population growth by their grazers and that nematode species differ in their sensitivity to changes in food type and food availability. The objectives of this study are (1) to quantify population growth rates of six bacterial-feeding nematode species across different isolates of soil bacteria; and (2) to characterize population growth rates across a range of food concentrations.

MATERIALS AND METHODS

Origin and isolation of bacterial-feeding nematodes and bacteria

In 1991 and 1992, cultures of bacterial-feeding nematodes were established from soil (Venette and Ferris, 1997) in the Sustainable Agriculture Farming Systems (SAFS) project (Temple *et al.*, 1994). Five of the isolated species were selected for these studies based on differences in reproductive capacity (Venette and Ferris, 1997). A laboratory culture of *Caenorhabditis elegans* (Maupas) Dougherty var. Bristol (wild type strain N2) was obtained as a comparative standard. Voucher specimens are deposited in the U.C. Davis Nematode Collection. Representatives of the Cephalobidae have the following accession numbers: *Acrobeloides bodenheimeri* (Steiner) Thorne (UCDNC 2908 and 2909), *A. buetschlii* (de Man) Steiner and Buhrer (UCDNC 3030) and *Cephalobus persegnis* Bastian (UCDNC 3031). Members of the Rhabditidae include: *Bursilla labiata* (Völk) Andrássy (UCDNC 3028 and 3029), *C. elegans* (UCDNC 3032), and *Rhabditis cucumeris* (Marcinowski) Andrássy (UCDNC 3033). Gnotobiotic cultures of all nematodes were maintained on nematode growth medium (NGM; Sulston and Hodgkin, 1988) with *Escherichia coli* strain OP50 as described by Venette and Ferris (1997).

In 1994, bacteria were isolated from SAFS plots using serial dilution and plating (Postgate, 1969) onto 0.1× nutrient agar (NA; Difco, Detroit, MI, U. S. A.). Five morphologically unique isolates were purified and maintained on 1× NA. Bacteria were submitted to Microbe Inotech Laboratories (St. Louis, MO, U. S. A.) for identification using standard GC-FAME (gas chromatographic fatty acid methyl esterase) and Biolog® procedures (Biolog, Hayward, CA, U. S. A.). One isolate was identified by the Department of Plant Pathology at North Dakota State University using staining and physiological assays (Gerhardt, 1981; Anonymous, 1984; Liao and Wells, 1987; Schaad, 1988; Klement *et al.*, 1990). The five bacterial isolates were *Bacillus megaterium* (GC subgroup A), *B. megaterium* (GC subgroup B), *B. polymyxa*, *Brevibacterium linens*, and *Streptomyces halstedii scabies*.

Nematode population growth rates across food types

For each bacterial isolate, 30 replicates of 0.1× 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 bacterial suspension in sterile, deionized water. Dishes, containing ~10 ml of 0.1× NAC, were incubated at room temperature until a bacterial lawn was clearly established (2–5 d). As a comparative standard in each trial, an additional 5 replicates were prepared with a suspension of *E. coli* as the sole food source. Plates with *E. coli* were incubated at 35°C until use.

When the bacterial isolate established a lawn, nematodes were surface sterilized using a procedure modified from Grewal (1991). Nematodes were rinsed from the surface of stock cultures with 10 ml of sterile deionized water. One ml of 0.01% "Thimerosal" (w:w sodium ethyl mercurithiosalicylate, Sigma, St. Louis, MO, U. S. A.) was added to the liquid containing the nematodes. The solution was gently agitated for 30 s then centrifuged for 2 min at 740 *g*. The supernatant was discarded, and the nematodes were resuspended in 10 ml of sterile deionized water. A single male and/or a fourth-stage juvenile, depending on the reproductive strategy of the species, was aseptically transferred to each dish. Condensate was allowed to evaporate in a laminar flow hood. Dishes were sealed with laboratory film and arranged randomly in an upright incubator at 20.2 ± 0.2°C.

Each replicate (i.e. each Petri dish) was examined daily for the presence of eggs. The onset of oviposition was set at the time of the observation prior to the detection of eggs and marked the beginning of time for determination of population growth rates. Following the emergence of juveniles, the total number of vermiform nematodes throughout the agar and on the edge of the Petri dish was counted daily for 3 d (Venette and Ferris, 1997). Replicates were discarded if they became contaminated, if a parent nematode moved onto the edge of the dish, or if the juvenile was male. The procedure was repeated for all 30 nematode × bacterium combinations.

To ensure that calculations of population growth rate were based on observations made while populations were in an exponential growth phase, logistic growth functions were fitted to the nematode counts (Venette and Ferris, 1997). For each nematode species, separate functions were determined for the bacterial isolates and the *E. coli* controls. Observations with nematode population sizes greater than one-half of the average carrying capacity (K/2), or observations made after the predicted amount of time required for a population to grow to K/2, were excluded from further analysis.

To calculate the intrinsic rate of population increase (r), population counts for each replicate were divided by the initial number of parents and ln-transformed. Data were then used in a linear regression against time, measured in hours since the onset of oviposition. The slope of the resulting line provided an estimate of r. The transformation, e^{24r}, provided an estimate of λ, the finite rate of increase d^{-1}. Differences between the rate of growth on *E. coli* and the bacterial isolate were determined using multiple linear regression and by coding food source as a qualitative independent variable. For each rate, 95% confidence intervals (CI) were determined. Confidence intervals for nematode population growth rate on *E. coli* were determined from the average rates of growth estimated in each of the five control trials.

For the bacterium that did not support population increase, λ was set at one, and 95% CI were approximated using the reciprocal of the duration of the total life course (Ferris *et al.*, 1996a; Hunt *et al.*, 1987). To determine whether bacterial size may have prevented ingestion, the bacterium was Gram-stained. Fifty filamentous strands and spores, respectively, were measured using a Leitz Ortholux II compound microscope connected to computer video imaging system (Java™). Body lengths and stoma widths for 25 individuals of *C. elegans* collected from a fresh stock culture were also measured. The distributions of hyphal length and stoma width were compared using Fisher's t-test.

Population growth across food concentrations

Changes in nematode population growth rate were measured relative to changes in food availability following procedures established for bacteria (Monod, 1949; Koch, 1979). The number of bacteria were controlled by limiting nutrients in the media. Nutrient concentrations were selected based on the outcome of a test for osmotic stress. Dialysis tubing was sealed with 5 ml of 1×, 0.1×, 0.001× nutrient broth (5.0 g Bacto-peptone, 3.0 g yeast extract in dilute saline, 1.0 g NaCl l^{-1} deionized H_2O), dilute saline, sucrose solution (454 g l^{-1} deionized H_2O; hypertonic control), or deionized water (isotonic control). Three replicates were prepared for each solution. Tubing with solution was weighed and soaked in 1.5 l of deionized water for 48 h. Tubing was then blotted dry and weighed. Osmotic stress was measured as the proportional increase in weight. The trial was repeated. Data were pooled and analyzed using analysis of variance and Duncan's means separation test.

Media selected were 0.1, 0.01, 0.001, and 0× NAC. For each medium, only the concentrations yeast extract and peptone varied. Concentrations of NaCl, cholesterol, and Bacto-agar were constant as described above. For each medium, a set of 30 replicates was prepared with *E. coli* (as described above). For each set, an additional 5 replicates were prepared using 0.1× NAC. All Petri dishes were incubated at 35°C for 18–24 h.

For each nematode species, parent individuals were prepared and transferred to Petri dishes as described above. Petri dishes were sealed and incubated at 20.1 ± 0.2°C. Each replicate was examined daily for the presence of eggs and the number of vermiform individuals counted. Nematode counts from the different media were analyzed together to estimate the response of population growth rate to changes in food availability. The process was repeated for five of the nematode species. Stock cultures of *A. bodenheimeri* were lost following an infestation by mites and bacteria.

To derive appropriate data transformations to characterize the effect of food availability on nematode population growth rate, we followed rationale applied to bacteria (Monod, 1949). For bacteria, a simple relationship may exist between population growth rate and the concentration of an essential substrate (Fig. 1a). Frequently, the "Monod" equation,

$$r(C) = \frac{r_{\max} C}{K_m + C} \qquad (1)$$

applies (reviewed in Simkins and Alexander, 1984), although other hyperbolic functions may also fit the data (Monod, 1949). In the model, $r(C)$ is the rate of population growth at substrate concentration, C; r_{max} is the intrinsic rate of population increase under non-limiting conditions; and K_m is a coefficient describing the concentration at which the growth rate is one-half of r_{max}. If the concentration of an essential substrate can be maintained (as in a chemostat) and no other factor constrains growth, populations will grow exponentially at a characteristic rate. A series of superimposed curves describes population growth with different, constant concentrations of substrate (Fig. 1b). In a statistical sense, the changing slopes represent an interaction between substrate concentration and time.

Two transformations were developed and applied to the data to determine coefficients for the Monod and for an alternative hyperbolic equation. For both analyses, following the rationale of Koch (1979), we express the concentration of food (C) as

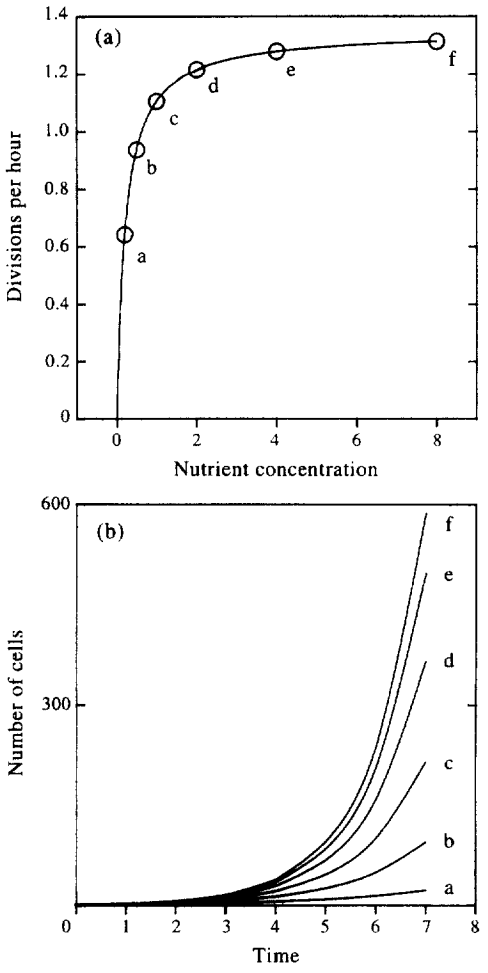

Fig. 1. (a) Idealized Monod kinetics describing bacterial population growth rate as a function of nutrient concentration, $r(C)$. (b) Predicted growth of populations at different, but constant, nutrient supplies. Rates of growth follow the relationship in (a).

the amount of bacteria (total colony forming units [CFUs]) per number of individual nematodes at the time of the previous observation (N_{t-1}). No adjustments were made for the species or developmental stage of the individuals. In time, as the number of nematodes increases, by definition, the concentration of food per nematode decreases. To find a transformation that would yield parameters for Eq. 1 from linear regression, we began by substituting Eq. 1 into an exponential growth model,

$$N_t = N_0 e^{\left(\frac{r_{max}C}{K_m + C}\right)t} \tag{2}$$

where N_t is the number of vermiform nematodes at time t; N_0 is the number of parent nematodes; e is the natural base; and t is the number of hours since the onset of oviposition. From the equation, it follows that

$$\ln\left(\frac{N_t}{N_0}\right) = \left(\frac{R_{max}C}{K_m + C}\right)t. \tag{3}$$

After taking the reciprocal of both sides and rearranging,

$$\frac{1}{\ln\left(\frac{N_t}{N_0}\right)} = \left(\frac{1}{tC}\right)\left(\frac{K_m}{r_{max}}\right) + \left(\frac{1}{t}\right)\left(\frac{1}{r_{max}}\right). \tag{4}$$

Using this equation as the basis to transform nematode counts, food concentrations and time for linear regression, we find

$$\frac{1}{\ln\left(\frac{N_t}{N_0}\right)} = \left(\frac{1}{tC}\right)A + \left(\frac{1}{t}\right)B, \tag{5}$$

where A and B are coefficients determined from the regression analysis. From these coefficients,

$$r_{max} = \frac{1}{B} \text{ and } K_m = \frac{A}{B}.$$

Parameters for a more simple hyperbolic function were determined from another transformation,

$$\ln\left(\frac{N_t}{N_0}\right) = \left(\frac{t}{C}\right)A' + tB' \tag{6}$$

A' and B' are also coefficients determined from regression analysis. B' is approximately equal to r_{max}. Using this model,

$$r(C) = B' + \frac{A'}{C} \tag{7}$$

Data were transformed according to equations 2 and 6 and analyzed using linear regression. The Monod or hyperbolic model was selected based on the goodness of fit as measured by the coefficient of determination for a given species.

To use these models for nematodes, we have assumed that the number of bacteria is determined solely by nutrient availability and that bacteria consumed by the nematodes are replaced as remaining, viable cells multiply. If these assumptions are correct, when r becomes 0 ($\lambda = 1$), the number of bacteria per nematode should be constant regardless of the nutrient concentration of the media. To test the assumptions, when nematode populations were no longer growing, 3 to 4 weeks following the onset of oviposition, 5–20 replicates of 0.1× and 0× NAC, respectively, were selected randomly. Each replicate (i.e. each dish) was weighed. From each replicate, a block of agar (approximately 2.25 cm^2) was excised and aseptically transferred to 10 ml of sterile saline (8.5 g NaCl l^{-1} deionized H_2O). The solution was vigorously agitated for 3 min, followed by serial dilution. At appropriate dilutions, 1 ml was spread onto each of two Petri dishes containing 1× NA. Dishes were incubated at 35 C for 24 h after which CFUs were counted. After excising samples to enu-

merate bacteria, each replicate was weighed again and the remaining agar was macerated and suspended in water. Nematodes were counted in three 1-ml subsamples. Nematode and CFU counts were corrected to express values on a per replicate basis. CFUs per nematode were determined for each replicate. The process was repeated for all six nematode species.

RESULTS

Nematode population growth rates as affected by food type

All bacterial isolates, except *S. h. scabies*, supported nematode population growth (i.e. λ was ≥ 1; Fig. 2). Finite rates of population increase ranged from 12.26 (± 3.06) d^{-1} for *C. elegans* feeding on *B. polymyxa* to 1.18 ($\pm 2 \times 10^{-9}$) for *A. bodenheimeri* feeding on *B. linens*. The bacterial isolates did not have a consistent effect on all nematode species. For members of the Rhabditidae, *B. linens* supported a slower rate of population growth relative to the rate of growth on *E. coli* controls. The rates of population growth on the two isolates of *B. megaterium* (GC subgroup A and B) were similar for each nematode, respectively, with 95% confidence intervals about each rate overlapping, except for *A. buetschlii* and *C. persegnis*.

Across the range of suitable bacteria, nematode species differed in their sensitivity to changes in food source (Table 1). The geometric mean (GM) of population growth rates provides an indicator of sensitivity (Venette and Ferris, 1997). If the GM was > 1.0, a nematode species was capable of population growth despite variation in the type of food it consumed. The GM was > 1.0 for all species investigated. *Caenorhabditis elegans* exhibited the greatest variability in population growth rate (λ, d^{-1}) due to changes in food type; *A. bodenheimeri* had the least (Table 1). Exclusion of *S. h. scabies* from the analyses raised the GM by 4 to 33%.

When nematode species were presented with *S. h. scabies* as a food source, juveniles were frequently located in or near the bacterial lawn within 24 h ($> 30\%$ of all replicates). Beyond that time, if

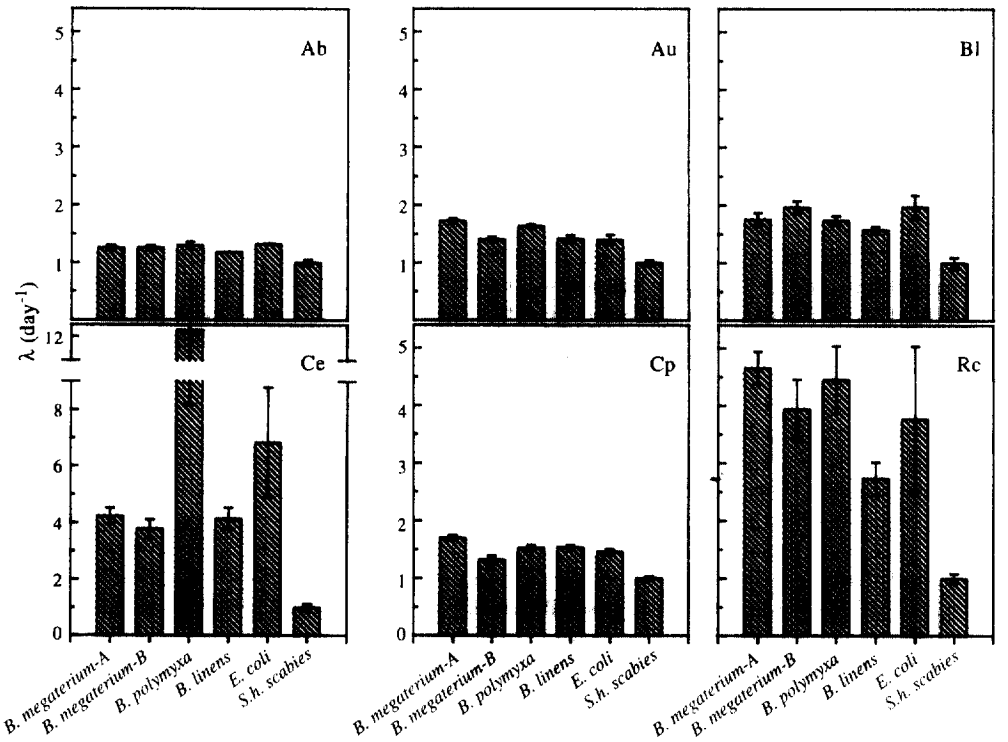

Fig. 2. Finite rates of population increase (λ, d^{-1}) for six species of bacterial-feeding nematodes (Ab, *Acrobeloides bodenheimeri*; Au, *A. buetschlii*; Bl, *Bursilla labiata*; Ce, *Caenorhabditis elegans*; Cp, *Cephalobus persegnis*; and Rc, *Rhabditis cucumeris*) determined on six bacterial isolates at 20°C. Error bars indicate 95% confidence intervals. Confidence intervals for *Escherichia coli* OP50, the comparative standard in each of the five trials, were determined by combining data from the different trials. Confidence intervals for *Streptomyces halstedii scabies* were approximated using the reciprocal of the duration of the life course.

Table 1. Geometric mean (GM) of population growth rates (λ, d^{-1}) for six nematode species across food types. The nematodes were separately fed *Bacillus megaterium* (GC subgroups A and B), *B. polymyxa*, *Brevibacterium linens*, *Escherichia coli* OP50, and *Streptomyces halstedii scabies* (Shs). Analyses consider the effect of including (+) or excluding (−) Shs because no nematode species was able to multiply on the isolate

	GM (+Shs)	GM (−Shs)
A. bodenheimeri	1.214	1.262
A. buetschlii	1.409	1.509
B. labiata	1.628	1.794
C. elegans	4.205	5.604
C. persegnis	1.405	1.504
R. cucumeris	3.072	3.845

nematodes could be found at all, individuals were typically observed in water droplets formed on the edge or surface of the Petri dish. Fourth-stage juveniles were seen alive and active up to 2 weeks after placement in the Petri dishes but, based on the size of the individuals, had not matured into adults. No eggs were produced when *S. h. scabies* was the only available bacterium.

The lengths of *S. h. scabies* in the filamentous form ranged from 4.1 to 32.3 μm and from 0.85 to 1.9 μm for elliptical spores. Across all vermiform stages, the width of the stoma of *C. elegans* ranged from 0.89 to 4.6 μm; the stoma width was larger than the length of a spore ($P = 0.03$) but smaller than the length of a filament ($P = 0.0001$; Fig. 3).

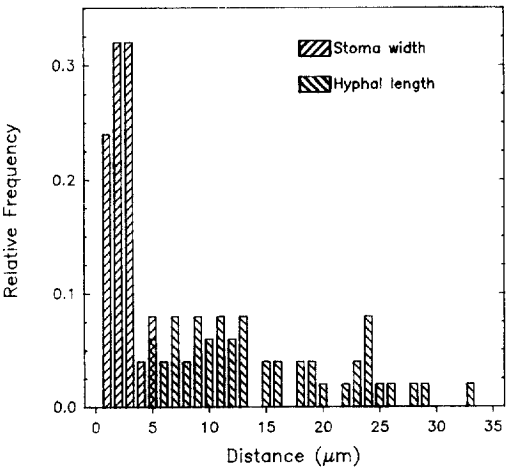

Fig. 3. Comparison of the stoma diameter of *Caenorhabditis elegans* with the hyphal length of *Streptomyces halstedii scabies*. Data for *C. elegans* include all vermiform stages of development.

Nematode population growth rates as affected by food availability

Changing nutrient concentrations to regulate the amount of *E. coli* did not significantly affect the osmotic potential of the media. For the range of nutrient concentrations used in this study, the average weight of fluid-filled dialysis tubes increased by less than 3% and was not different from the isotonic control ($P > 0.05$). The weight of the hypertonic controls increased 2.04-fold and was greater than any other treatment ($P < 0.05$). The weight of dialysis tubing filled with 1× NB increased by 38%, significantly less than the hypertonic control but greater than all remaining treatments.

The amount of *E. coli* initially available to the nematodes ranged from $4–8 \times 10^6$ CFUs on 0× NAC to $6–14 \times 10^8$ CFUs on 0.1× NAC. Across all nutrient concentrations, the number of *E. coli* available per nematode varied with species (Table 2) but typically ranged from 10^4 to 10^9 CFUs per nematode. Within this range of food concentrations, the hyperbolic function accounted for 95–97% of the total variability ($P < 0.0001$), while the Monod function accounted for 78–86% ($P < 0.0001$; Table 3).

Finite rates of population increase (λ, d^{-1}) decreased with reductions in food supply for *C. elegans* ($P < 0.0001$) and *R. cucumeris* ($P = 0.07$). In contrast, population growth rates increased with reduced food availability for *A. buetschlii* and *C. persegnis* ($P < 0.0001$). Within the observed range of food concentrations, nematodes approached maximal growth rates between 10^4 and 10^5 CFUs per nematode. Extrapolation from the hyperbolic model suggested that 10^3 bacteria must be available for populations of *C. elegans* and *R. cucumeris* to grow; similar extrapolations for *A. buetschlii* and *C. persegnis* yielded estimates less than zero (Table 2). The geometric mean of point estimates of the finite rate of population increase from 10^3 to 10^8 CFUs per nematode, inclusive, was calculated at integer increments of the exponent. The geometric mean of point estimates was 1.254 for *A. buetschlii*, 1.740 for *B. labiata*, 3.827 for *C. elegans*, 1.453 for *C. persegnis*, and 2.221 for *R. cucumeris*. Estimates were not available for *A. bodenheimeri*.

Table 2. Range of *Escherichia coli* OP50 concentrations available to bacterial-feeding nematodes across all media types, and threshold concentrations of bacteria required for population growth as extrapolated from a hyperbolic model of growth. Concentrations are measured in colony forming units per nematode

	Range		Predicted threshold concentrations from hyperbolic growth model
	minimum	maximum	
A. buetschlii	2.9×10^5	1.4×10^9	-1.2×10^5
B. labiata	9.7×10^4	6.9×10^8	8.2×10^3
C. elegans	2.0×10^4	1.4×10^9	5.1×10^3
C. persegnis	6.8×10^4	1.4×10^9	-3.3×10^4
R. cucumeris	2.0×10^4	1.4×10^9	2.1×10^3

Table 3. Parameters of models used to describe changes in the intrinsic rate of population increase (r, h^{-1}) with changes in food supply. The hyperbolic model follows Equation 7 in the text and the Monod model follows Equation 1

	Hyperbolic				Monod			
	N^a	A'	B'	R^2	N^b	K_m	r_{max} (h^{-1})	R^2
A. buetschlii	241	1984.3§	0.016§	0.96§	183	−8.0‡	0.013§	0.86§
B. labiata	158	−287.4	0.035§	0.92§	125	8291.2	0.038§	0.84§
C. elegans	410	−392.5§	0.077§	0.95§	306	8439.5+	0.088§	0.86§
C. persegnis	427	733.2§	0.022§	0.97§	319	−82 425.9§	0.018§	0.83§
R. cucumeris	320	−107.8*	0.052§	0.95§	238	−3610.9	0.048§	0.78§

[a]Includes observations at time 0.
[b]Excludes observations at time 0.
[c]§$P \leq 0.0001$, ‡$P < 0.001$, +$P < 0.01$, *$P < 0.1$, otherwise $P > 0.1$.

When populations stopped growing (i.e. reached the carrying capacity of the media), food concentrations ranged from 10^3 to 10^4 CFUs per nematode. The threshold concentration of food was similar for 0× and 0.1× NAC (Table 4).

DISCUSSION

Requirements for energy and elements such as carbon affect the abundance and dynamics of nearly all organisms. The balance of resource availability, requirements, and acquisition may account for the mechanisms underlying resource competition between species (Tilman, 1990). Our understanding of the resource requirements for bacterial-feeding nematodes is extremely limited. By understanding how populations react to changes in food type and abundance, we may better predict when and where particular species will predominate.

Food type

Bacterial-feeding nematodes ingest food without mastication by drawing suspended bacteria and organic molecules into the esophagus. The structure of the alimentary system suggests that members of the Rhabditidae feed non-selectively (Nicholas, 1962). Members of the Cephalobidae, especially *Acrobeles* spp., may utilize probolae to restrict the size of food entering the buccal cavity (Lee and Atkinson, 1977). Thus, size of microbial prey partially determines which bacterial species can sustain nematode growth (Nicholas, 1962).

Once bacteria are ingested, cuticularized plates in the posterior pharyngeal bulb may crush larger particles as the material is passed into the intestine (Lee and Atkinson, 1977). Enzymes are also secreted in the pharynx and intestine to aid digestion of carbohydrates, proteins, and fats (Müge, 1967). Nevertheless, 30–60% of the ingested bacteria may be defecated as viable cells (Chantanao and Jensen, 1969). Consequently, the recalcitrance of a bacterium may also affect its nutritive value for nematodes.

Previous research suggests that bacterial-feeding nematodes can utilize several microbial taxa to sustain population growth on agar (Table 5). Different media, temperatures, timing, variables (such as individual growth rates, fecundity, or maximum number of individuals observed), and qualitative or quantitative measurements complicate the comparison of results from various authors. In Table 5, although contrary instances may occur, prey are considered suitable if they supported population growth under some set of conditions. Prey did not necessarily support the same rate of population growth. Our results corroborate and supplement those findings.

Most of the bacteria considered in this study supported nematode development and reproduction. The effects of each isolate on population growth rates varied with the species of nematode. The most pronounced increase in population growth rate came from *B. polymyxa* fed to *C. elegans*. The slowest rate of population growth was observed when *B. linens* was fed to *A. bodenheimeri*. *Escherichia coli*, a common food source in laboratory studies, always supported adequate, but not necessarily maximal, rates of population growth for all species. Of the soil isolates, populations of nematodes grew most rapidly on *B. megaterium* or *B. polymyxa*. Interestingly, *B. polymyxa* is a predominant species in the rhizosphere (Lochhead, 1952). Although gen-

Table 4. Average number of colony forming units (CFUs) of *E. coli* and average number of nematodes ($\bar{x}$) per Petri dish observed when nematode populations reached carrying capacity on media varying in nutrient concentration

	0× NAC				0.1× NAC				Combined	
	CFUs	S.E.[a]	$\bar{x}$	S.E.	CFUs	S.E.	$\bar{x}$	S.E.	CFU $\bar{x}$	S. E.
A. buetschlii	2.3×10^6	2.9×10^5	9.4×10^2	1.0×10^2	5.4×10^7	1.1×10^7	2.1×10^4	1.2×10^3	2.7×10^3	3.7×10^2
B. labiata	3.8×10^6	2.9×10^6	2.4×10^2	1.3×10^1	2.6×10^8	7.2×10^7	3.7×10^3	3.7×10^2	4.1×10^4	1.2×10^4
C. elegans	2.2×10^5	5.4×10^4	9.8×10^2	1.0×10^2	9.2×10^7	1.1×10^7	1.2×10^4	4.2×10^2	5.0×10^3	9.8×10^2
C. persegnis	4.9×10^7	2.8×10^7	3.8×10^2	1.7×10^2	3.3×10^8	1.7×10^8	1.4×10^4	2.6×10^3	5.2×10^4	1.5×10^4
R. cucumeris	4.7×10^7	8.4×10^6	9.3×10^2	1.9×10^2	8.3×10^7	1.6×10^7	4.7×10^7	2.8×10^2	3.1×10^4	7.6×10^3

[a]S.E. = Standard Error.

Table 5. Reported capacity of several micro-organisms to support the population growth of several bacterial-feeding nematode species. A " – " indicates a non-suitable food source. A " + " does not imply the same rate of growth

Nematode Species	Acinetobacter calcoaceticus	Aerobacter aerogenes	Agrobacterium tumefasciens	Azotobacter sp.	Bacillus cereus	Bacillus megaterium	Bacillus sp.	Bacillus sp. (non-pigmented)	Bacillus sp. (pigmented)	Chlorella pyrenoidosa	Enterobacter ammigenus	Enterobacter cloacae	Erwinia amylovora	Erwinia carotovora	Escherichia coli	Flavobacterium sp.	Proteus vulgaris	Pseudomonas aeruginosa	Pseudomonas cepacia	Pseudomonas fluorescens	Pseudomonas maltophilia	Pseudomonas phaseolicola	Pseudomonas putida	Pseudomonas sp.	Saccharomyces cerevisiae	Sarcina lutea	Serratia liquefaciens	Serratia marcesens	Staphylococcus aureus	Vitreoscilla sp.	Zymobacillus macerans	Source
	Prey Species																															
Acrobeles kotingotingus					+																											a
A. buetschlii										-					+										-	+			+			b
A. nanus															+																	c
Acrobeloides sp.															+				+													d, e
A. syrtisus					+																											a
Caenorhabditis briggsae															+	+	+							+				+			+	f, g, h, i
C. elegans	+				+		+				+	+			+			+			+			+				+				g, j, k
C. remanei															+												+					k
Caenorhabditis sp.																			+													e
Cephalobus nanus															+																	l
C. persegnis															+																	m
Cylindrocorpus curzii		+																														n
C. longistoma		+																														n
Diplogaster nudicapitatus				+		+		-	-						+		+			+				+					-		o, p
Mesodiplogaster biformis															+																	p
M. lheritieri																			+													e
Mesorhabditis littoralis					+																											a
Mononchoides potohikus					+																											a
Panagrolaimus australis					+																											a
Panagrolaimus sp.															+																	d
Panagrellus redivivus															+																	q
Pelodera sp.															+				+													e, r
Pelodera strongyloides															+																	s
P. teres															+																	p
Pristionchus lheritieri			+										+	+								+		+				+				t
Rhabditis maupasi				+		+		-	-						+		+			+				+					-		o
R. oxycerca															+																	d
R. pellio															+																	g
Rhabditis sp.																			+													e
R. terricola															+																	p
Zeldia punua					+																											a
Zeldia sp.															+																	k

[a]Yeates, 1970; [b]Nicholas, 1962; [c]Sohlenius, 1973; [d]Rad, 1991; [e]Anderson and Coleman, 1982; [f]M. P. Briggs, unpubl. M.Sc. Thesis, Stanford University, 1946; [g]Dougherty, 1953; [h]Zuckerman *et al.*, 1969; [i]Nicholas *et al.*, 1973; [j]Grewal, 1991; [k]van der Knapp *et al.*, 1993; [l]Popovici, 1973; [m]Popovici, 1972; [n]Chin and Taylor, 1970; [o]Sohlenius, 1968; [p]Sohlenius, 1969a,b; [q]Cryan *et al.*, 1963; [r]Marchant and Nicholas, 1974; [s]Yarwood and Hansen, 1968; [t]Chantanao and Jensen, 1969.

eral information is available on the effects of *Bacillus* spp. on nematode population growth rates, information of the effects of particular species is scarce (Sohlenius, 1968; Grewal, 1991).

Certain isolates of *Bacillus* do not support nematode population growth (Table 5). The reason nematodes cannot reproduce when fed these isolates is not clear, although some species (e.g. *B. thuringiensis*) may produce toxins which adversely affect nematodes (Meadows *et al.*, 1990). *Bacillus* spp. form endospores, potentially resistant to digestion by nematodes, when conditions do not favor vegetative growth. In our experiments, all *Bacillus* spp. supported nematode population growth. These experiments were conducted on nutrient rich media with actively growing bacteria. At this time, bacteria capable of endospore formation were likely to be in a vegetative phase of growth.

The only bacterial isolate in our study that could not be used as a food source was *S. h. scabies*. Like other actinomycetes, this bacterium has a filamentous growth form and produces asexual conidia. The length of a filament was typically much greater than the width of the stoma of *C. elegans* (Fig. 3). *Caenorhabditis elegans* was used for comparison because of its large tubular stoma. Other species used in this study have stomas that are narrower than that of *C. elegans* at comparable points of development. Consequently, other nematode species were unlikely to ingest *S. h. scabies*. There was no indication that *S. h. scabies* had any other adverse effect on the nematodes.

All nematode species used in this investigation could be considered generalist feeders because all ingestable bacteria provided adequate nutrition. However, *C. elegans* and *R. cucumeris* were generally more sensitive to changes in food type and quantity than the members of the Cephalobidae (Table 1); *B. labiata* was intermediate. These groupings are remarkably similar to our previous estimates of thermal sensitivity (Venette and Ferris, 1997) and are consistent with the colonizer-persister (c-p) groupings of Bongers (1990). Rhabditidae (c-p 1) are considered enrichment opportunists because of their ability to rapidly exploit growth of presumably all bacterial populations. Cephalobidae (c-p 2) are less opportunistic and are assumed to maintain relatively consistent numbers despite variation in food or environment.

Food availability

The relationship between food concentration and population growth rate is poorly understood for bacterial-feeding nematodes. Schiemer *et al.* (Schiemer, 1982b, 1983; Schiemer *et al.*, 1980) provide the only quantitative assessments of nematode

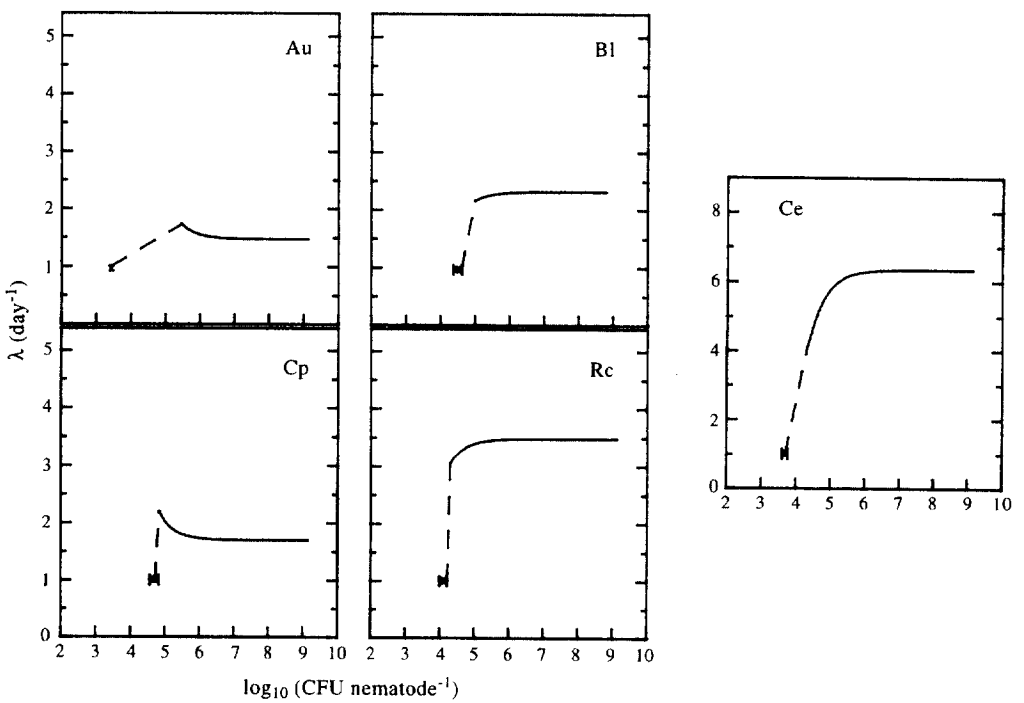

Fig. 4. Finite rates of population increase (λ, d^{-1}) for five species of bacterial-feeding nematodes (Au, *A. buetschlii*; Bl, *Bursilla labiata*; Ce, *Caenorhabditis elegans*; Cp, *Cephalobus persegnis*; and Rc, *Rhabditis cucumeris*) as affected by changes in food concentration (colony forming units of *Escherichia coli* per nematode) at 20°C. X indicates the observed food concentration when population growth stopped ($\lambda = 1$) on both 0× and 0.1× NAC. Bars around X reflect standard error. Bars that appear to be absent are obscured by the symbol. Solid lines indicate bacterial concentrations observed for measurement of λ.

population growth rate across a range of food concentrations. In our investigation, the response in population growth rate to changing concentrations of bacteria more closely followed a general hyperbolic model than the Monod function (Table 3). Unlike the results of Schiemer (Schiemer, 1982b, 1983), and Schiemer *et al.* (1980), population growth rates did not necessarily remain constant or decline with reductions in food availability. Within the range of concentrations observed, the rate of population growth increased slightly, but significantly, for *A. buetschlii* and *C. persegnis*. Rates of population growth for *C. elegans* and *R. cucumeris* followed a more typical pattern and declined as intraspecific competition for food increased (Fig. 4).

Reduced rates of population growth at surplus food concentrations have been observed in other organisms including *Daphnia* (Porter *et al.*, 1983) and rotifers (Halbach, 1972). For rotifers, reduced growth may have resulted from a loss of feeding efficiency and/or from toxic products produced by the prey (reviewed in Schoenberg, 1985). Certain nematode species also produce fewer larvae (Sohlenius, 1973), develop more slowly (Sohlenius, 1969a), or have a slower rate of population change (Popovici, 1973) on media with greater nutrient content presumably supporting more bacteria but not exerting osmotic stress on the nematodes.

In our study, populations of nematodes grew most rapidly when food concentrations ranged from 10^5 to 10^6 bacteria individual^{-1}. These observed thresholds closely match the predictions of Ferris *et al.* (1997) who calculated that *A. buetschlii*, *B. labiata*, *C. elegans*, and *R. cucumeris* must consume on the order of 10^6 cells d^{-1}, and *C. persegnis* must consume 10^5 cells d^{-1} at 20°C to support population growth at a rate that is not constrained by food availability.

Eventually, as expected, population growth rates of all nematodes declined as food availability declined. This transition point occurred outside the range of food concentrations observed while monitoring the instantaneous change in population growth rates for *A. buetschlii* and *C. persegnis*. Consequently, minimum food requirements predicted from the Monod and hyperbolic functions for these species are unreliable. Using the better-fitting hyperbolic model, we calculate that *C. elegans* requires 5×10^3 bacteria individual^{-1} and *R. cucumeris* requires 2×10^3 bacteria individual^{-1} to sustain a minimum degree of population growth. Food concentrations, measured when the populations had reached the carrying capacity of the media, indicate that nematode species require 10^3–10^5 bacteria individual^{-1} to maintain population growth (Fig. 4, Table 4). In comparison to reported estimates for *C. briggsae*, our estimates are considerably lower than 10^6–10^7 cells individual^{-1} (Nicholas *et al.*, 1973) but are similar to 8×10^4 cells individual^{-1}, converted from Schiemer (1982b).

As discussed by Schiemer (1983), the functions relating population growth rate to food concentration identify the range of food concentrations to which each species is best adapted. *Acrobeloides buetschlii* maintains population growth at extremely low food concentrations, but with a slight increase in food availability, *C. elegans* reproduces more rapidly. As food concentrations continue to increase, *R. cucumeris* also gains advantage over *A. buetschlii*. *Cephalobus persegnis* never has a comparative growth advantage over any other species studied due to food availability. However, the discussion of relative population growth rates of each species only pertains to 20°C.

Nutritive requirements, and hence the relationship to food availability, will be affected by changes in temperature. The temperature 20°C favors the growth of *C. elegans* and *R. cucumeris* populations; as temperatures rise to 25 or 30°C and ample food remains available, population growth rates for other species become maximal (Venette and Ferris, 1997). So, the reproductive advantage of *R. cucumeris* and *C. elegans* when surplus food is available will switch as temperatures, rather than food, begin to constrain population growth.

Implications for nematode dynamics and nutrient cycling

In earlier investigations, we monitored the population dynamics of 12 bacterial-feeding nematode species in organically-farmed tomato plots. From April to June 1993, numbers of *B. labiata* and *R. cucumeris* increased 15-fold and 4-fold, respectively, over initial population numbers, while numbers of *A. bodenheimeri*, *A. buetschlii*, and *C. persegnis* remained relatively constant (Ferris *et al.*, 1996b). During this period, bacterial numbers varied from 8–14×10^7 cells g^{-1} dry soil (extrapolated from 1994 data, Erica Lundquist, personal communication). Although we could ascribe the dynamics of *R. cucumeris* to changing soil temperatures (Venette and Ferris, 1997), we have been unable to explain the constrained growth of the cephalobid nematodes over the entire growing season. Inter- and intra-specific competition for bacteria now seem to play a considerable role.

Competition for food among bacterial-feeding nematodes is likely to fluctuate seasonally and will be most intense in the middle-to-end of the growing season. By April, the typical start of the tomato-growing season, nematode numbers are low because soils were dry and fallow during the previous fall. The cold, wet condition of the soil from November to May is also not conducive to nematode population growth. In April, following incorporation of a leguminous cover crop, microbial populations increase and provide ample food (Ferris *et al.*, 1996b). Due to innate

differences in reproduction at lower temperatures, rhabditid nematodes rapidly exploit the bacteria. As soil temperatures warm and favor development of the cephalobid nematodes, interspecific competition with the Rhabditidae will continue to constrain the growth of cephalobid populations.

The effects of competition for food might be lessened if more bacteria were available to bacterial-feeding nematodes. We have speculated about agronomic methods to augment bacteria and nematode populations during critical phases of plant growth (Ferris *et al.*, 1996b). By manipulating populations of bacterial grazers, more N might be mineralized from the microbial biomass. Providing carbon, nitrogen, or water in the fall when soils are typically dry and fallow should create suitable conditions for the growth of bacteria and, subsequently, bacterial-feeding nematodes.

Management of free-living nematode populations, either by creating suitable habitat or introducing select species, requires information about the physiological requirements and tolerances of the species of interest. *Acrobeloides bodenheimeri*, *A. buetschlii*, and *C. persegnis* are least affected by changes in temperature (Venette and Ferris, 1997), food type, or food availability. As a result, these nematode species should occur across a wider range of soil conditions. Although *B. labiata*, *C. elegans*, and *R. cucumeris* are more likely to predominate when environmental conditions suit their reproduction, the Cephalobids are also more likely to establish reproductive populations when introduced into soils despite variation in the environment.

Acknowledgements—We would like to thank Robin Lamppa and Dr James Venette for identification of *Brevibacterium linens*; Dr Valerie Williamson for *C. elegans*, and Carol Johnson for information on microbial kinetics.

REFERENCES

Alexander M. (1977) *Introduction to Soil Microbiology*, 2nd edn., John Wiley, New York.

Anderson R. V. and Coleman D. C. (1982) Nematode temperature responses: a niche dimension in populations of bacterial-feeding nematodes. *Journal of Nematology* **14**, 69–76.

Anonymous (1984) *Difco Manual, Dehydrated Culture Media and Reagents for Microbiology*, 10th edn., Difco Laboratories Incorporated, Detroit.

Atlas R. M. and Bartha R. (1993) *Microbial Ecology, Fundamentals and Applications*. The Benjamin/Cummings Publishing Company, Menlo Park.

Bongers T. (1990) The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* **83**, 14–19.

Cayrol J. C., Frankowski J. P. and Quiles C. (1987) *Cephalobus parvus* as a carrier of *Rhizobium japonicum* in field experiment on soybean cultures. *Revue de Nématologie* **10**, 57–59.

Chantanao A. and Jensen H. J. (1969) Saprozoic nematodes as carriers and disseminators of plant pathogenic bacteria. *Journal of Nematology* **1**, 216–218.

Chin D. A. and Taylor D. P. (1970) Observations on molting in species of *Cylindrocorpus*. *Nematologica* **16**, 1–5.

Cryan W. S., Hansen E., Sayre F. W., Martin M. and Yarwood E. A. (1963) Axenic cultivation of the dioecious nematode *Panagrellus redivivus*. *Nematologica* **9**, 313–319.

Dougherty E. C. (1953) Some observations on the monoxenic cultivation of certain rhabditid nematodes. *Journal of Parasitology* **39**, 32.

Ferris H., Lau S. and Venette R. C. (1995) Population energetics of bacterial-feeding nematodes: respiration and metabolic rates based on carbon dioxide production. *Soil Biology & Biochemistry* **27**, 319–330.

Ferris H., Eyre M., Venette R. C. and Lau S. S. (1996a) Population energetics of bacterial-feeding nematodes: stage-specific development and fecundity rates. *Soil Biology & Biochemistry* **28**, 271–280.

Ferris H., Venette R. C. and Lau S. S. (1996b) Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems, and their impact on soil fertility. *Applied Soil Ecology* **3**, 161–175.

Ferris H., Venette R. C. and Lau S. S. (1997) Population energetics of bacterial-feeding nematodes: carbon and nitrogen budgets. *Soil Biology & Biochemistry* **29**, 1183–1194.

Freckman D. W. (1982) Parameters of the nematode contribution to ecosystems. In *Nematodes in Soil Ecosystems*, ed. D. W. Freckman, pp. 81–97. University of Texas Press, Austin.

Freckman D. W. (1988) Bacterivorous nematodes and organic matter decomposition. *Agriculture, Ecosystems and Environment* **24**, 195–217.

Gerhardt P. (1981) *Manual of Methods for General Bacteriology*. American Society for Microbiology, Washington.

Grewal P. S. (1991) Influence of bacteria and temperature on the reproduction of *Caenorhabditis elegans* (Nematoda: Rhabditidae) infesting mushrooms (*Agaricus bisporus*). *Nematologica* **37**, 72–82.

Halbach U. (1972) Einfluß der Nahrungsqualität auf die Populations-dynamik des planktischen Rädetieres *Brachinus calyciflorus* im Labor und im Frieland. *Verhandlungen der Deutschen Zoologischen Gesellschaft* **65**, 83–88.

Hunt H. W., Coleman D. C., Ingham E. R., Ingham R. E., Elliott E. T., Moore J. C., Rose S. L., Reid C. P. P. and Morley C. R. (1987) The detrital food web in a shortgrass prairie. *Biology and Fertility of Soils* **3**, 57–68.

Ingham R. E., Trofymow J. A., Ingham E. R. and Coleman D. C. (1985) Interaction of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. *Ecological Monographs* **55**, 119–140.

Klement Z., Rudolph K. and Sands D. C. (1990) *Methods in Phytobacteriology*. Acadêmiai Kiadó, Budapest.

Koch A. L. (1979) Microbial growth in low concentrations of nutrients. In *Strategies of Microbial Life in Extreme Environments*, ed. M. Shilo, pp. 261–279. Dahlem Konferenzen, Berlin.

Lee D. L. and Atkinson H. J. (1977) *Physiology of Nematodes*. Columbia University Press, New York.

Liao C. H. and Wells J. M. (1987) Association of pectolytic strains of *Xanthomonas campestris* with soft rots of fruits and vegetables at retail markets. *Phytopathology* **77**, 418–422.

Lochhead A. G. (1952) Soil microbiology. *Annual Review of Microbiology* **7**, 187–206.

Marchant R. and Nicholas W. L. (1974) An energy budget for the free-living nematode *Pelodera* (Rhabditidae). *Oecologia* **16**, 237–252.

Meadows J., Gill S. S. and Bone L. W. (1990) *Bacillus thuringiensis* strains affect population growth of the free-living nematode *Turbatrix aceti*. *Invertebrate Reproduction and Development* **17**, 73–76.

Monod J. (1949) The growth of bacterial-cultures. *Annual Review of Microbiology* **3**, 371–393.

Müge S. G. (1967) Preliminary data on feeding and the nutritional physiology of *Panagrolaimus rigidus*. In *English Translation of Selected East European Papers in Nematology*, eds B. M. Zuckerman, M. W. Brzeski and K. H. Deubert, pp. 24–28. University of Massachusetts, East Wareham.

Nicholas W. L. (1962) A study of a species of *Acrobeloides* (*Cephalobidae*) in laboratory culture. *Nematologica* **8**, 99–109.

Nicholas W. L., Grassia A. and Viswanathan S. (1973) The efficiency with which *Caenorhabditis briggsae* (*Rhabdtidae*) feeds on the bacterium, *Escherichia coli*. *Nematologica* **19**, 411–420.

Popovici I. (1972) Studies on the biology and population development of *Cephalobus persegnis* (Nematoda. Cephalobidae) in agar culture. *Pedobiologia* **12**, 123–127.

Popovici I. (1973) The influence of temperature and of nutrient medium on populations of *Cephalobus nanus* (Nematoda, Cephalobidae). *Pedobiologia* **13**, 401–409.

Porter K. G., Gerritsen J. and Orcutt J. D. Jr. (1983) Functional response and fitness in a generalist filter feeder: *Daphnia magna* (Cladoceran: Crustacea). *Ecology* **64**, 735–742.

Postgate J. R. (1969) Viable counts and viability. In *Methods in Microbiology, Vol. 1*, eds J. R. Norris and D. W. Ribbons, pp. 611–628. Associate Press, New York.

Rad G. R. (1991) Feeding comparison of *Rhabditis oxycerca* (Nematoda: Rhabditidae). *Nematologica* **37**, 481–482.

Schaad N. W. (1988) *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 2nd edn. American Phytopathological Society, St. Paul.

Schiemer F. (1982a) Food dependence and energetics of freeliving nematodes. I. Respiration, growth and reproduction of *Caenorhabditis briggsae* (Nematoda) at different levels of food supply. *Oecologia* **54**, 108–121.

Schiemer F. (1982b) Food dependence and energetics of freeliving nematodes: II. Life history parameters of *Caenorhabditis briggsae* (Nematoda) at different levels of food supply. *Oecologia* **54**, 122–128.

Schiemer F. (1983) Comparative aspects of food dependence and energetics of freeliving nematodes. *Oikos* **41**, 32–42.

Schiemer F., Duncan A. and Klekowski R. Z. (1980) A bioenergetic study of a benthic nematode, *Plectus palustris* De Man, 1880, throughout its life cycle. II. Growth, fecundity and energy budgets at different levels of bacterial-food and general ecological considerations. *Oecologia* **44**, 205–212.

Schoenberg S. A. (1985) Reduced fitness at surplus food levels in *Daphnia*: an artifact? *Ecology* **66**, 1086–1088.

Simkins S. and Alexander M. (1984) Models for mineralization kinetics with the variables of substrate concentration and population density. *Applied and Environmental Microbiology* **47**, 1299–1306.

Sohlenius B. (1968) Influence of microorganisms and temperature upon some rhabditid nematodes. *Pedobiologia* **8**, 137–145.

Sohlenius B. (1969a) Studies on the population development of *Mesodiplogaster hiformis* (Nematoda, Rhabditida) in agar culture. *Pedobiologia* **9**, 243–253.

Sohlenius B. (1969b) The monoxenic cultivation of some rhabditid nematodes. *Oikos* **20**, 287–293.

Sohlenius B. (1973) Growth and reproduction of a nematode *Acrobeloides* sp. cultivated on agar. *Oikos* **24**, 64–72.

Sulston J. and Hodgkin J. (1988) Methods. In *The Nematode Caenorhabditis elegans*, ed. W. B. Wood, pp. 587–606. Cold Spring Harbor Laboratory, Cold Spring Harbor.

Temple S. R., Friedman D. B., Somasco O., Ferris H., Scow K. and Klonsky K. (1994) An interdisciplinary, experiment-station based participatory comparison of alternative crop management systems for California's Sacramento valley. *American Journal of Alternative Agriculture* **9**, 65–72.

Tilman D. (1990) Mechanisms of plant competition for nutrients: the elements of a predictive theory of competition. In *Perspectives on Plant Competition*, eds J. B. Grace and D. Tilman, pp. 117–141. Academic Press, New York.

van der Knapp E., Rodriguez R. J. and Freckman D. W. (1993) Differentiation of bacterial-feeding nematodes in soil ecological studies by means of arbitrarily-primed PCR. *Soil Biology & Biochemistry* **25**, 1141–1151.

Venette R. C. and Ferris H. (1997) Thermal constraints to population growth of bacterial-feeding nematodes. *Soil Biology & Biochemistry* **29**, 63–74.

Yarwood E. A. and Hansen E. L. (1968) Axenic culture of *Pelodera strongyloides* Schneider. *Journal of Parasitology* **54**, 133–136.

Yeates G. W. (1970) Studies on laboratory cultures of dune sand nematodes. *Journal of Natural History* **4**, 119–136.

Yeates G. W., Bongers T., de Goede G. M., Freckman D. W. and Georgieva S. S. (1993) Feeding habits in soil nematode families and genera: An outline for soil ecologists. *Journal of Nematology* **25**, 315–331.

Yeates G. W. and Coleman D. C. (1982) Nematodes and decomposition. In *Nematodes in Soil Ecosystems*, ed. D. W. Freckman, pp. 55–80. University of Texas Press, Austin.

Zuckerman B. M., Castillo J. M., Duebert K. H. and Gunner H. B. (1969) Studies on a growth supplement for *Caenorhabditis briggsae* from freeze dried bacteria. *Nematologica* **15**, 543–549.