ENERGY RELATIONS IN NEMATODES WITH PARTICULAR REFERENCE TO APHELENCHUS AVENAE BASTIAN, 1865

by

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ABSTRACT

An energy budget for Aphelenchus avenae feeding on Botrytis cinerea at 26°C, in the laboratory has been determined by estimation of the calorific values of ingestion (using cinematographic techniques), growth, reproduction and respiration (using cartesian divers). Calorific values were established by bomb calorimetry.

Growth at 26°C, 15°C and 8°C and reproduction at 26°C and 15°C under different culture conditions has been studied. The rate of food ingestion has been shown not to vary with the time of day. The oxygen consumption per weight relationship of A. avenae at 26°C has been studied. From the energy budget efficiency of A. avenae as a converter of energy has been calculated.

The population structure of A. avenae in various cultures has been studied. Using ideal egg production curves and the ideal mortality rate of various ages of A. avenae a model of population numbers has been derived and compared with the actual numbers obtained.

A survey was made of the calorific values of nematodes from various habitats and nutritional states.

The calorific values of various tissues of Ascaris lumbricoides has been determined and dicussed in relation to their chemical composition.

The effects of starvation on the production of viable eggs by A. avenae females has been studied, and discussed in relation to energetics.

Finally, the relevance of energetics to the study of host - parasite relationships, and as a means of assesing pathogenicity, efficiency of parasitism and efficiency of a parasite is discussed.

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GENERAL INTRODUCTION

The first law of thermodynamics requires that exactly the same quantity of energy entering an organism is eventually surrendered by it. Energy that enters biological systems as solar energy in autotrophs or food in heterotrophs, is transformed into energy which can be used to accomplish work (activity, growth, reproduction) by respiratory processes. From the second law of thermodynamics it follows that energy is lost at each food transfer. Energetics is concerned with the energy transformations which occur within an organism, between organisms, or between organisms and the physical environment in which they exist. The calorie is used as a unit of measurement for energy.

The present study was undertaken to apply the principles of energy transfer to nematodes and to their nutritional relationships.

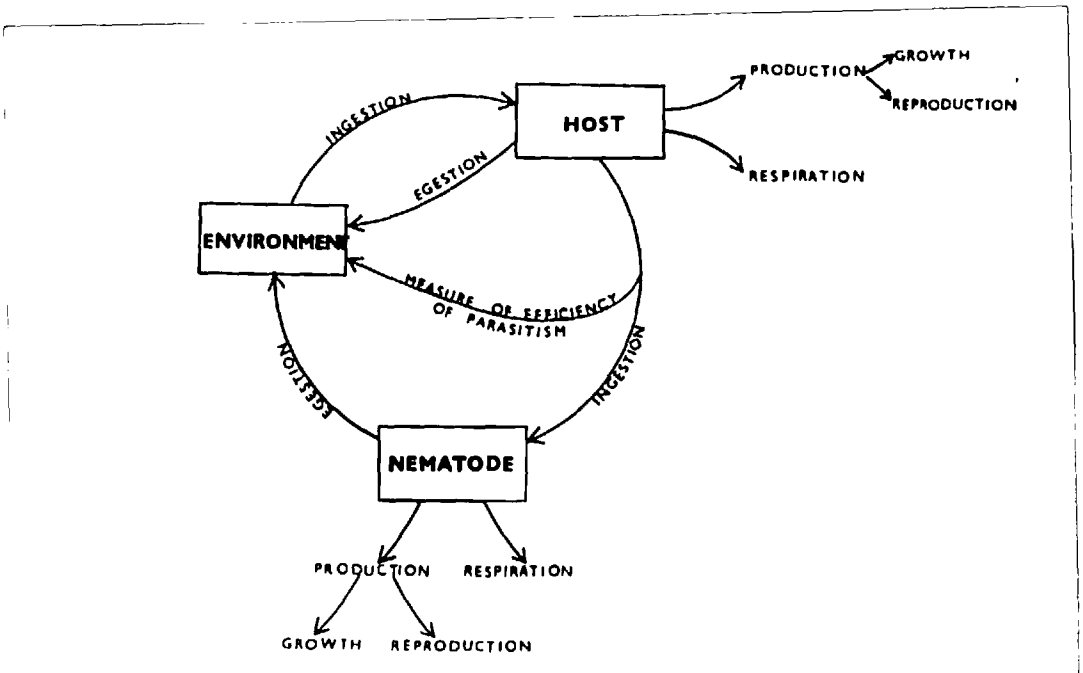
In addition to the fundamental significance of energy budgets, and its contribution to comparative energetics and physiolgy, a knowledge of nematode energetics may have a relevance to the energetics of paratism and pathology. To collect data on the energetics of life processes such as feeding, growth, respiration and reproduction together with populations and mortality, in nematodes may be to consider a nematode-host relationship in a new perspective.

There are very many technical difficulties in obtaining reliable energetics data for host-parasite systems, but the plant root or fungal feeding nematode Aphelenchus avenae cultured on the fungus Botrytis cinerea was selected, as the most suitable model (Fig. 1). The whole system was controlled under laboratory conditions, and the results obtained in this way, while possibly applicable to, are not directly comparable with field conditions.

A quantitative energetics analysis was made by constructing a budget

Fig. I.

ENERGY FLOW THROUGH A HOST-PARASITE SYSTEM



for the fungus and the nematode. There are two parts to the construction of an energy budget: 1) The productivity, i.e. the total number or biomass of individuals; 2) The energy budget of each individual.

An energy budget is constructed by obtaining estimates of: 1) Ingestion (I), gross energy intake or heat of combustion of food; 2) Egestion (E), ingestion less assimilation; 3) Assimilation (A), metabolizable energy available for life processes; 4) Respiration (R), energy cost of maintenance, work and activity; 5) Net production (P), energy contained in organic material synthesized by the organism including growth, reproduction and exuviae; and mortality and biomass of the population in calories. This terminology is adapted from Wiegert (1964).

Thus $A = I - E = R + P$.

Where the terms were not obtained directly they were calculated by substituting data available, in the above formula. The reliability on an energy budget depends on the number of parameters which may be directly measured. But to measure all the parameters is often technically impossible.

Slabodkin (1961) stated that, "there is a minimum set of biochemical components required for an animal's survival." If the energy supply available to an organism is in excess of the maintenance requirements, the additional energy will be used for reproduction or growth, but will usually not be stored in the organism as higher calories per gram. If this is so the calorific value per unit weight of an organism will depend on the maintenance energy and the availability of food. Eggs and diapausing stages of organisms are known to have a higher calorific value per unit weight (Slabodkin & Richman 1961) this being related to their maintenance during anabiosis.

Nematodes are the most divergent in habit of the Metazoa occupying

many ecological nitches. A survey of calorific values of nematodes from different habitats was made to compare the effects of different foods, environments and nutritional states on the calorific value of nematodes. This included parasites of vertebrates and invertebrates, fungi and higher plants. Among the selection were infective non-feeding stages dependent upon food reserves, and eggs and cysts of plant nematodes.

HISTORICAL REVIEW

Studies of energy and energy flow in organisms have gained much interest in recent years. Ivlev (1939) used the term "coefficient of energy consumption" to describe the quantity of energy transformed in a given link of a nutritive chain in aquatic animals.

Lindemann (1942) initiated the 'trophic - dynamic' approach to energy flow within ecosystems. His concept was applied in the field by Odum (1957), Slobodkin (1960), Engelmann (1961, 1966), Teal (1962), Golly (1960), Wiegert (1964, 1965), and others; and in the laboratory by Richmann (1958), Slobodkin (1959) and others.

Nielsen (1949) made a qualitative and quantitative study of the biology and ecology of soil nematodes, and his study may be regarded as the closest to nematode energetics made so far.

Energetics studies are based on a synthesis of other biological and physiological data such as respiration, growth, nutrition and reproduction. While not previously applied to energetics systems, these fields have of course been investigated with respect to nematodes. These will be revised separately.

Respiration

Respiratory rates of free - living and plant parasitic nematodes were studied by Nielsen (1949), using a Cartesian diver (Holter & Linderstrøm - Lang 1943), at 16°C. Santmyer (1956) studied large numbers of Panagrellus redivivus, over a wide temperature range (5°C to 40°C), using a differential micro-respirometer (Cunningham & Kirk, 1940). Rohde (1960), found that respiratory rates of certain plant parasitic nematodes were affected by CO_2 tensions, and that after removal from the host the rate declined progressively until death, although this may have been little more than the measurement of dying.

Weiser & Kanswisher (1960) studied the relationship of weight and surface/volume relations, with rate of respiration of the marine nematode Enoplus communis. The irregularities in the weight and respiration rate curves were correlated with moulting. They concluded that the surface/volume ratio, did not control oxygen consumption in this species.

Nielsen (1961) calculated the respiration of free - living nematodes per hectare per year, for three areas in Denmark. The calculations were based on laboratory data he obtained in 1949.

Bhatt and Rhode (1965) showed an increase in the respiratory rate of quiescent Anguina tritici when placed in water. He found that adult Ditylenchus dipsaci had a higher respiratory rate than many other plant parasitic nematodes.

Hämmen (1967) using a modification of Warburg & Krippahl's (1959) method, measured the oxygen consumption of Panagrellus redivivus in air. He reported a respiratory quotient of 0.734 $\pm$ 0.028 which is higher than that reported by Santmyer (1956), which was obtained in the absence of CO_2.

There are very many studies on the respiratory rate of animal parasitic nematodes. The effects on respiration of anaerobic and aerobic conditions, partial pressures of oxygen, and CO_2 concentration on adult and infective stages of animal parasitic nematodes have been studied by Davey (1938), von Brand (1942, 1947), Laser (1944), van Grembergen, van Damme & Vercruysse (1949), Rogers (1949), Bair (1955), Schawbe (1957), Roberts & Fairbairn (1965), Eckert (1967), and Barrett (1969b). von Brand (1943), Fernando (1963), Wilson (1965), and Barret (1969), studied the effects of temperature on the oxygen uptake of infective larvae of some animal parasitic nematodes.

The effect of light on the respiratory rate of third stage larvae of Nippostrongylus brasiliensis and Haemonchus contortus was studied by Wilson (1966), who reported an increase due to photoactivation.

The effectsof osmotic pressure (von Brand, 1960b; Schwabe, 1957; Fernando, 1963; and Barret, 1969b); pH (von Brand, 1943; Swhwabe, 1957; Eckert, 1967; and Barret 1969b) and inorganic ions (von Brand, 1943; and Beudin, 1949) have also been studied.

Rogers (1948), and Barrett (1969a) studied the effects of aging on oxygen consumption of infective stages of Nippostrongylus braziliensis and Strongyloides ratti. Passey and Fairbair (1957) found changes in the rate of respiration during development of Ascaris lumbricoides eggs. von Brand (1942) found that the oxygen consumption of larval Eustrongylides ignotus decreased by 50 per cent of the prestarvation rate in four days. The oxygen consumption of Trichinella spiralis was similarly effected in 22 hours (von Brand et al 1952).

The oxygen consumption/weight relationship of animal parasitic nematodes has been considered by von Brand (1934, 1942, 1952), Krueger (1936, 1940), and Glockling and Fairbairn (1952).

Nutrition

Quantitative data on ingestion assimilation or egestion of food by plant parasiti nematodes has not been directly assessed. Nielsen (1961) calculated the quantity of bacterial and plant material ingested by nematodes in a hectare of land per year. The estimates were obtained from respiratory equivalents.

Of the studies dealing with the method of food ingestion of soil inhabiting nematodes, Christie & Arndt, (1936); Fisher & Evans (1967), and Sutherland (1968) have considered aspects of the feeding process of Aphelenchus avenae.

Aphelenchus avenae has been reported to be a fungal as well as a

facultative plant parasite. Steiner (1931, 1936) published photographs of A.avenae females and eggs within tomato roots and claimed that "in this instance the nematode was a plant parasite." (1936).

The efficacy of A.avenae in biological control of phytogenic fungi has been demonstrated by Arndt & Christie (1937), Rhoades &Linford (1959), Mankau & Mankau (1962), Barker (1964), and Klink and Barker (1968). This gave rise to the opinion that A.avenae was merely a fungal feeder.

Goodey (1951), Lordello (1958), Mello (1958), Winslow (1960), and Decker (1962), were of the opinion that A. avenae was a facultative plant parasite.

This was further substantiated by Barker (1963, 1965)who demonstrated the ability of A.avenae to multiply on tobacco, carrot, periwinkle and tomato callus tissues.

Chin and Estey (1967) showed that A. avenae not only fed on the mycelium of various fungi but also on the root hairs of certain agricultural plants.

Growth and Population Dynamics

Only a few studies giving day to day growth curves of nematodes have been made (Bird, 1959; Blake,1962; Burdett, Bird & Fisher, 1963). Weiser & Kanswisher (1960) obtained the growth curve for Enoplus communis by determining the average weight of individuals in monthly samples. Growth of nematodes has mainly been studied by measuring the size of different stages in the life cycles, together with the length of the development periods of each stage. Heckler (1962) gave similar dimensions in a study of the development of A. avenae on the fungus Pyrenochaeta terrestris at 28°C.

Population dynamics of nematodes have been extensively studied by Jones (1956), Peters (1961), Seinhorst (1965,1966, 1967a,1967b), Oostenbrink (1966), and others. Wallace (1963) has reviewed the work

on the effects of the environment and host crop on the population dynamics of plant parasitic nematodes.

Townshend (1964) studied the population increase of A. avenae on different fungal hosts, on agar culture.

Pillai & Taylor (1967, 1968) reported the effects of temperature on the biology, host preference and host suitability (assessed as population increase and morphometrics) of some mycophagous nematodes including A. avenae.

Reproduction

A few studies on the number of eggs laid by individual nematodes have been made by Tyler (1938), Linford & Oliveira (1940), Wallace (1960, 1963), French & Barraclough (1961). But the numbers given may be inaccurate since the numbers were estimates and not obtained by continual observation throughout the nematode's life span.

The rate of egg laying has been based on total egg counts from single females or rates of egg laying over short periods by Tyler (1933), for Meloidogyne sp.; Yuksel (1960), and French & Barraclough (1961), for Aphelenchoides ritzemobosi; Rhoades &Linford (1961), for Paratylenchus projectus; and Loos (1962) for Radopholus similis.

Fisher (1968, 1969) obtained egg-production curves for A. avenae on cultures of Rhizoctonia solani, showing that the rate of egg-laying changes with age. Therefore the rate of egg-laying has little meaning unless the age of the female is specified.

To establish an energy budget of an organism the weights of individuals and their calorific values must be determined. Nielsen (1949), Volz (1951), and Andrassy (1956) have given methods by which weights of nematodes may be geometrically determined.

No calorific values for nematodes have been published.

GENERAL METHODS

A. Choice of the nematode for energetics studies

It was first necessary to obtain a system where the transfer of energy from the 'host' to the 'parasite' under known and controlled conditions could be studied.

Large numbers of nematodes were necessary for calorimetry, and different stages of the nematode were required for the establishment of energy budgets of each stage in the life-cycle. Standard inoculations of the nematode were required to assess the effects on the host. The culture of plant and animal parasitic nematodes is difficult under controlled conditions, for it is difficult to observe the parasite *in vivo* and maintain if *in vitro*.

Aphelenchus avenae was chosen because: a) It is easily cultured in the laboratory on fungi enabling a precise statement of the conditions of the system; b) It completes its life-cycle in 6 days at 25°C to 28°C (Heckler, 1962; Pillai & Taylor, 1967) and c) It's population increase is very rapid in fungal culture, (Townshend, 1964; Pillai & Taylor, 1968); d) It is also known to feed on plant roots, and may be a plant parasite (Chin & Estey, 1966) and so give the study a direct bearing on phytonematology.

B. The establishment of a nematode-fungal system

In order to find a good culture system various media and fungi were investigated. To evaluate the energy loss to the fungus by the presence of the nematode it was necessary to separate the fungus from the culture medium after nematode feeding. Three species of fungi were investigated: a) *Rhizoctonia solani*, b) *Trichoderma viridi*, c) *Botritis cinerea*. The following combination of factors were sought in choosing the system:

a) A fungus which allowed high population build up of *A.avenae*

b) A medium on which the fungi grew well and supported a good population of nematodes.

c) A medium from which the fungus could be separated.

To these ends a number of culture media and fungi were tested.

1) Culture of fungi and A.avenae on cellophane

Cellophane of grade PT300 was cut to fit exactly over potato dextrose agar (PDA) in 9 cm. petri-dishes. Cutting was made easier by washing the cellophane in boiling distilled water for 10 minutes. Each piece of cellophane was layered between moist filter paper and sterilized at 15 lb./sq.in. for 15 minutes. The cellophane was layered on the PDA in a sterile inoculating chamber and the fungal inoculum placed on it. In the system the fungus obtained its nutrient through the permeable cellophane (Kerr & Flentje 1957). This technique is not suitable for plant pathogenic fungi which produce cellulase, causing the break-down of the cellophane layer. Therefore B.cinerea and R.solani could not be cultured on cellophane. Trichoderma viridi was successfully cultured in this manner. But when nematodes were introduced, they passed under the edges of the cellophane, carrying fungal spores, which then established colonies directly on the agar. For this reason the cellophane system was rejected. With further modifications this technique may provide a good method of nematode culture, as it also facilitates the extraction of the nematodes.

2) Culture of fungi and A.avenae on liquid media

The fungi were grown in malt extract broth and ozapek dox liquid medium in 50 ml. flasks. Nematodes were inoculated onto the cultures after one week of incubation at 26°C. Inactive worms were recovered from the bottom of the flasks when tested one week after inoculation. This may have been caused through either an oxygen deficiency or a lack of food in

the bottom of the flask, as the fungus grew on top of the liquid.

Two modifications of the above technique were tried, using the same nutrients.

a) Sand was moistered with the liquid media and inoculated with fungi. The fungus grew well on the sand and could be separated from it by agitation in water. Some reproduction of A.avenae was obtained in this culture.

b) The fungus was inoculated onto 10 ml. of liquid nutrient in 9 cm. petri-dishes, in an attempt to concentrate the fungus and increase the oxygen. The nematodes were inoculated when the fungus had colonised the plate. It was observed that the nematodes found difficulty in obtaining a purchase for feeding on the fungal hyphae, which were suspended in liquid. The population increase in liquid culture was tested against nutrient agar culture by inoculating 3 adult A.avenae onto each of six plates; three with PDA and B.cinerea and three with malt extract broth and B.cinerea. An equal volume of total extract from each plate was counted two weeks after inoculation of A.avenae. Increase in population of A.avenae on the liquid medium was approximately a third of that obtained on the agar medium. Table 1.

3) Culture of fungi and A.avenae on potato dextrose agar (PDA) and malt extract agar (MEA).

Two plates each, with each medium and B.cinerea was inoculated with three A.avenae each, and extracted after 2 weeks incubation. An equal volume of total extract from each plate was counted. Table II. Population increase of A.avenae when tested on B.cinerea grown on PDA and MEA was

Table I

Number of A.avenae recovered 2 weeks after inoculation onto B.cinerea on malt extract broth (MEB) and PDA. (An equal volume of total extract from each plate was counted.)

Plate no.	Number of A.avenae	
	MEB.	PDA.
1	987	2217
2	840	2405
3	623	1918
Total	2450	6540
Mean	816	2180

Table II

Number A.avenae recovered 2 weeks after inoculation onto B.cinerea on MEA and PDA. (An equal volume of total extract from each plate counted)

Plate no.	Number of A.avenae	
	MEA.	PDA.
1	1394	1795
2	1838	1964
Total	3232	3759
Mean	1616	1879

essentially the same.

Most experiments were carried out on potato dextrose agar. Experiments requiring the separation of fungus from the medium were carried out using liquid media. Botrytis cinerea was subsequently used for all experiments for the following reasons:

a) The high reproductive rate of A.avenae on B.cinerea (Townshend, 1964).

b) The comparative ease of culture and rapid growth of B.cinerea (measured as area covered).

c) The sparely distributed hyphae in young colonies allowed easy observation of nematodes in culture.

The population of A.avenae, originating from a single female was routinely cultured in 9 cm. petri plates on B.cinerea grown on PDA.

C. Surface sterilization of Aphelenchus avenae

Aphelenchus avenae which were required for large inoculations had to be surface sterilized to prevent contamination of cultures. Hibitane diacetate (from I.C.I.) (0.5% solution) was used as recommended by Peacock (1959). An aqueous suspension of A.avenae was pipetted onto the outside of an overturned sintered glass crucible, the inside of which was plugged with tissue. The water rapidly seeped through the sintered glass onto the tissue. The tissue was removed and the Hibitane solution was pipetted onto the nematodes on the sintered surface. The tissue was replaced after 2 - 3 minutes and the Hibitane solution then soaked through. The nematodes were rinsed a few more times with Hibitane, followed with many rinses with sterile (auoclaved) water, the tissue being replaced when soaked. Finally the sintered glass crucible was overturned onto a watch glass containing sterile water, to such a level that it just touched

the sintered surface. The whole procedure took approximately five minutes with very little loss of nematodes. The operation was carried out in a sterile inoculating chamber. When tested on agar plates these nematodes showed no contamination.

D. Preparation of nematodes for calorimetry

Large (minimum weight of 3 mg.), debris free samples of dried nematodes were required to obtain calorific values of nematodes.

A modification of Gibbons and Grandison's (1966) method of separating nematodes from debris by layering aqueous nematode suspensions over sucrose solutions was used.

Best results were obtained with a 40% sucrose solution without injury to the nematodes. A 20 ml. portion of 40% sucrose solution was poured into a 50 ml. centrifuge tube and 10 ml. of a suspension of nematodes were carefully added along the side of the tube. The tubes were centrifuged for 10 minutes at 3500 r.p.m. The debris then fell to the bottom and a band of clean nematodes were left at the interface of water and sucrose. Plate 1.

The nematodes were pipetted into a crucible and washed with running distilled water, the water being drawn off through a sintered glass stick attached to a filter pump. The nematodes were quickly washed clean of sucrose and left in a dense suspension in distilled water. The washing procedure took only a few seconds. The nematodes that were stuck onto the sintered glass were recovered by suspending the filter stick over the crucible so that the sintered surface just touched the water (Plate 2). The efficiency of filter sticks in recovering A.avenae after washing was tested.

Ten ml. of a uniform suspension of nematodes were measured into four

Plate 1.

SEPARATION OF NEMATODES FROM DEBRIS BY CENTRIFUGATION IN 40% SUCROSE SOLUTION.

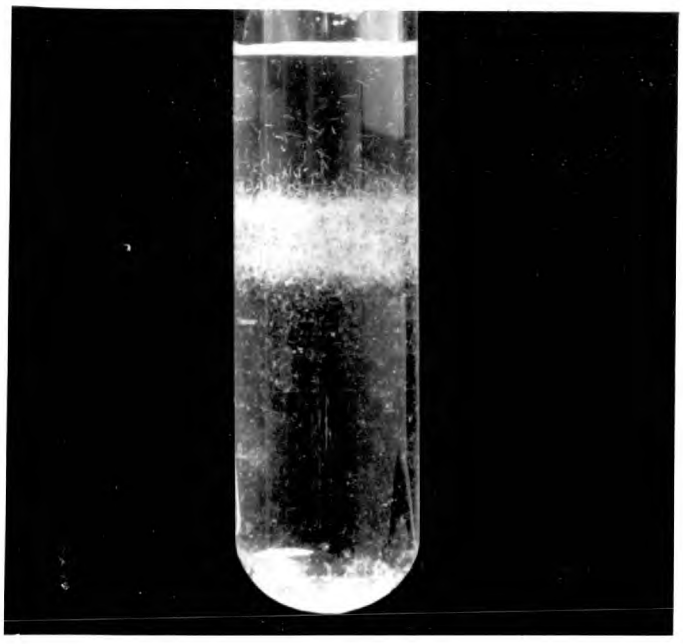


Plate 2.

FILTER STICK USED IN CHANGING NEMATODES FROM SUCROSE SOLUTION TO DISTILLED WATER.



crucibles. Three of the samples were washed using filter sticks. The nematodes in three 0.15 ml. samples of the one 'non-washed' sample and each of the three washed samples were counted. The volume of the three washed samples were not concentrated equally after washing, but an estimate of the number of nematodes in 10 ml. of solution per sample is given in Table III. The efficiency of recovery of A.avenae was 84%.

Samples for calorimetry were dried in crucibles coated with silicone "repelcote"(From Hopkin & Williams Ltd.) which greatly enhances the removal of nematodes, after vacuum or oven drying. All materials were dried for at least 24 hours. Pellets of 5 to 50 mg. were made by pressing the dried material in a tablet maker. (From Gallenkamp & Co. Ltd.)

E. Calorimetry

A bomb calorimeter of the type described by Phillipson (1964) in conjunction with a Pye scale lamp galvanometer was used in determining the energy content of the biological material. (Plate 3) Samples were weighed on a Cahn-gram electric balance or on an Oertling chemical balance. Pelleted material was weighed on a weighed platinum pan, which was then placed on the sample holder of the calorimeter such that the platinum wire connected to the firing circuit touched the pellet, (Fig. 1 Plate 3). The calorimeter was then closed, flushed and filled at 30 atmospheres of oxygen. It was placed on the holder in contact with the termocouples which were connected to the recorder, and the firing circuit connected. The apparatus was allowed to equilibrate in temperature (shown by the stability of the galvanometer reading), and fired.

The apparatus was calibrated using thermochemical benzoic acid (British chemical standard no. 190j) of 6.319 Kcals/gr. The samples were weighed on a Cahn gram electrobalance, calibrated to weigh to 0.001 mg.

Table III

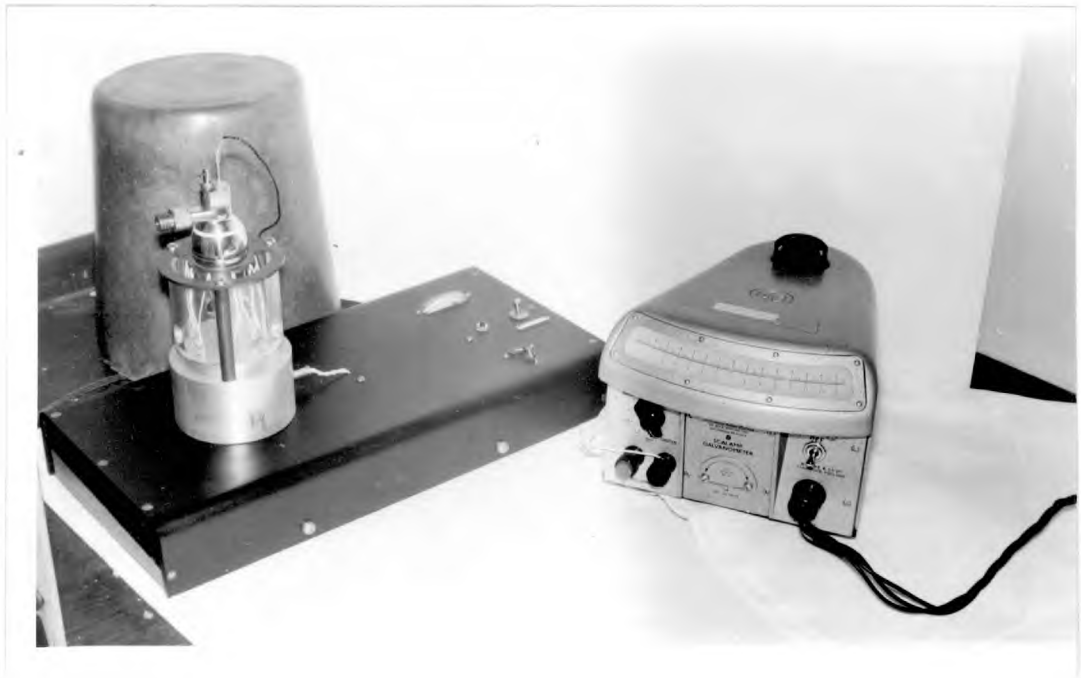
Efficiency of sintered glass sticks for recovery after washing of nematodes.

Number of *A.avenae* in 10 ml. of suspension				
Count replicate	Non-washed sample	Washed samples		
		1	2	3
1	69	45.5	51.0	51.0
2	70	52.8	69.0	60.0
3	63	56.5	75.0	69.0
Total	202	154.8	195.0	180.0
Efficiency of recovery		77.0%	97.0%	89.0%

Plate 3.

BOMB CALORIMETER CONNECTED TO FIRING CIRCUIT

AND RECORDER.



with 100 mg. counter balancing. Two ranges of the galvanometer were calibrated:

a) '0.5' range for samples between 4 mg. and 90 mg.

b) '1.0' range for samples between 2 mg. and 5.5 mg.

The scale reading per 100 calories was calculated for reading obtained from samples of differing weights of benzoic acid as outlined by Phillipson (1964) (Tables IV and V). The '0.5' range gave a mean value (divisions/100 cals.) of 1.7366 $\pm$ se.0.0283; and the '1.0' scale gave a mean value of 33.1113 $\pm$ se.1.6798 divisions per 100 calories. The '1.0' scale was difficult to operate as the galvanometer took a long time to stabilize. Thus the '0.5' range was considered the most reliable.

Table IV

Calibration of calorimeter

Mv. scale '0.5'

weight of sample (ash free) (mg)	calculated calorific* value of sample	scale reading (division)	divisions/ 100 cals.
12.848	81.187	1.50	1.8476
14.648	92.561	1.65	1.7826
26.528	167.630	3.00	1.7897
20.696	130.778	2.20	1.6822
37.308	235.749	3.95	1.6755
36.280	229.253	4.15	1.8102
7.364	46.533	0.70	1.5043
10.988	69.433	1.05	1.5122
34.276	216.590	3.60	1.6621
19.952	126.677	2.30	1.8243
14.072	88.921	1.50	1.6869
14.736	93.117	1.60	1.7183
11.608	73.351	1.20	1.6360
6.676	42.186	0.65	1.5408
13.980	252.634	4.70	1.8604
84.340	532.944	10.05	1.8858
62.460	394.685	7.10	1.7989
70.360	444.605	8.20	1.8384
87.960	555.819	10.40	1.8711
48.680	307.609	5.70	1.8530
36.980	233.677	4.20	1.7974

Mean scale reading / 100 cals. = 1.7366 $\pm$ se.0.0283

*Calorific value of benzoic acid = 6.319 Kcal./gram.

Table V

Calibration of calorimeter

Mv. scale '1.0'

weight of sample (ash free)	calculated calorific value of sample (cal./gr.)	scale reading (division)	divisions/ 100 cals.
2.968	18.7548	5.10	27.193
5.096	32.2016	11.25	34.936
3.860	24.3913	8.80	36.078
5.032	31.7972	10.80	33.965
4.072	25.7310	9.65	37.503
2.156	13.6238	3.95	28.993

Mean scale reading / 100 calories = 33.1113 $\pm$ se.1.6798

PART I

INDIVIDUAL ENERGY BUDGETS

Section I

INGESTION

A. Introduction

Aphelenchus avenae feeds by penetrating the host cell walls with its stylet. Then by the pulsations of the muscular oesophagus and its bulb (metacorpus), and valve, the cell contents are sucked in. Preliminary observations established that A. avenae fed on all ages of Botrytis cinerea hyphae. The size of fungal cells fed on by A. avenae is variable as is also the proportion of cell contents ingested. Therefore the volume of the cell had no relation to the volume of cell contents ingested.

Pulsation of the medium bulb and the streaming of cell contents into the stylet was observed immediately after penetration of the cell wall. As ingestion follows penetration (Christie & Arndt, 1936; Linford, 1937; Fisher & Evans, 1967) there is no time for secretions to be injected or take effect (Fisher & Evans, 1967).

Ingestion of Botrytis cinerea cell contents was assessed quantitatively by assuming that at each pulsation the valve fills up with cell contents to the same extent as it opens and that the fluid is sucked back into the gut when the valve contracts. Observation of the valve action during feeding suggested that this was a valid assumption.

The volume of B. cinerea cell contents passing through the valve per day was determined.

The calorific value of B. cinerea cell contents was established by extraction of cell contents and bomb calorimetry.

B. Volume of food ingested by Aphelenchus avenae.

The quantity of food ingested by A. avenae was calculated by estimating the volume of food ingested per second of the time the nematode was feeding. The volume of the open valve (a) and the rate of pulsation

per second (b) were measured. This was then related to the time spent feeding per day (y). An equation could therefore be derived for the volume of food ingested i.e. (a) x (b) x(y).

Observations on feeding were made by placing an A. avenae, of known age on a 2 mm. thick, small disc of B. cinerea on PDA, on a slide. A cover slip was then placed on the preparation. Observations were made at X400 or X 1000 magnification.

1. The rate of pulsation and the volume of the medium valve.

The pulsation of the medium value of A. avenae during feeding is too rapid to be accurately counted at normal speed. Cine films were taken of the different ages, at 24 frames per second using a Vinten 16 mm. camera. The films were projected at 8 frames per second at which speed the pulsation rate could be counted. Films were made of all stages from newly hatched second stage larvae (L_2) to adult females (Table VI).

An eye piece scale was included in the microscope and a micrometer slide was filmed at the same magnification as the nematode, at the end of each day, for reference. By viewing the film on a film editor, measurements of the internal diameter of the fully open valve were taken in microns. The valve dilates to form a spherical cavity, the volume of which was calculated from the measurements of its diameter (Table VI).

The length and width of the medium bulb were measured across the medium valve to determine when it stopped growth. Measurements were taken from different ages of nematodes using a calibrated eye piece scale. Measurements were made of a few newly hatched (day 1) and 4, 5, 6, 7, 10, 16 and 24 day old A. avenae (Table I in appendix). It was apparent from these measurements that the bulb and presumably the valve stopped growth after the 7th day. Therefore films were taken of a few A. avenae of each day upto the 6th day and of an adult over 7 days old. Data regarding the

Table VI

INGESTION

Day	Volume of Valve (a) μ^3	Mean No puls/sec (b)	Volume of Food Ingest/s. in μ^3 (axb) = x	Feeding Time/15m Period Secs. Y	Vol. Ingested/ 15 m period XY μ^3	Vol. Ingested for 24 hours XYx4x24 μ^3	Vol. Ingested per 24 hrs. in ml.	Cals. Ingested Per Day
1	34.5496	8.14	281.2337	16.62	4674.1041	448713.9936	44871399^{-14}	0.0000396613
2	67.4838	7.47	504.1040	25.99	13101.6630	1257759.6480	125775965^{-14}	0.0001111721
3	84.3652	7.50	632.7390	15.66	9908.6927	951234.4992	95123450^{-14}	0.0000840786
4	147.8714	6.50	961.1641	10.11	9717.3691	932867.4336	93286743^{-14}	0.0000824552
5	200.6264	6.83	1370.2783	54.60	74817.1952	7182450.7392	718245074^{-14}	0.0006348496
6	200.6264	6.54	1312.0967	45.15	59241,1660	5687151,9360	568715194^{-14}	0.0005026816
7	227.7503	7.21	1642.0797	14.40	23645.9477	2270010.9792	227001098^{-14}	0.0002006440
8	227.7503	7.21	1642.0797	23.98	39377.0712	3780198.8352	378019884^{-14}	0.0003341279
9	227.7503	7.21	1642.0797	37.33	61298.8352	5884688.1792	588468818^{-14}	0.0005201417
10	227.7503	7.21	1642.0797	-	-	-	-	-
16	227.7503	7.21	1642.0797	30.80	50516.0548	4855301.2608	485530126^{-14}	0.0004291552
21	227.7503	7.21	1642.0797	17.82	29261.8603	2809138.5888	280913859^{-14}	0.0002482969
24	227.7503	7.21	1642.0797	36.47	59886.6467	5749118.0832	574911808^{-14}	0.0005081587

$\mu^3 = 10^{-12}$ ml

1 ml = 88.389 cals.

rate of pulsation and volume of value from the 7th day onwards were taken from the film of the adult (Table VI).

2. Duration of feeding.

The time spent in feeding by A. avenae was noted by observing nematodes of different ages, for 15 min. each. The duration of each feed was noted. The duration of feeding per 15 min. was observed at room temperature (22°C) and in a constant temperature room at 26°C. One and two day old A. avenae were observed during the night as well as during the day to test any possible periodicities. In between all observations nematodes were incubated at 26°C so that the rate of maturity would remain constant. The total time spent in feeding and the number of feeds per 15 min. period of 1 and 2 day old A.avenae at approximately 22°C and 26°C during day time and at approximately 22°C at night is given in Table VII. From the figures given in Table VII it is apparent that the L_3 spend longer in feeding than the L_2. But there is no effect of the three given conditions on the time spent feeding on either age. All further observations of feeding were made at room temperature, during day time. The total time spent in feeding and the number of feeds per 15 min. period of day I to day 24 A. avenae is given in Table VIII and Fig. II. The volume of food ingested per 24 hours by each age of A. avenae was determined, as explained earlier (Table VI).

C. Calorific value of a given volume of Botrytis cinerea cell contents.

Method:

B. cinerea was grown on 100 ml. of standard potato dextrose broth in each of 12, 250 ml. conical flasks, for 1 week at 26°C.

The mat of mycelium was removed from the culture flasks, washed in distilled water and the surface moisture removed by mopping with tissue.

Table VII

The total time spent feeding and the number of feeds per 15 min. period of 1 (L_2) and 2 (L_3) day old A. avenae at 22°C at various times of day.

	Day at 22°C		Day at 26°C		Night at 22°C	
L_2 number	Total feeding time	no. of feeds	Total feeding time	no. of feeds	Total feeding time	no. of feeds
1	18.8	2	16.5	2	19.9	2
2	22.4	2	7.4	3	13.6	3
3	9.0	1	19.9	7	20.9	3
4	5.8	2	24.4	4	15.0	3
5	6.6	5	6.5	3	7.3	2
6	30.9	3	10.2	4	13.4	3
7	26.9	6	-	-	-	-
Mean	17.2		14.2		15.0	
L_3 number						
1	10.8	3	45.6	5	41.7	15
2	25.1	4	38.8	7	30.2	2
3	28.9	5	6.8	3	22.8	9
4	20.4	4	12.3	4	31.4	13
5	13.1	6	47.6	10	34.8	15
6	23.3	10	24.7	7	19.6	4
7	46.5	10	10.2	4	17.4	3
8	6.6	3	12.7	2	9.7	2
9	59.2	11	-	-	-	-
Mean	25.9		24.9		25.9	-

Table VIII

The total time spent feeding and the number of feeds per 15 min. period of different ages of A. avenae.

Worm no.	Day 1		Day 2		Day 3	
	Time in sec.	no. of feeds	Time in sec.	no. of feeds	Time in sec.	no. of feeds
1	18.8	2	10.8	3	29.0	3
2	22.4	2	25.1	4	53.2	10
3	9.0	1	28.9	5	44.7	8
4	5.8	2	20.4	4	0	0
5	6.6	5	13.1	6	0	0
6	30.9	3	23.3	10	0	0
7	22.9	6	46.5	10	13.3	5
8	-	-	6.6	3	0	0
9	-	-	59.2	11	0	0
Mean	16.62	-	25.99	-	15.60	-
S.E.	3.65	-	5.70	-	7.13	-
	Day 4		Day 5		Day 6	
1	2.6	1	29.1	6	3.4	2
2	1.9	1	63.2	16	15.9	5
3	0	0	117.2	21	37.3	11
4	66.3	13	35.1	12	78.4	27
5	0	0	16.6	3	74.1	19
6	0	0	66.4	21	61.8	18
7	0	0	-	-	-	-
Mean	10.11	-	54.60	-	45.15	-
S.E.	10.12	-	14.78	-	12.75	-

contd./-

Table VIII contd./-

Worm no.	Day 7		Day 8		Day 9	
	Time in sec.	no. of feeds	Time in sec.	no. of feeds	Time in sec.	no. of feeds
1	7.3	4	42.3	29	14.7	11
2	25.8	10	17.4	8	87.2	30
3	20.6	6	11.0	10	32.6	20
4	0	0	31.4	12	25.8	10
5	32.7	18	26.6	14	29.5	7
6	0	0	15.2	8	34.2	9
Mean	14.40	-	23.98	-	37.33	-
S.E.	5.68	-	4.78	-	10.37	-
	Day 16		Day 21		Day 24	
1	29.0	9	18.8	10	43.0	2
2	37.4	10	0	0	18.0	6
3	65.0	15	52.4	27	86.0	4
4	44.6	28	0	0	35.2	7
5	0	0	7.3	2	0	0
6	8.8	7	26.0	3	36.6	3
Mean	30.80	-	17.82	-	36.47	-
S.E.	9.73	-	8.18	-	11.79	-

Fig. II.

TIME SPENT FEEDING BY DIFFERENT AGES OF A. AVENAE.

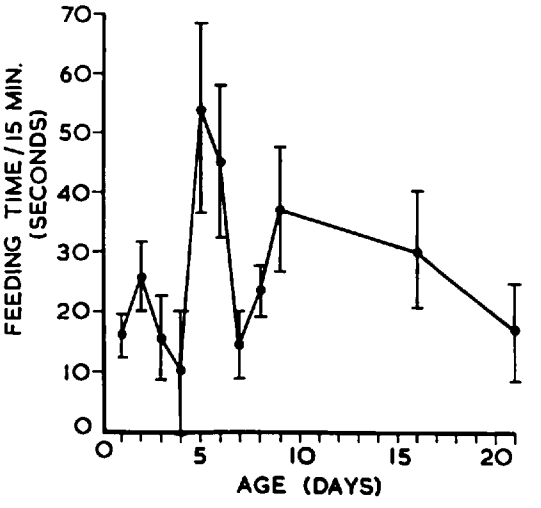
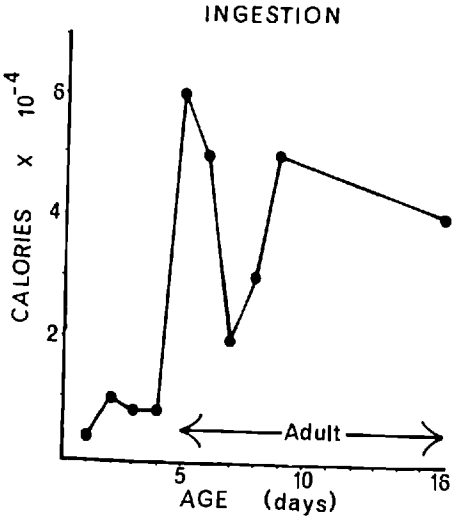


Fig. III.

CALORIES INGESTED PER DAY BY DIFFERENT AGES OF A. AVENAE.



The mycelium was cooled to about 4°C cut up into approximately 2 cm. square pieces and macerated in a vibro-mixer. The fungus container was surrounded with ice. The well macerated parts of the mycelium were introduced into 15 ml. centrifuge tubes and centrifuged at 3000 r.p.m. for 15 minutes in a refrigerated centrifuge maintained at 4°C. Drops of the supernatant were checked on a slide under a microscope to varify the absence of cell walls. With a graduated syringe, 1 ml. of the supernatant was pipetted into each of 2 weighed ampules. They were freeze dried for 48 hours and vacuum dried over P_2O_5 and weighed again. The dry weight of 1 ml. of cell contents was thus established (Table IX).

The remaining fungal material was mixed with cold water and re-centrifuged as before. The supernatant was checked under the microscope, collected and kept in the refrigerator. The procedure was repeated (on the same day) until a large quantity of material had been collected. Finally the bulk of cell contents was dried, as before, pelleted and the calorific value established by bomb calorimetry.

Extreme care was taken to keep the material cold, to minimise enzyme reaction through - out the procedure until the material was dried.

Results:

Weight of 1 ml. of B. cinerea cell contents = 0.0252 g.

Calorific value of B. cinerea cell contents = 3507.5 calories/g.

∴ 1 ml. of B. cinerea cell contents = 88.389 calories.

D. Discussion.

The calorific value of food ingested per day was calculated by knowing the volume of food ingested and the calorific value of a unit volume of food (Table VI).

The mean number of pulsations per second of the medium value of the different ages, is not widely variable. The volume of the valve and the

Table IX

Weight of 1 ml. of B. cinerea cell contents

Wt. of ampule empty	Wt. of ampule with 1ml. of fell contents	∴wt. of 1 ml. of cell content
13.0724 g.	13.0974 g.	0.0250 g.
13.0370 g.	13.0624 g.	0.0254 g.
		Mean = 0.0252 g.

time spent in feeding is dependent on the age, and has most bearing on the change in daily ingestion (Fig. III).

A few 3 and 4 day old A. avenae from the sample were found to be in a moult, and as such were not feeding during the time of observation. This depressed the mean value for the time spent feeding, and thereby ingestion per day. 7 and 21 day old A. avenae also had a low mean feeding time, when compared to other adults (Table VI). This may not be significant as the nematodes that were feeding on these days did not show a decreased feeding time, when compared to other feeding adults. The comparatively low mean, may be due to the inclusion of 2 A. avenae which did not feed in the 15 min. observation periods on both these days. These results illustrate tendencies which require statistical verification. The time spent in feeding varies greatly between A. avenae of the same age. The standard error of the mean is thereby very large.

Section II

REPRODUCTION

A. Introduction

When cultured on Botrytis cinerea at 26°C, Aphelenchus avenae males were not observed. Females are mostly parthenogenetic. Therefore it was possible to study egg production by isolating a single fourth - stage larvae onto a culture, and counting the progeny at intervals.

The number of eggs produced per day by progressively ageing females was thus obtained. Then the dry weight was calculated by measuring the volume of newly laid eggs, their specific gravity and water content. Calories per gram dry weight was obtained by bomb calorimetry.

For the purpose of determining a daily energy budget of A.avenae experiments on B.cinerea on PDA at 26°C were chiefly used. Other culture media and temperatures were tried for purposes of comparison and for reference to population dynamics data.

Using these techniques data was collected, enabling an energetic expression of reproduction to be made.

B. The rate of egg production of Aphelenchus avenae.

The rate of egg production of A.avenae was observed on B.cinerea under different conditions. 1. Sand moistered with czapek dox liquid medium, at 26°C. 2. Potato dextrose agar (PDA) at 26°C. 3. PDA at 15°C.

1. Egg production on sand and czapek dox medium, at 26°C.

Botrytis cinerea was grown at 26°C in a conical flask with 100 gm. of autoclaved sand moistened with 10 ml. of standard czapek dox liquid. After a weeks growth, 0.2 to 0.3 gm. of sand with B.cinerea was put into each of 15 watch glasses with a fourth stage A.avenae larvae. The watch glasses with their covers sealed with silicone grease (Edward's high vacuum grease) were sterilized at 15 lb./sq.in. for 15 minutes, before

introduction of fungal cultures. The cultures were incubated at 26°C.

The number of eggs and larvae produced was counted one week after inoculation of the 4th stage larva and subsequently at 3 to 4 day intervals (Table X). The eggs and larvae were extracted by breaking up the sand and fungus in a 2.5 cm. diameter nylon seive of 195 µ pore size, placed on a counting dish filled with water. The original adult female was transferred into a fresh culture at every count. The sand and fungus on the seive was emptied into another counting dish and further examined. The latter procedure was later abandoned since the examination did not reveal any more eggs or larvae. Some of the adult nematodes were lost at every extraction. Sometimes broken pieces of the female were seen. For this reason more frequent counts of egg laying could not be taken. It was necessary to transfer the female onto a fresh culture frequently for the following reasons:

a) So that the original female could always be dinstinguished from its progeny.

b) The change in rate (if any) of egg production could be observed.

c) The cultures were extracted on every third and fourth day alternatively so that results could be collectively related to the population dynamics data which were taken weekly.

2. Egg production on potata dextrose agar, at 26°C.

A fourth stage larva was inoculated onto a 1.5 cm. diameter disc of PDA with one week's growth of B.cinerea in a solid watch glass which had been autoclaved. Twenty such cultures were incubated at 26°C. After 4 days each disc was washed into a 2.5 cm. seive which was placed on a counting dish filled with water. The seives were allowed to stand for at least 30 minutes. Then the agar was squashed through the seive so that

Table X

Reproduction of A. avenae at 26°C on B. cinerea on sand and czapek dox medium.

Time		no. of females observ.	Total progeny		Mean progeny/female	
Days after inoculation	Actual age of female (days)		Eggs	larvae	Eggs	larvae
7	11	12	310	292	25.8	24.3
10	14	9	211	73	23.4	8.1
14	18	8	112	136	14.0	17.0
17	21	4	26	21	6.5	5.2
21	25	2	16	7	8.0	3.5

Table XI

Reproduction of A. avenae at 26°C on B. cinerea on PDA (combined results of two trials)

Time		no. of females observ.	Total progeny		Mean progeny/female	
Days after inoculation	Actual age of female (days)		Eggs	larvae	Eggs	larvae
4	8	20	245	3	12.3	0.2
7	11	17	474	177	27.9	10.4
11	15	19	375	402	18.2	21.2
14	18	15	146	82	9.7	5.5
18	22	7	24	4	3.4	0.6
21	25	4	1	2	0.3	0.5

any eggs or larvae remaining would fall through. The originally inoculated female was transferred onto a fresh culture.

As with the sand and czapek dox culture the original A. avenae was not always recovered at each extraction. The procedure was repeated to obtain sufficient records of egg laying for the whole length of the life of the female. In the second trial, readings were taken from the 11th day onwards, although the female was transferred to fresh cultures as usual before that day. The results of the trials are combined and given in Table X1.

Daily egg production data was necessary for determining the daily energy budget of A. avenae. From the counts of eggs and larvae produced, taken every 3or 4 days, daily egg production was deduced by my observations that eggs take 2 days to hatch at 26°C. Then the larvae counted on the 3rd or 4th day were taken as the count of eggs produced on the 1st and/or 2nd day of the period tested. The eggs counted were produced on the 3rd and/or 4th day of the period. When total produce of 2 days was counted together, an average was taken for each day of egg production (Fig. 1V). No females were recovered from cultures after 28 days at 26°C.

3. The above procedure was repeated incubating the cultures at 15°C. Development of A. avenae at 15°C is not so rapid as at 26°C (Fig. V11, in Section 111) and therefore counts were taken only once a week (Table X11).

C. Calorific value of eggs produced / day.

To convert the numerical data on egg production to calories of eggs produced / day, the calorific value of an average egg had to be established. The number of individuals contained in a pellet used for calorimetry could not be accurately stated. Therefore the weight of an egg had to be established. The weight of eggs was obtained by measuring the volume and specific gravity.

Fig. IV.

CALORIES OF EGGS PRODUCED PER DAY BY AN AVERAGE FEMALE.

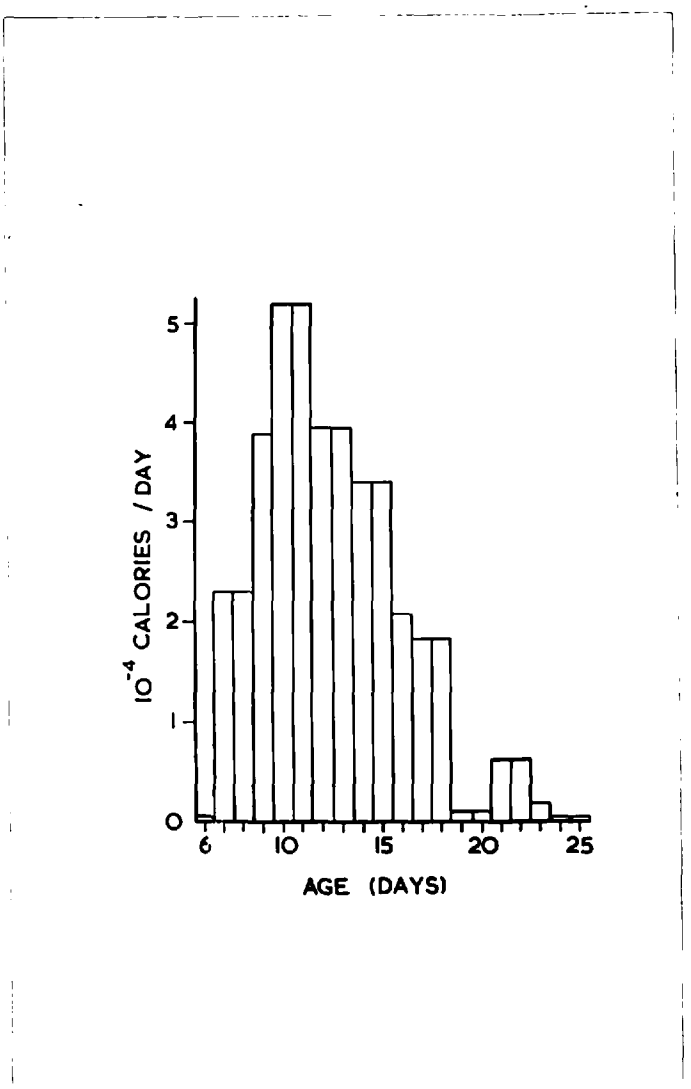


Fig. V.

CUMULATIVE EGG LAYING RATE OF A. AVENAE UNDER VARIOUS CONDITIONS

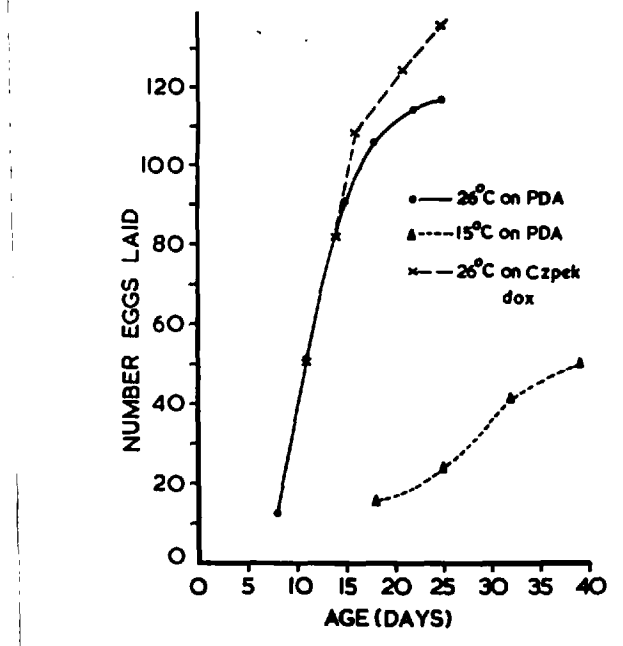


Table XII

Reproduction of *A. avenae* at 15°C on *B. cinerea* on PDA. (Combined results of two trials)

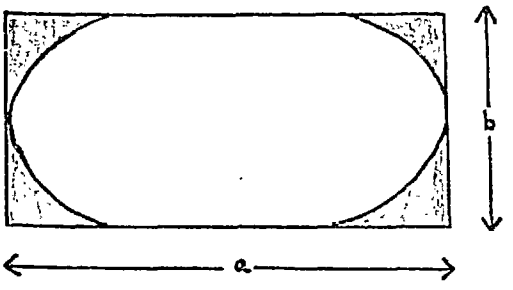
Time		no. of females observ.	Total Progeny		Mean progeny/female	
Days from inoculation	Days, actual age of female		Eggs	larvae	Eggs	larvae
14	18	26	295	101	11.3	3.9
21	25	16	145	66	9.1	4.1
28	32	6	61	14	10.2	2.3
35	39	1	6	3	6.0	3.0

1. The volume of newly laid Aphelenchus avenae eggs.

Aphelenchus avenae females were picked from one to two week old culture (where there is excess food), and incubated in tap water in a watch glass for approximately 1 hour. The eggs laid during this period were collected onto microscope slides (approximately 5 per slide). Sufficient water was introduced so that the eggs did not get squashed when the coverslip was put on. A few strands of glass-wool were put into the preparation as an added precaution. The coverslip was sealed with vaseline to prevent evaporation. Camera lucida drawings were made of the outline of the eggs on graph paper. A micrometer scale of known length was drawn on each page of the paper used at the same magnification as the eggs.

The eggs are elliptical in shape and on observation of the drawing it was clear that the eggs could be described by (1) drawing a rectangle around it (Fig. VI) and (2) by taking the volume of the square about the shorter side (b) of the rectangle less the sphere which fits within this square, (radius = $\frac{1}{2}$b) the volume of the rectangle which is not occupied by the egg is found, (shaded in Fig. VI)

Fig. VI Diagram of egg



and (3) by subtracting the ultimate value obtained from (2) (i.e. square - sphere) from the volume of the rectangle, the volume of the egg was obtained (Table II in appendix).

Although there is a simpler method of calculating the volume of an

oblong, the above method was considered more accurate as the egg is not always a perfect shape and the parameters required for the method used were very easily obtained from the drawings.

2. Density of Aphelenchus avenae.

The density of A. avenae eggs, larvae and adults was found by using a gradient of ficoll solutions. Ficoll was used in preference to other solutions because of its very low osmotic pressure at high concentrations (Fig. I in appendix).

From preliminary observations and from previous work (Ellenby, 1968; Andressey, 1956) it was apparent that the density of A. avenae was more than 1.0 g/ml. Solutions of densities of 1.01 g/ml. to 1.06 g/ml. were prepared from a graph provided by Pharmacia Ltd. of the density per concentration (weight / volume) of Ficoll (Table III & Fig. 1 in appendix).

Using a hypodermic syringe 5 ml. each of solutions of densities 1.03, 1.02, 1.01 g/ml. were layered on each other in a 25 ml. centrifuge tube. When layering solutions, the hypodermic syringe was kept on the side of the tube at the level of liquid and raised gradually as the level of liquid rose in the tube. Using a Pasteur pipette 0.5 ml. of a dense aqueous suspension of A. avenae (all stages) was pipetted on top of the Ficoll gradient. Another tube containing a gradient of solutions of densities 1.06, 1.05, 1.04 g/ml. was prepared as above. The tubes were centrifuged for 10 minutes at 3000 r.p.m.

On centrifugation the nematodes in the first tube dropped to the bottom indicating that they were of a higher density than 1.03 g/ml. In the second tube a few nematodes settled at each interface indicating that the density was 1.04 to 1.06 g/ml. All stages were found at each interface, therefore this does not show that the different stages have different densities.

To test further, an A. avenae aqueous suspension was layered in tubes containing ficoll of 1.04, 1.05 and 1.06 g/ml. respectively. The distance from the surface that the nematodes dropped was observed at 5 and 10 minutes (Table XIII). In solution of density 1.04 most nematodes sank in 10 minutes. In solution 1.05 a few sank in 10 minutes but most were at the interface (water & Ficoll). The density of A. avenae was taken to be 1.05.

3. Water content of Aphelenchus avenae

The water content of A. avenae eggs, larvae and adults was determined by using Myers' (1967) method. Essentially, this consisted of passing water saturated air for 10 minutes over nematodes in weighed sintered glass crusibles (Plate 4). The crusibles were then weighed and dried at 50°C until a constant weight was obtained. The wet and dry weights of samples of A. avenae was thus obtained and the percentage dry weight calculated (Table XIV).

4. Calorific value of one Aphelenchus avenae egg

A large number of eggs are required for the establishment of calorific values. Calculating from my results, a minimum of 735,000 eggs would have been required to obtain a reading on the bomb calorimeter. This estimate does not account for the loss during processing into a pellet for calorimetry. Although 10 - 14 day cultures contain a large number of eggs it was not possible to obtain the number required for pellets. Therefore the calorific value obtained from the mixed sample of A. avenae was used to obtain the calorific value of an egg. From the calculations given in Table II in appendix, the calorific value of one A.avenae egg is 0.3718^{-4} cal./g. $\pm$ se. 0.0734^{-4}.

Using the mean calorific value of A. avenae eggs. calories of eggs produced per day was determined (Fig. IV).

Table XIII

Distance dropped by A.avenae in 5 and 10 min. in solutions of ficoll of different densities.

Density of ficoll soln (g/ml.)	Max. distance dropped in 5 min.	Max. distance dropped in 10 min.	Position of concentration of A. avenae 10 min.
1.04	3.0 mm	11.0 mm	3.0 mm
1.05	2.0 mm	10.0 mm (few)	at interface
1.06	2.0 mm (very few)	10.0 mm (very few)	at interface

Table XIV

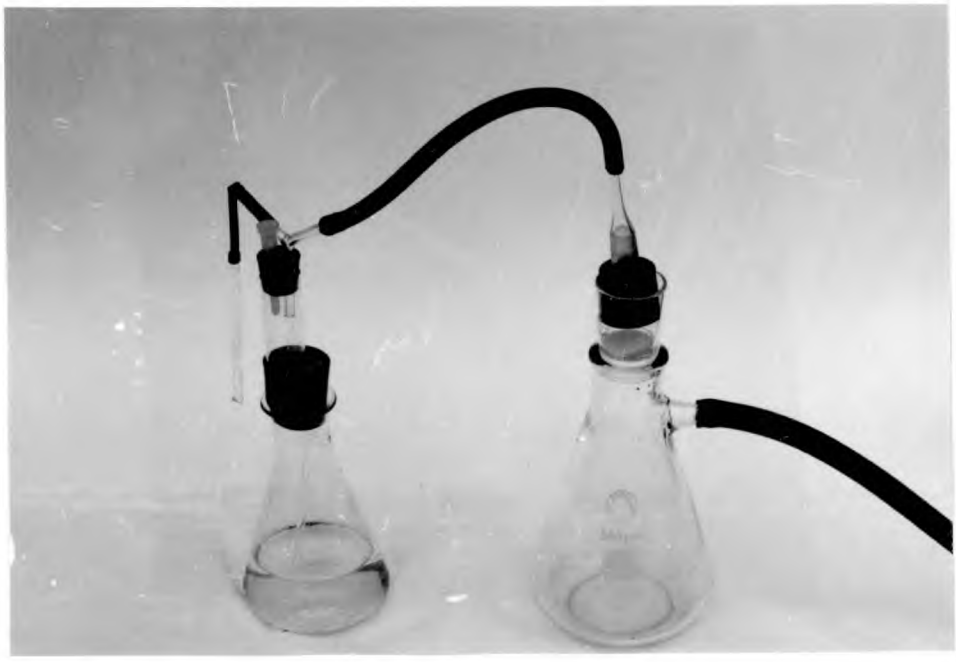
Percentage water content of Aphelenchus avenae

	Replicates			
	1	2	3	4
Weight of crucibles	10.2274 g.	10.8776 g.	11.0131 g.	11.9196 g.
wt. of crucibles + wet A.avenae	10.2406 g.	10.8878 g.	11.0568 g.	11.9717 g.
∴ Wet wt. of A.avenae	0.0132 g.	0.0102 g.	0.0437 g.	0.0521 g.
wt. of crucible + dry A.avenae	10.2302 g.	10.8805 g.	11.0207 g.	11.9259 g.
wt. of crucibles	10.2274 g.	10.8776 g.	11.0131 g.	11.9196 g.
∴ dry wt. of A.avenae	0.0028 g.	0.0029 g.	0.0076 g.	0.0063 g.
∴ % dry wt. over wet wt.	21.21%	28.43%	17.39%	12.09%

Average dry wt. = 19.78%
Water content = 80.22%

Plate 4.

MYER'S METHOD OF ESTIMATING WATER CONTENT OF NEMATODES.



D. Discussion

The rate of egg production at 26°C varies throughout the life of the female. This was also observed by Fisher (1968, 1969). Egg laying starts on the 6th day reaching a maximum on the 9th or 10th day, and tailing off from then as shown in Fig. V. The length of life of A. avenae is approximately 28 days, when cultured at 26°C. Egg laying continues at a very slow rate, almost upto death (Fig. V).

At 15°C A. avenae lives longer (Table XII and Fig. V) but the female matured, and started egg laying much later than at 26°C. Therefore the length of life of the adult female is probably the same at 15°C and at 26°C.

Section III

GROWTH

A. Introduction

The growth of second stage larvae through to adults of Aphelenchus avenae was estimated by calorimetric estimation of dry weight, at various points during development. Direct calorimetric estimations of growth was not possible because of the small size of A. avenae.

To obtain a calorific expressions of growth it was first necessary to obtain the weights of each age group. The method described by Andrassy (1956) was used to obtain weights from linear measurements of length and width. Andrassy derived his formula by first obtaining the volume and the specific gravity of nematodes. The volume was obtained by dividing the body into three geometric sections (fostrum, cylinder and cone) and summing their volumes. The specific gravity was obtained by immersion in salt solutions and diluted sulphuric acid, of various concentrations. From this detailed calculation, Andrassy derived an "emperical key-number" and thereby simplified formulae for the calculation of volumes and weights of nematodes. When compared to the more detailed calculation the simplified method showed at most an error of $\pm$ 5%. The simplified formula, $G = \frac{a^2 \times b}{16 \times 100,000}$ (where a = greatest body width, b = length of the body and G = body weight expressed in micrograms (μg)), was used to obtain wet weights of Aphelenchus avenae

Two methods were attempted in order to obtain the linear measurments of growth of A. avenae:-

1. Record of individual A. avenae growth rate by photography of the same nematode at daily intervals.
2. Camera lucida drawings of batches of known aged A. avenae,

extracted from cultures at daily intervals.

The water content of A. avenae was obtained by Myers' (1967) method as explained in section II (p.50). The calorific value of A. avenae was obtained from samples containing all stages, by bomb calorimetry.

The calories required for growth per day was determined for the total lifetime of A. avenae females.

The growth rate was studied at 26°C. Some records of growth at 15°C and 8°C were kept for comparison.

B. Measurement of Aphelenchus avenae growth at 26°C.

1) Recorded by photography.

From a petri-dish with one weeks growth of Botrytis cinerea on 2 mm. layer of PDA ,1 cm. diameter discs were cut and placed on each of 20 flamed slides. A newly hatched larva was placed on each disc and a 2.5 cm. diameter flamed cover-slip was placed on top.

The nematodes were photographed at 24 hr. intervals at magnifications of X400 and/or X100. In this way the growth of individual nematodes was recorded.

The slide cultures were placed in 14.5 cm. diameter, petri-dishes with a plug of cotton-wool soaked with autoclaved water, so that the cultures remained moist. The cultures were incubated at 26°C.

A photograph of a slide micrometer was taken each day and with each film used. Each film was printed with a constant enlargement such that the photograph of the scale would be at the same magnification as the photographs of the nematodes. A map measurer was used in obtaining the length of the nematode (measured along the middle). The divisions on this were then converted into microns by measuring the photograph of the scale.

The measurements obtained from the photographs are smaller than expected from preliminary observations (Table IV in appendix). The length of newly hatched larvae are about 50 µ smaller than has been recorded by Hechler (1962). Undulations of the nematode body in the three dimensional plain (when lying over hyphae for instance) would not be apparent on a photograph. The photographs were sometimes slightly out of focus at points along the length of the body. This probably caused the error.

The experiment was not completed, and the data were not converted into weights because of this possible confusion.

2) Recorded by Camera lucida drawings.

With a 1.5 cm. diameter cork borer discs of PDA with one week's growth of Botrytis cinerea were cut. The discs were transferred, one into each of a batch of solid watch glasses, which had previously been sterilized in steam at 15 lb./sq. in. (121°C). Silicone high vacuum grease (Edwards Ltd.) which does not melt at the autoclave temperature was used to seal the covers on to the watch glasses.

Eggs of Aphelenchus avenae were collected from 10 to 14 day cultures on B. cinerea. The eggs were incubated in water for 18 hours at 26°C. The larvae hatching out were placed individually onto the discs of B. cinerea in the watch glasses. The larvae were inoculated onto each disc, and they were incubated for the required number of days, at 26°C.

Newly hatched larvae were carefully straightened by warming in water, on a microscope slide. Camera lucida drawings were made such that the length and the greatest width could be determined.

Each day nematodes from 3 discs of B. cinerea were extracted, on 2.5 cm. diameter nylon seives and placed on a counting dish. The nematodes collected were heat relaxed and drawings were made as described

above.

Drawings of micrometer scales were made on each page of drawing paper used, at the same magnification and Camera lucida setting as used for the nematodes.

A daily record of growth was kept in this manner, from newly hatched larvae to 10 day old adults. From then on a less frequent record was kept until the 24th day. The nematodes were transferred every third or fourth day (after maturation) into new cultures so that the original nematodes could be recognised from their progeny, and to assure that there was an abundance of food.

Andrassy's (1956) method was used to convert these linear measurements into weights. As described in his method the rounded tail end of A. avenae was extended to a point before the length was measured. Using a CDC 6600 computor the linear measurements of each nematode were converted into wet weights (Table V in appendix).

The percentage dry weight of 19.78 and the calorific value of 5453.2 calories/gr. dry weight (as established in Section II) was used to convert the individual wet weights into calories for each A. avenae individual. Then the standard error for the calorific value of A. avenae of each age group was calculated (Table V in appendix).

A record of growth at 15°C and 8°C was kept. At 15°C measurements were taken of 4, 8, 12 and 24 day old A. avenae. At 8°C measurements were taken of 4, 8, and 24 old A. avenae (Table VI & VII in appendix).

C. Discussion

The growth of A. avenae at 26°C follows a sigmoid curve which reaches a platue after day 9 Fig. VII. Growth is very rapid from hatching (L_2) to the 5th day when adults may be present. The three moults from L_2 to adult follow in rapid succession.

At 15°C development is much slower, taking 8 days to become fourth stage larvae. At 8°C development is even slower. Adults were not present even on the 24th day (Fig. VII).

The average calorific increase of A. avenae per day per individual with age and the cumulative growth curve is given in Fig. VIII and Fig. IX, respectively. As expected from the cumulative growth curve there is a rapid increase in the calories aquired per day per individual until day 7. The drop in calorific input between day 5 and 6 is probably due to moulting in many individual A. avenae. This last moult which produces the adult takes longer, and is therefore more frequently observed than the other moults. Between day 7 and 8 there is apparently no increase in calorific input, although there is an increase between day 8 and 9. Egg laying started mainly on day 7 and the cessation of growth between day 7 and 8 may be related to the building up of reserves for the peak egg laying period which follows. The calorific value of individuals were deduced from linear measurements and a common calorific value per gram of A. avenae. Therefore an increase in calorific value which does not correspond with an increase in size would not be appreciated by the method used.

Fig. VII.

GROWTH AS INCREASE IN WEIGHT OF A. AVENAE IN TIME

AT DIFFERENT TEMPERATURES

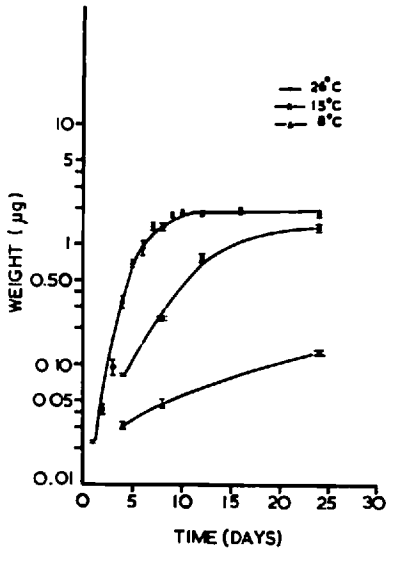


Fig. VIII .

DAILY CALORIFIC INCREASE OF A. AVENAE WITH AGE

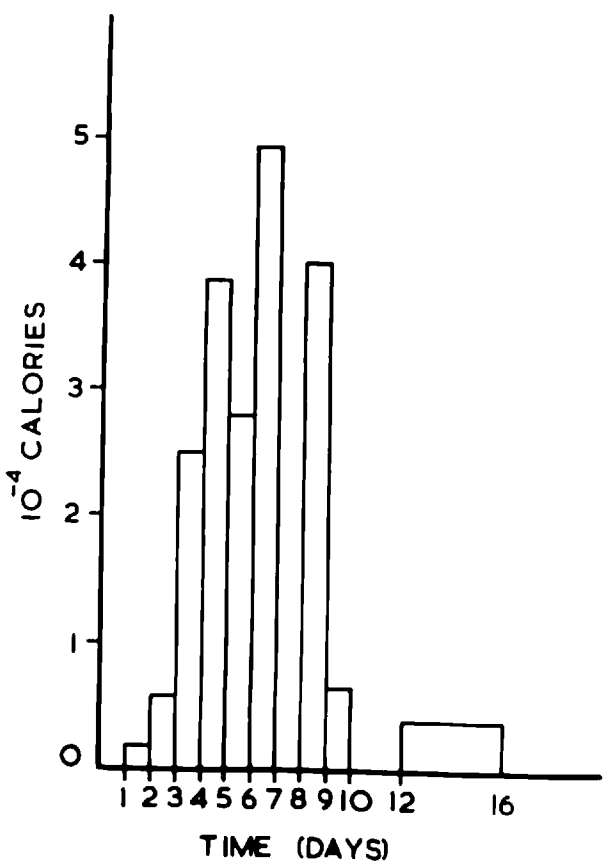
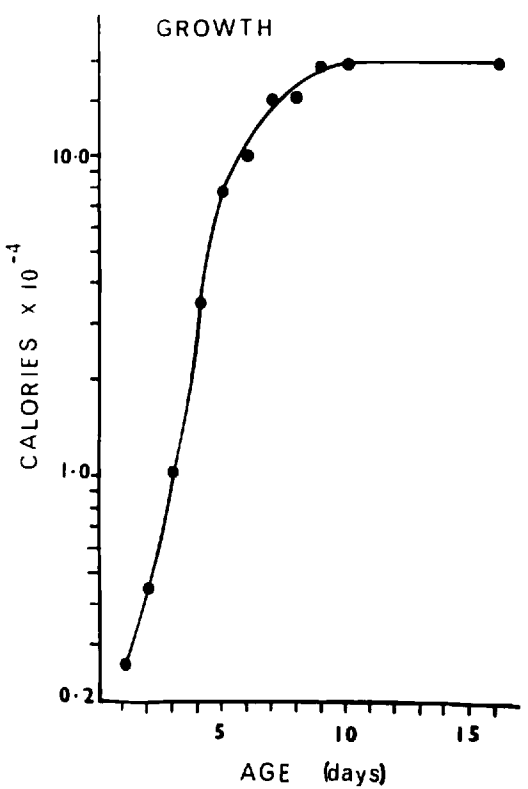


Fig. IX

CUMULATIVE CALORIFIC INCREASE OF A. AVENAE



Section IV

RESPIRATION

A. Introduction and Methods

The amount of energy required for respiration by the various stages of Aphelenchus avenae was investigated.

Because of the small size of A. avenae, especially of the early larval stages, a very sensitive apparatus had to be used to record the oxygen consumption of individuals during larval development, and as adults.

A capillary micro-respirometer described by Tobias & Gerard (1941) and modified by Call (*) was used without success. The changes in volume obtained in the flasks containing nematodes was not sufficiently consistent and often varied no more than the changes in the thermo-barometric flask. A large number of A. avenae (approximately 100) adults had to be used to obtain results. As it is not possible to obtain such large numbers of a known age on any one day, the respiration of various ages could not be established by this method.

A Gilson respirometer and Shandon's oxygen electrodes were also used without success.

A Zeuthen's (1950) cartesian diver was constructed and used in accordance with instructions given by Klekowski (1967). Reliable values of oxygen consumption of all stages of A. avenae from eggs to adults were obtained using this method. The divers were of 1 to 3.0 µl gas volume, with fitted stoppers. Each diver contained nematodes in a small drop of water, which was separated from a 0.1 N NaOH solution by a bubble of air of a known specific volume for a given diver. A hollow glass stopper was inserted in the neck of the diver. The diver was floated in a flotation vessel filled with 0.1 N NaOH. Each flotation

* Unpublished

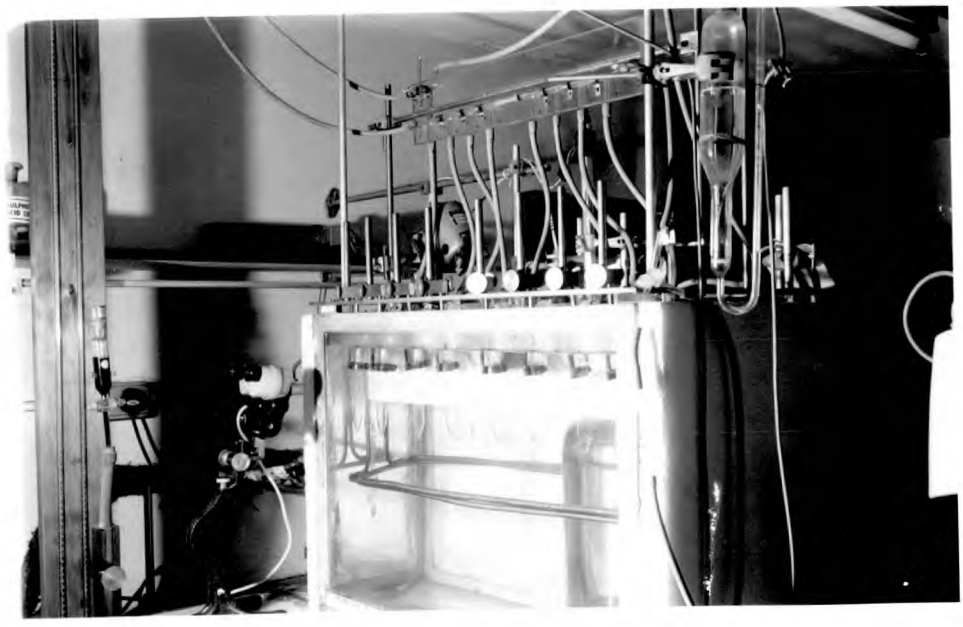
vessel was connected with a manifold which was connected at one end with a manometer filled with Brodie's solution. The pressure was regulated by coarse and fine adjustment-screws and a large syringe (Plate 9).

All experiments were carried out at 26°C. Divers were made to float at a level marked on the flotation vessel and the pressure recorded. The changes in pressure required to float the vessel at the same level at various times was recorded. The pressure changes in time was plotted on graph paper and an estimate of the slope of the line obtained by using a transparent ruler with parallel lines. The average pressure difference per hour was thus obtained from the graph. By using Charles' Law the volume change (i.e. the oxygen consumption) per hour was calculated.

Various ages of A.avenae were obtained by inoculating newly hatched larvae onto cultures of Botrytis cinerea on PDA, and extracting at required time intervals. The cultures were incubated at 26°C and the oxygen consumption recorded at 26°C. Freshly laid eggs upto 18 hours and 18 hours to hatching age (48 hours) were obtained by incubating adult females in water in watch glasses for 18 hours. The eggs laid were collected and either used immediately for respiratory studies or incubated at 26°C for a further 18 hours. When eggs with larvae developed within them were obtained. Respiratory studies were made on these two arbitrarily aged groups of eggs, and on newly hatched larvae to adults at daily intervals. To obtain a reliable rate of oxygen consumption, it was necessary to use a minimum of 20 eggs or 20, 1, 2 or 3 day old larvae per diver. Divers in my experiments usually held in excess of this number. Between 9 and 20 individuals were placed in each diver, for studies on older A. avenae.

Plate 5.

CARTESIAN DIVER APPARATUS



B. Results and Discussion

Table XV shows the respiratory rate for the various ages of A. avenae expressed as µl of O_2 per individual per hour, and as calories used by individuals per day. As explained by Brody (1964), Southwood (1966) and others the range of calorific equivalents of O_2 when varing substrates are used in respiration, is 4.7 to 5.0 kilo-calories per litre which is within the limits of experimental error. Therefore the Respiratory Quotieht was not determined but a value of 4.825 kcal per litre was used in converting oxygen consumption to calories lost in respiration.

The calories lost in respiration by the various ages of A. avenae is plotted in Fig. X. Oxygen consumption per individual increases with age. The eggs and newly hatched larvae (not fed) have a low oxygen consumption, with perhaps a slight increase in rate in eggs prior to hatching. Some 4 and 5 day old A. avenae are in the last moult. The variation between samples on these days may be due to the sample containing varing proportions of nematodes just before, after or during a moult. The batch of nematodes plotted in Fig. X as between day 4 and 5 were all in a moult and hand-picked from a culture containing all ages. From Fig. X it appears that oxygen consumption is related to the size of A. avenae as it follows the growth curve (Fig. VII). An allometric plot (Zeuthen 1953; von Brand, 1960) of oxygen consumption against weight is drawn in Fig. XI. The line of best fit include the values for eggs but exclude the extreme points on day 4. The slope of the line, b, in the logarithmic plot is the power to which the body weight must be raised to describe the relationship between body weight and metabolic rate. In my analysis b = 1.0. The increase in metabolism therefore parallels weight. The surface area is not likely to be a limiting

Table XV

Respiration of Aphelenchus avenae at 26°C

Age	Mean wet wt. *1 µg	Sample no.	µl O_2/individual/hr.	Cals. respired/day *2
Eggs (0-18hrs)	0.034	1	0.8992	0.104127^{-4}
		2	0.3795	0.034792^{-4}
		3	0.3025	0.035030^{-4}
		4	0.5585	0.064674^{-4}
Eggs (18 hrs-hatching)	0.034	1	1.7222 (51 eggs 10 hatched)	0.199431^{-4}
		2	0.7789 (51 eggs 13 hatched)	0.088807^{-4}
Day 1 (L_2)	0.024	1	0.8308	0.096207^{-4}
		2	0.9907	0.114723^{-4}
		3	0.4874	0.056441^{-4}
Day 2 (L_3)	0.042	1	1.3380	0.154940^{-4}
		2	1.4259	0.165119^{-4}
		3	1.7088	0.197879^{-4}
Day 3	0.095	1	2.5691	0.297502^{-4}
		2	2.8954	0.335287^{-4}
		3	2.7587	0.322768^{-4}
Day 4	0.33	1	3.8150	0.441777^{-4}
		2	3.1042	0.359466^{-4}
		3	6.6211	0.766723^{-4}
		4	8.1994	0.949491^{-4}
Day 4 - 5		1	10.5198	1.218193^{-4}
Day 5	0.68	1	14.1972	1.644036^{-4}
		2	9.6422 (many in moult)	1.116567^{-4}
		3	14.9352	1.729496^{-4}
		4	14.5715	1.687380^{-4}
Day 6	0.93	1	19.1426	2.216713^{-4}
		2	29.9261	3.465442^{-4}
		3	18.2486	2.113188^{-4}
		4	25.6667	2.972204^{-4}

Table XV contd./-

Age	Mean wet wt. *1 µg	Sample no.	µl O_2/individual/hr	Cals. respired/day *2
Day 6 starved to day 7		1	15.8621 (25 eggs)	1.836831^{-4}
Day 7	1.39	1	34.5504 (44 eggs)	4.000936^{-4}
Unaged females		1	33.4524 (56 eggs)	3.873788^{-4}

*1 as established in Sections II and III

*2 4.825 Kcal / litre (Brody, 1964)

Fig. X.

CALORIES RESPIRED BY AN INDIVIDUAL PER DAY BY VARIOUS AGES OF A. AVENAE AT 26°C.

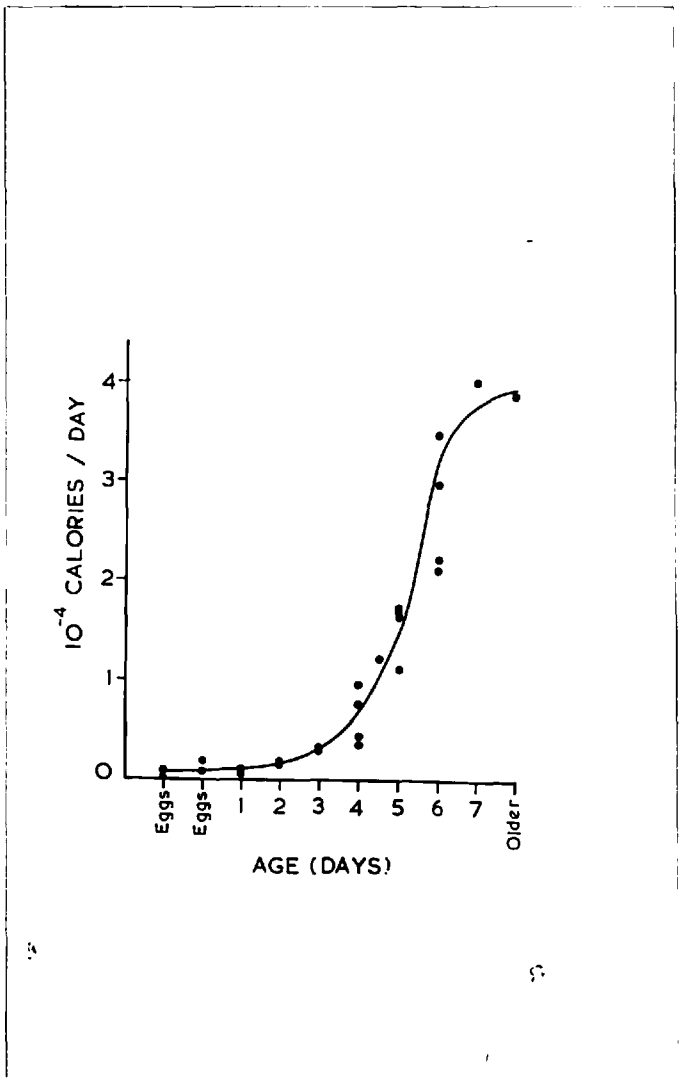
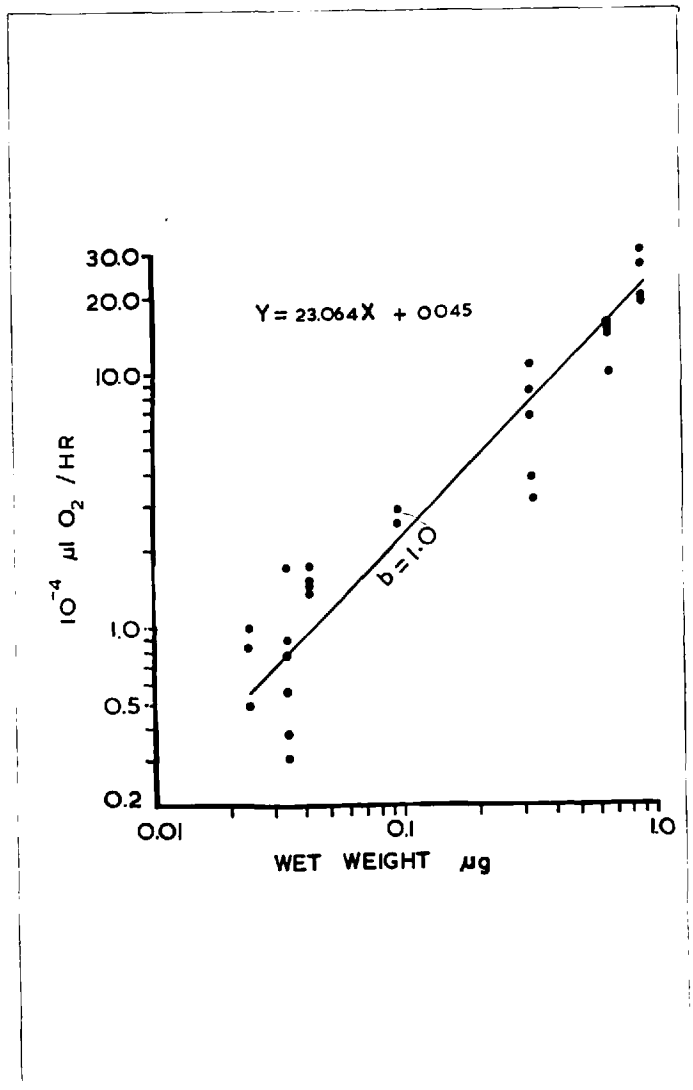


Fig. XI.

ALLOMETRIC PLOT OF OXYGEN CONSUMPTION / SIZE RELATIONSHIP OF VARIOUS AGES OF A. AVENAE.



factor in the respiration of these small animals.

Von Brand (1960a) made an interspecific analysis of Nielsen's (1949) data according to the allometric procedure and obtained a curve with a b - value of 0.87, for nematodes varing in weight from 0.0002 to 0.40 mg. In my intraspecific analysis of larvae to adults of A. avenae, varing in size from 0.00002 to 0.0013 mg. a slightly steeper slope is obtained.

Zeuthen (1953) made interspecific comparisons of various species (from eggs to adults) and found that three phase curves result, with the b - value being close to 1.0 during a middle phase of the life-cycle and lower during an initial and end phase. The life cycle of A. avenae at 26°C occupies only 5 to 6 days and therefore changes associated with development may not be appreciated when readings are taken on groups which on any day contain nematodes which may be slightly more or less developed than others.

The respiratory rates of adults over 6 days old is not accurately recorded (Table XVI) since the females lay many eggs within the divers, thus confusing the results, by the presence of eggs in the divers.

Section V

DISCUSSION, INDIVIDUAL ENERGY BUDGET

An energy budget may be constructed from the estimates of igestion, growth, egg-production and respiration. Fig. XII shows the calories ingested and used in growth, reproduction and respiration on any particular day in the life of A. avenae at 26°C. Feeding commenced as soon as the larva hatched and reached a peak feeding level on about the 5th day. Growth was also very rapid during this period with the larva maturing into an adult on about the 5th day. Egg production started on the 6th day, and there was a tendency for the rate of feeding to be greatly reduced during this period. There was a slight loss in the average bio-mass of the adults between day 7 and 8, corresponding with the start of egg laying by the majority of adults. The calorific intake on day 7 was 2.0064^{-4} (Table XVI) with a decrease in biomass of 0.2858^{-4} calories between day 7 and 8.

Growth ceased on the 10th day and egg-production reached a peak level during this period. Ingestion varied from day to day during adult life. Egg production was reduced to 0.1115^{-4} calories by the 19th day, but ingestio continued at approximately the same rate as during the peak egg laying period. Since net production (growth + egg production) was low after day 19, the energy ingested was either mostly not assimilated or the cost of maintenance (respiration) must have risen. The adult therefore, became more inefficient either in assimilation or in respiration as it aged.

From Fig. XII it appears that gross production (= respiration + net production = assimilation) may be in excess of ingestion on some days. Growth was initially measured as an increase in volume, and by determining an average calorific value (of all ages), it was converted to calories. Therefore an increase in calorific value of the standing crop without an increase in volume may cause an error. The energy ingested on one day

Fig. XII.

MEAN INDIVIDUAL ENERGY BUDGET OF A. AVENAE PER DAY AT 26°C.

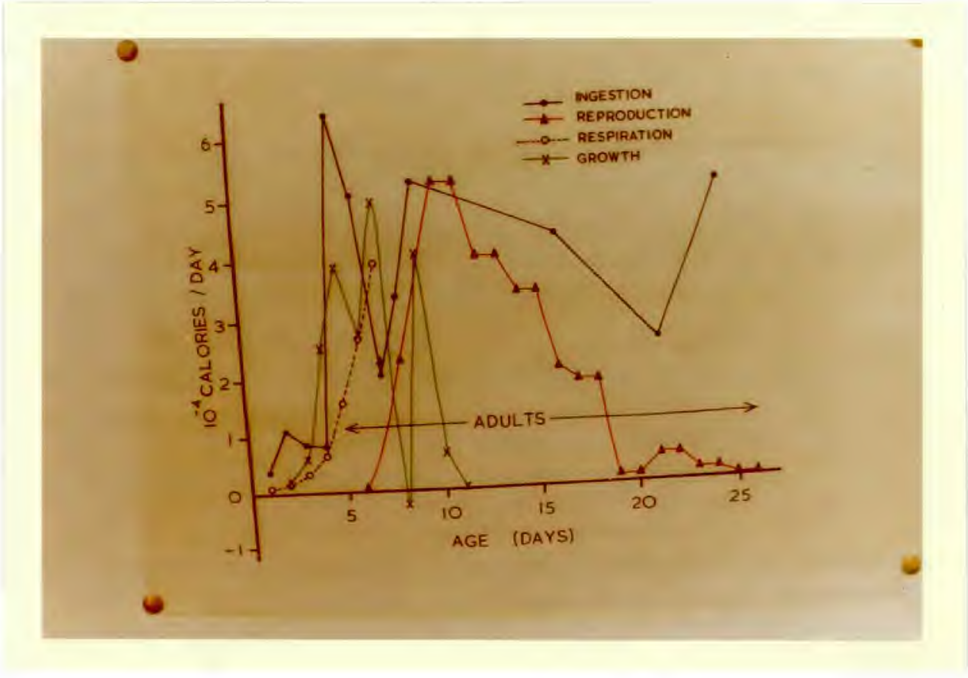


Fig. XIII.

NET PRODUCTION EFFICIENCY OF A. AVENAE AT 26°C.

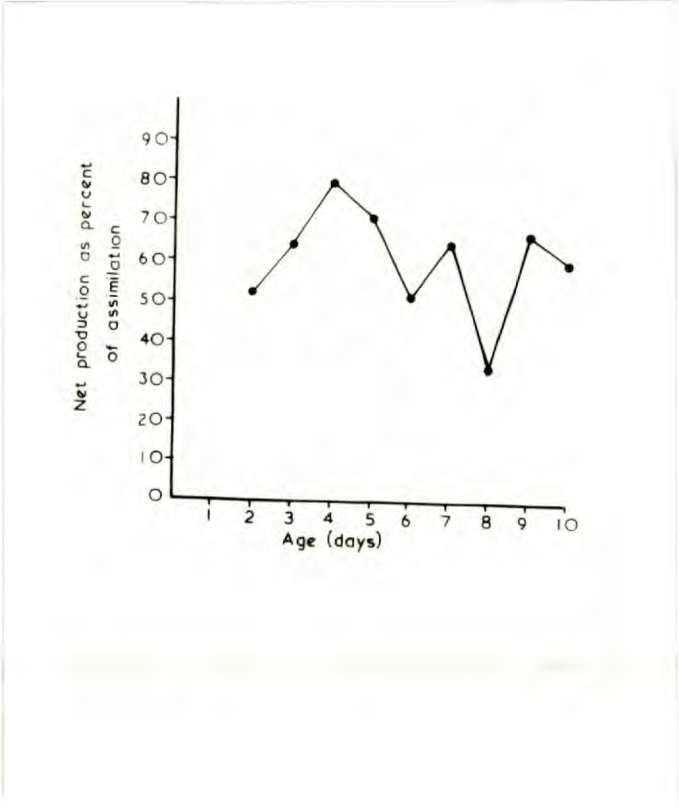


Table XVI

Mean individual energy budget per day of A. avenae

Age in days	Ingestion in 10^{-4} calories	Growth in 10^{-4} calories	Egg-production in 10^{-4} calories	Respiration in 10^{-4} calories
1	0.3966	-	0.0000	0.0891
2	1.1117	0.1903	0.0000	0.1726
3	0.8408	0.5693	0.0000	0.3164
4	0.8245	2.5094	0.0000	0.6294
5	6.3484	3.8519	0.0000	1.5444
6	5.0268	2.7921	0.0558	2.6919
7	2.0064	4.9267	2.2791	3.9374*
8	3.3413	-0.2858	2.2791	3.9374
9	5.2014	4.0484	3.8667	3.9374
10	-	0.6355	5.2052	3.9374
11	-	0.0000	5.2052	-
12	-	0.0000	3.9411	-
13	-	0.0000	3.9411	-
14	-	0.0000	3.3834	-
15	-	0.0000	3.3834	-
16	4.2915	0.0000	2.0449	-
17	-	0.0000	1.8218	-
18	-	0.0000	1.8218	-
19	-	0.0000	0.1115	-
20	-	0.0000	0.1115	-
21	2.4829	0.0000	0.6321	-
22	-	0.0000	0.6321	-
23	-	0.0000	0.1859	-
24	5.0816	0.0000	0.0558	-
25	-	0.0000	0.0558	-

* Average respiration of 7 day and older adults.

and used on suceeding days for processes such as egg production may not by appreciated in an individual daily budget as presented in Fig. XII. Therefore a comulative energy budget was constructed (Table XVII) by summing the ingestion or production of one day to that of the proceeding days, as outlined by Klekowski & Zyromska-Rudzka (1967). From this analysis, ingestion, growth, egg-production and cost of maintenance upto a given day may be obtained. There was no significant change in standing crop after day 10 (Fig. VII, Section III) and so the cumulative production of body tissue remained constant at 19.2378^{-4} cal. An average value of respiration was obtained from Table XV. From day 7 to day 10 the value was obtained from respiratory studies on day 7 and older, unaged but egg-laying females. No correction has been made for the respiration of eggs produced within divers, since neither time of laying nor respiration of eggs *in utero* is known.

Ingestion was not measured continually after day 9. When measured after day 9, daily ingestion varied between 2.4829^{-4}cal. and 5.0816^{-4} cal. (on day 21 and 24 respectively) and therefore it was not possible to obtain a reliable average figure to cumulate per day. Therefore cumulative values for ingestion are only available upto day 9. It is apparent that cumulative assimilation (= gross production) exceeded cumulative ingestion from day 4 onwards. This error is probably caused by a low estimate of ingestion. The duration of feeding (Section I) was estimated by observation of *A. avenae* in culture on slides with a cover-slip placed on the preparation. If the weight of the cover-slip was on the nematode feeding may have been impeded. If the cover-slip was replaced by a cover of cellophane better results may have been obtained.

The gross production efficiency, $k = \frac{P}{I}$; (Ivlev 1939 a, b) cannot be assessed without accurate values of ingestion. Net production efficiency

Table XVII

Cumulative energy budget

Age (days)	Ingestion (I) 10^{-4} calories	Growth 10^{-4} calories	Egg Prodcn. 10^{-4} calories	Net Prodcn.*1 10^{-4} calories	Respiration 10^{-4} calories	Assimilation *1 10^{-4} calories
1	0.3966		0.0000	0.0000	0.0891	0.0891
2	1.5083	0.1903	0.0000	0.1903	0.2617	0.4520
3	2.3491	0.7596	0.0000	0.7596	0.5781	1.3377
4	3.1736	3.2690	0.0000	3.2690.	1.2075	4.4765
5	9.5330	7;1209	0.0000	7.1209	2.7519	9.8728
6	14.5488	9.9130	0.0558	9.9688	5.4438	15.4126
7	16.5552	14.8397	2.3349	17.1746	9.3812	26.5558
8	19.8965	14.5539	4.6140	19.1679	13.3186	32.4865
9	25.0979	18.6023	8.4807	27.0830	17.2560	44.3390
10		19.2378	13.6859	32.9237	21.8748	54.7985
11		19.2378*	18.8911			

* No significant growth after the 10th day.

*1 excluding exuviae.

Contd./-

Age (days)	Ingestion (I) 10^{-4} calories	Growth 10^{-4} calories	Egg Prodcn. 10^{-4} calories	Net Prodcn.*1 10^{-4} calories	Respiration 10^{-4} calories	Assimilation *1 10^{-4} calories
12		19.2378	22.8322			
13		19.2378	26.7733			
		19.2378	30.1567			
15		19.2378	33.5401			
16		19.2378	35.5850			
17		19.2378	37.4068			
18		19.2378	39.2286			
19		19.2378	39.3401			
20		19.2378	39.4516			
21		19.2378	40.0837			
22		19.2378	40.7158			
23		19.2378	40.9017			
24		19.2378	40.9575			
25	105.00	19.2378*	41.0133	60.2511		

$k_2 = \frac{P}{A}$, (Ivlev 1939 a, b) is calculated in Table XVIII from values given in Table XVI. The exuviae fraction was not determined and therefore there is a small error in the net production quoted and assimilation quoted of the moulting stages. Fig. XIII describe the efficiency of A. avenae as a converter of energy. It increased in the larval stages from 52% in the youngest larva to 80% in the fourth-stage larva. A minimum net production efficiency of 34% is obtained on day 8, caused by the loss in biomass between day 7 and 8.

From the energy budgets constructed the net loss to the host for one day by supporting a given aged nematode or the cumulative net loss of supporting that nematode upto any age of its life may be obtained. Since my estimate of ingestion for A. avenae is low, the net loss assessed by assimilation of A. avenae is a minimum. For example a 9 day old adult assimilated 11.8525^{-4}cal. (Table XVIII) on day 9 and 44.3390^{-4}cal. (Table XVII) through out life. This is a minimum estimate of the net cost to the fungus of supporting one 9 day A. avenae. Gross loss to the fungus is probably even more than what is ingested.

Table XVIII

Net production efficiency of A. avenae/day.

Age	Net production (P) (G + egg production) 10^{-4} calories	Assimilation (P + R) 10^{-4} calories	% net production efficiency $(\frac{P}{A} \times 100)$
1	-	0.0891	-
2	0.1903	0.3629	52
3	0.5693	0.8857	64
4	2.5094	3.1388	80
5	3.8519	5.3963	71
6	2.8479	5.5398	51
7	7.2058	11.1432	65
8	1.9933	5.9307	34
9	7.9151	11.8525	67
10	5.8407	9.7781	60

PART II

POPULATION ENERGETICS

Section 1

POPULATION DYNAMICS AND EFFECTS OF APHELENCHUS AVENAE ON BOTRYTIS CINEREA

A. Introduction

Studies were made on the population energetics of Aphelenchus avenae in culture, and the effects of populations on fungal productivity.

The population growth of A. avenae on Botrytis cinerea was studied on the following three culture media at 26°C:-

1. Potato dextrose agar.
2. Sand and Czapek dox liquid.
3. Potato dextrose broth.

The effects of A. avenae on fungal productivity was assessed when the fungus could be separated from the medium.

B. Studies on potato dextrose agar

Population fluctuation of A. avenae on B. cinerea was studied on PDA. A standard inoculum of B. cinerea was inoculated into each of 12 sterile, 9 cm. petri-dishes with 20 ml. of PDA. A standard inoculum of B. cinerea was obtained by growing the fungus for 2 weeks in 100 ml. of Czapek dox liquid in 250 ml. flasks at 26°C. Standard amounts of fungus were cut by using a 1 cm. diameter cork borer. One disc of fungus was used per plate. The cultures were incubated in a constant temperature room at 26°C for 1 week.

After one week's growth at 26°C B. cinerea covered the surface of the agar in the plate. A fourth stage larva of A. avenae was inoculated onto the plates one week after the initial fungal inoculum. Each week, thereafter 3 plates were extracted for three days on 8 cm. diameter nylon seives of 195 μ pore size. The whole of the nematode extract was counted at the end of week one and two. In the third and fourth weeks, the

numbers obtained were too large to count the whole extract. Therefore the extract was diluted and made upto 250 ml., and 5 ml. replicates of an even suspension (obtained by blowing air into the suspension for 30 seconds) was counted. Three counts were made from each replicate plate on weeks three and four. The eggs, the three larval stages and the adults were counted separately. The linear measurements given for growth by Heckler (1964) for the different stages of A. avenae was used in distinguishing the different age groups. A calibrated microscope with a graduated eye piece was used in making the counts.

Table VIII in the appendix presents the counts of A. avenae for the four weeks. On weeks three and four the 3 replicate counts of the 3 replicate plates are given. Fig. XIV gives the total population increase over the four weeks in culture. The fungus is destroyed after the third week after nematode inoculation. The number of eggs counted in week four was much lower than on week three, showing that egg production by the adult female is decreasing.

C. Studies on sand and Czapek dox liquid

The population growth of A. avenae on B. cinerea and the gross effect on B. cinerea was studied in sand and Czapek dox liquid cultures.

Sand seived through a 2 mm. sq. seive was washed, dried and 100 mg. weighed into each of 52, 250 ml. conical flasks. The flasks were then sterilized at 15 lb./sq. in. (121°C) for 1 hour. The flasks were allowed to cool and 20 ml. of standard Czapek dox liquid, sterilized for 20 min. at 10 lb./sq. in. was measured into each flask. A standard inoculum of B. cinerea obtained as described above (p. 79) was added to each flask. The cultures were then incubated at 26°C.

After one week the fungus from 4 flasks was killed by adding 20 ml. of 0.5% Hibitane diacetate. After 20 min. the fungus was washed off the

Fig. XIV.

POPULATION CHANGES IN THE DIFFERENT STAGES OF A. AVENAE ON B. CINEREA CULTURES ON PDA.

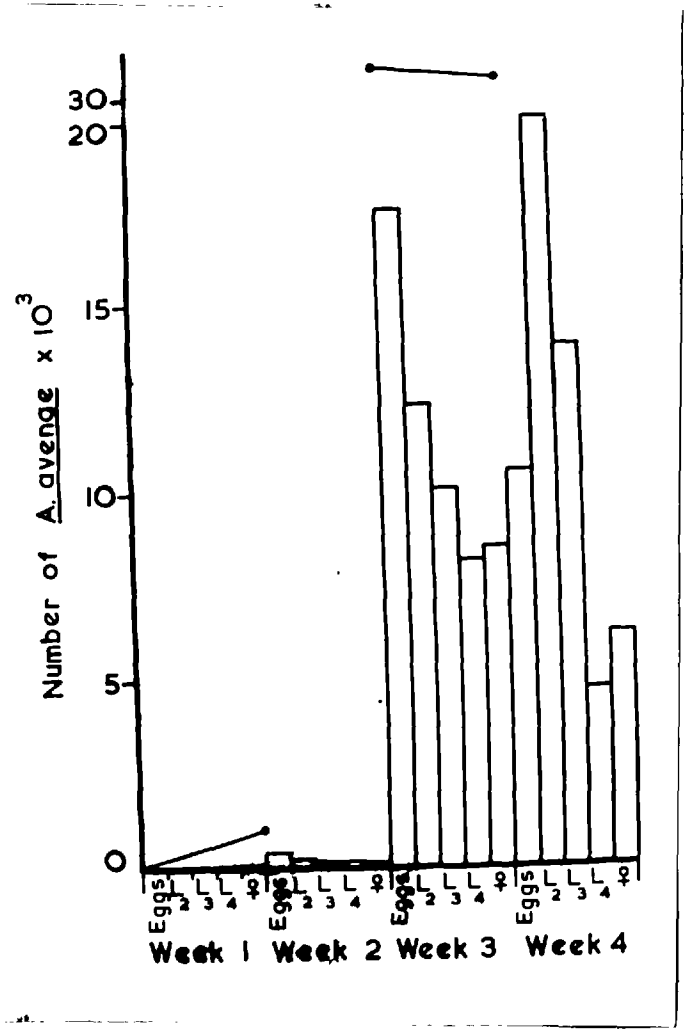
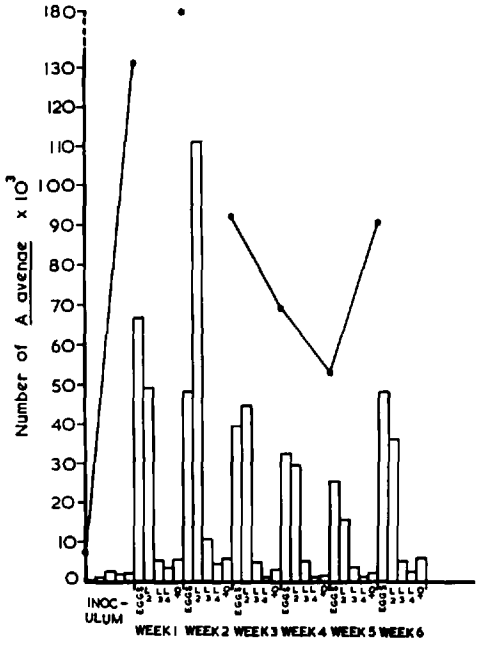


Fig. XV

POPULATION CHANGES IN THE DIFFERENT STAGES OF A. AVENAE ON CULTURES OF B. CINEREA ON SAND AND CEZPEK DOX MEDIUM.



sand by agitation with water. The fungus floated to the top and was decanted off. Approximately 400 ml. of water with the fungus was collected from each flask. The fungus was concentrated from this by centrifugation. The fungus with a small quantity of sand was dried in an oven at 55°C (in crusibles coated with 'Repelacoat'), and stored over calcuim chloride for assessment of calorific value.

To half of the remaining 48 flasks, 1 ml. of a suspension of surface sterilized A. avenae in sterilized water, was added. The total number and the number of different stages in the inoculum was assessed by counting six replicates of an even suspension of 0.15 ml. on a Mcmaster slide (Table IX in the appendix).

Each week thereafter, eight flasks four with nematodes and four with fungus only, were layed out on Whitehead trays (1965) with a twofold layer of muslin which facilitated the removal of the fungus after extraction of nematodes. After 3 days extraction, the nematode extract was poured into a 1000 ml. measuring cylinder, made upto 600 ml. and 6 samples of 0.15 ml. counted on a Mcmaster slide (Table IX in the appendix).

As described before the flasks were always treated with Hibitane diacetate before extraction. After the extraction of A. avenae, the fungus was washed off the sand and dried. Eight samples of B. cinerea, four of which had been infested with A. avenae, were processed each week.

It was not possible to remove all the sand, adhering to the fungus without loss of fungus. The dry material containing sand could not be pelleted and the calorific value was not obtainable by bomb calorimetry. Instead the weight of B.cinerea in each sample was assessed by treatment with chromic acid solution (Ivlev 1934). The sample of sand plus B. cinerea was weighed in a procelain crucible, treated with repeated

changes of chromic acid (to remove the fungus), washed, dried and the sand reweighed (Table XIX).

Fig. XV gives the population fluctuation of A. avenae on B. cinerea. It was observed that the fungus had declined after the second week of nematode inoculum. Table XIX gives the weight of the fungus with and without nematode infestations for 6 weeks after inoculation with A. avenae. The weight of the fungus decreases in time but there is no difference in the weight of infested and uninfested B. cinerea. Although the fungus is apparently destroyed in the infested cultures, the hyphal cell walls still remain.

Table XIX

Weight of B. cinerea infested and not infested with A. avenae over 6 weeks on sand and Czapek dox liquid at 26°C (A. avenae inoculated at the end of 1 week)

Time after inoculation	Weight of B. cinerea (not infested) in grams replicates				Weight of B. cinerea (after infestation) in grams replicates			
	1	2	3	4	1	2	3	4
Week 1	0.1223	0.1106	0.1735	0.2474				
	Mean = 0.1780				Nematode inoculum added			
Week 2	0.2220	0.1344	0.0932	-	0.1103	0.2356	0.1104	0.2934
	Mean = 0.1499				Mean = 0.1874			
Week 3	0.1355	0.0762	0.2307	0.0831	0.1592	0.1192	0.1507	-
	Mean = 0.1314				Mean = 0.1430			
Week 4	0.0848	0.0936	0.0884	0.1779	0.0877	0.1196	0.0879	-
	Mean = 0.1112				Mean = 0.0984			
Week 5	0.1139	0.0730	0.0790	0.1049	0.1461	0.1204	0.0983	0.0674
	Mean = 0.0927				Mean = 0.1080			
Week 6	0.0835	0.1085	0.0825	0.0550	0.0956	0.0596	0.0641	0.1036
	Mean = 0.0824				Mean = 0.0807			
Week 7	0.0558	0.0560	0.0943	-	0.1015	0.0666	0.0802	0.1008
	Mean = 0.0687				Mean = 0.0873			

D. Studies on potato dextrose broth.

The population growth of A. avenae on B. cinerea and the effect of A. avenae population, on spore production, growth and calorific value of B. cinerea was studied on potato dextrose broth.

Ten ml. of standard potato dextrose broth was measured into each of the 50 sterile petri-dishes. A standard inoculum was added to all/plates. The inoculum was prepared by growing B. cinerea for 2 weeks in PDB. A cork borer of 1.0 cm. diameter was used to cut uniform discs, and 2 discs were used per plate. The cultures were incubated at 26°C. Care was taken to incubate the culture plates level on a flat surface, so that the liquid medium was evenly distributed on the plate. After 1 week, plates with uneven distribution of fungus were discarded. Onto each of half the number of remaining plates I fourth-stage larva was inoculated.

Six plates, 3 with A. avenae and 3 with only B. cinerea were extracted 2 weeks after nematode inoculation and at weekly intervals thereafter for 2 weeks (Table X in the appendix). Nematodes were extracted on a twofold layer of muslin placed on a 8 cm. nylon seive of 195 μ pore size.

Before extraction, 1 cm. discs of B. cinerea were cut from each culture and introduced into a test-tube containing a diluted soap solution. The tube was shaken vigorously and the number of spores in each tube estimated (Table XX).

After extraction of the nematodes the B. cinerea was collected from the seives, placed on weighed plastic-plates, dried at 55°C and weighed. A measurement of growth of B. cinerea, infested and non-infested with A. avenae was thus obtained (Table XXI).

B. cinerea from the 3 A. avenae infested plates were pooled, as were also the 3 non-infested B. cinerea on weeks 3 and 4. The calorific value of each group established the change (if any) of the calorific value of

Table XX

Spore counts of B. cinerea, infested and not infested with A. avenae on PDB cultures at 26°C.
(0.15 ml. of 0.10 ml. suspension of spores in soapy water counted).

Week 2 Count no.	B. cinerea (not infested) Plate Replicate			B. cinerea (after infestation) Plate Replicate		
	1	2	3	1	2	3
1	341	332	171	94	730	761
2	254	329	153	108	776	902
3	299	321	170	186	583	800
Total	894	982	494	388	2089	2463
	Mean/0.45 ml. = 790			Mean/0.45 ml. = 1647		
Week 3 Count no.	Plate Replicate			Plate Replicate		
	1	2	3	1	2	3
1	360	92	425	502	76	182
2	403	127	540	409	67	220
3	385	85	586	286	30	193
Total	1148	304	1551	1197	173	595
	Mean/0.45 ml. = 1001			Mean/0.45 ml. = 655		
Week 4 Count no.	Plate Replicate			Plate Replicate		
	1	2	3	1	2	3
1	560	423	252	18	370	378
2	519	382	305	36	383	293
3	574	385	203	12	370	354
Total	1653	1190	760	66	1123	1025
	Mean/0.45 ml. = 1201			Mean/0.45 ml. = 738		

Table XXI

Weight of B. cinerea infested and not infested with A. avenae over 4 weeks on PDB at 26°C (A. avenae inoculated at the end of an initial 2 weeks growth of B. cinerea). First reading taken 2 weeks after A. avenae 1 L_4) inoculation.

Time after inoculation	Weight of B. cinerea (not infested) in grams plate replicates			Weight of B. cinerea (after infestation) in grams plate replicates		
	1	2	3	1	2	3
Week 2	0.0607	0.0666	0.0670	0.0660	0.0761	0.0614
	Mean = 0.0647			Mean = 0.0678		
Week 3	0.0570	0.0740	0.0710	0.0481	0.0724	0.0415
	Mean = 0.0673			Mean = 0.0540		
Week 4	0.0970	0.0687	0.0523	0.0541	0.0560	0.0673
	Mean = 0.0726			Mean = 0.0591		

Table XXII

Calorific value of B. cinerea infested and not infested with A. avenae for 3 and 4 weeks on PDB at 26°C.

Time after inoculation	kcal/g. of B. cinerea (not infested) in grams sample replicate			kcal/g. of B. cinerea (after infestation) sample replicate		
	1	2	3	1	2	3
Week 3	4.39	4.09	4.32	3.97	4.18	4.39
	Mean = 4.27			Mean = 4.15		
Week 4	.40	4.13	3.94	3.92	4.61	4.36
	Mean = 4.16			Mean = 4.30		

infested and non-infested B. cinerea with increasing A. avenae infestation, and age of fungus (Table XXll).

Fig. XVI shows the gradual increase in population of A. avenae in PDB. There is a tremendous increase in the number of second stage larvae in the fourth week indicating that the population is still increasing. It was observed that the fungus was still healthy in patches, and probably capable of supporting larger populations.

There is great variation in the spore counts of equally treated cultures (Table XX). There is a slight decrease in the number of spores produced in B. cinerea cultures infested with A. avenae for 3 and 4 weeks. Table XXI shows that there is no difference in the weight of B. cinerea with time or with infestation. Table XXII shows that the calorific value of B. cinerea does not vary with infestation with A. avenae. The calorific value of B. cinerea cell wall (Section I) was found to be 4.48 Kcal/g. (mean of 3 samples). Therefore the destruction of B. cinerea by A. avenae which feed on the cell contents (Section I) is not likely to be shown in the calorific value estimations.

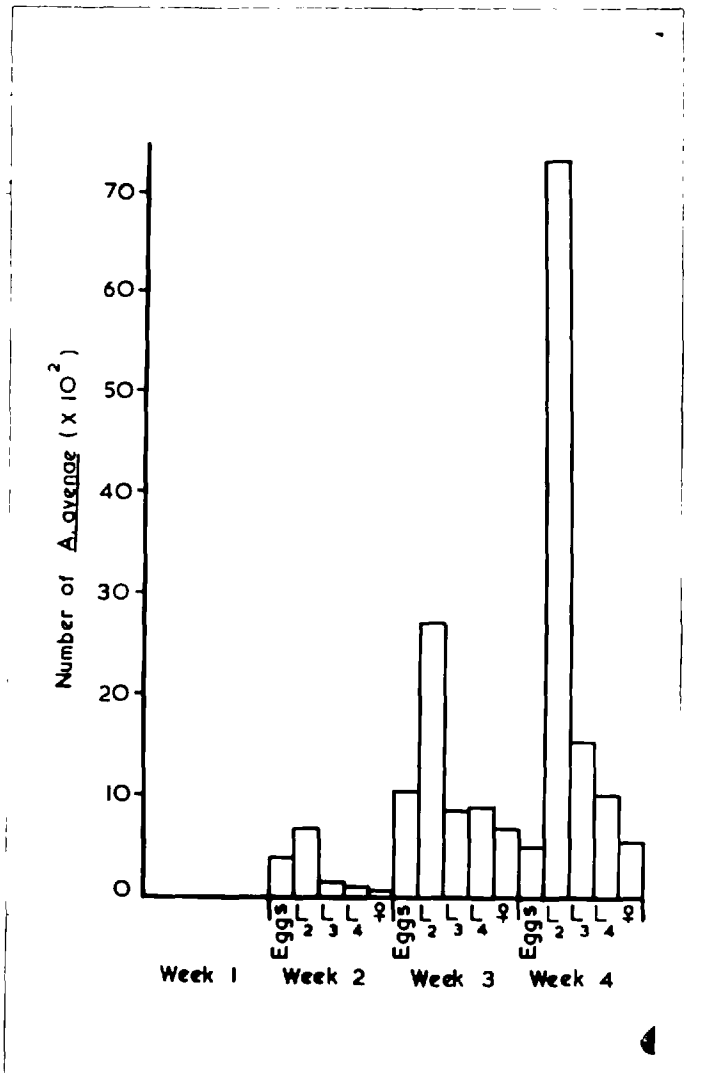
E. Discussion.

The initial inoculum of A. avenae was greatest in the sand and Czapek dox liquid cultures, and the maximum numbers of A. avenae were obtained in 2 weeks. The population increase on PDB was much slower than on PDA when the cultures were given the same initial A. avenae inoculum (Fig. XIV and XVI).

The weights of B. cinerea were not altered by infestation with A. avenae. There was no difference in the calorific value of infested and non-fested B. cinerea. Therefore the gross energy loss to the fungus could not be established.

Fig. XVI.

POPULATION CHANGES IN THE DIFFERENT STAGES OF A. AVENAE ON B. CINEREA ON PDB.



Section II

MORTALITY

A. Introduction

The mortality of eggs and different stages of A. avenae on B. cinerea was observed in cultures containing individual nematodes, when there was excess food and no effects of crowding. The mortality factor obtained under these 'ideal' conditions were designed to exclude the population effects. The conditions of culture excluded contamination and also the possibility of predation of the nematodes. The causes of death are inherent disease, and factors such as the blockage of the stylet with ingested food (Doncaster, personal communication), inability of females to lay eggs (the eggs then hatch within the female which eventually die), or experimental procedure such as handling of nematodes.

The mortality of eggs of A. avenae was established by direct observation. The mortality of newly hatched larvae to 7 day old females was tested by an indirect method.

B. Mortality of A. avenae eggs.

Females were incubated in water for 4 hours and the eggs deposited were collected. The mortality of eggs was tested at 26°C in tap water and cultures of B. cinerea.

1. Mortality of eggs in tap water.

Ten newly laid eggs were introduced into sterilized watch glasses containing autoclaved tap water. The number of eggs hatched was observed after 4 days incubation at 26°C (Table XXIII).

2. Mortality of eggs in PDA cultures.

Into each of 4 sterile watch glasses 1.5 cm. disc of PDA with B. cinerea were introduced and 10 newly laid eggs were inoculated. After 4 days the discs were washed onto 2.5 cm. diameter seives of 195 µ pore

Table XXIII

Mortality of A. avenae eggs in water. Counts taken 4 days after inoculating eggs. (Eggs which did not hatch within 4 days taken as dead)

Replicate	Eggs	Larvae	% Mortality
1	0	10	10
2	1	9	10
3	1	9	10
4	1	9	10
Average mortality = 7.5%			

Table XXIV

Mortality of A. avenae eggs on B. cinerea on PDA. Counts taken 4 days after inoculating eggs.

Replicate	Eggs	Larvae	% Mortality
1	1	7	12.5
2	0	10	0
3	1	7	12.5
4	0	10	0
Average mortality = 6.3%			

size and the larvae and eggs extracted and counted (Table XXIV).

C. Mortality of A. avenae newly hatched larvae to adults.

A single nematode of a known age was introduced onto 20 watch glasses each with 1.5 cm. disc of PDA with one weeks growth of B. cinerea. Twenty-four such cultures were prepared for each age of A. avenae from day 1 to day 7. A. avenae of known age was obtained for the experiment by inoculating newly hatched A. avenae onto B. cinerea cultures, and extracting at required ages.

The cultures covered and sealed with silicone grease were incubated at 26°C for 2 - 3 weeks and the number of cultures which were colonised by A. avenae were counted. If the culture contained A. avenae, the originally inoculated nematode must have developed to an adult and laid eggs. Therefore mortality was expressed as the number of nematodes which developed and produced viable eggs. The number surviving (i.e. establishing colonies) of the nematodes inoculated when they were one day old may be less than the number surviving of the nematodes inoculated when they were 2 days old because the one day old larvae have longer to live before laying eggs. Conversly, the number surviving of the A. avenae inoculated when they were 7 days old may be greater than any other age tested. Then by subtracting the percentage which did not establish colonies of x + I day old A. avenae inoculum from that of x day old A. avenae inoculum, the mortality at age x may be calculated.

D. Discussion

Table XXV shows that 8.3% of 7 day old nematodes did not establish cultures. Since these stages are already of egg laying age (Part I, Section II) the 8.3% loss is that which is caused by the experimental conditions, and is not the mortality of the nematodes. Six day old nematodes probably fell into the same category. Between day 1 and day 6,

Table XXV

% of A. avenae of different ages which established colonies.

Age of A. avenae inoculated in days	No. inoculated	No. cultures colonised	% cultures not colonised
1 (L_2)	24	20	16.7
2 (L_3)	24	21	12.5
3	24	21	12.5
4	24	21	12.5
5	24	20	16.7
6	24	22	8.3
7	24	22	8.3

8.4% (16.7% less 8.3%) mortality occurs. Percentage mortality for individual days (of age) cannot be confidently stated without much greater replication.

It is concluded that percentage mortality of eggs is 6.3%. Of the larvae produced 8.4% do not reach maturity on B. cinerea cultured on PDA at 26°C.

Section III

DISCUSSION, POPULATION ENERGETICS

By comparing population growth rate of A. avenae in culture on B. cinerea on PDA (Fig. XIV) with the 'ideal' population potential as calculated from the ideal egg production rates (Part I, Section II) and 'ideal' mortality rates (Part II, Section II), the effects of numbers on population growth may be evaluated.

The 'ideal' population potential of one A. avenae was calculated by first obtaining from Table XI (Part I, Section II), the number of eggs produced by an average female as it ages, and calculating the number of eggs and larvae that would be present at the end of 1, 2, and 3 weeks (Table XXVI). The number of eggs counted at the end of 4, 11 and 18 days may have hatched into larvae on days 7, 14 and 21 respectively. But since there is a 6.3% mortality of eggs (Part II, Section II) only 93.7% of eggs would be larvae at the end of each week (Table XXVI). The rate of egg production changes according to the age of the female. In Table XXVI the total eggs and larvae produced is given as the 1st, 2nd and 3rd rate of production for the 1st, 2nd and 3rd week of life of the female, respectively.

Therefore, if a culture was inoculated with a single fourth-stage larva, at the end of the first week 1 female and its progeny of 27.9 eggs and 22.1 larvae will be found, since the young female is producing according to the 1st rate of production (Table XXVI). In Table XXVII the original female is termed the 1st age group and the females developing subsequently termed 2nd age group, 3rd age groups and so forth. At the end of the 2nd week the 1st age group female should produce 53.5 eggs (at the 2nd rate of production) of which 43.8 should have hatched and 9.7 should still be eggs. Of the 22.1 larvae

Table XXVI

Average progeny produced per week by ageing female.

Age of female in days	No. eggs	No. larvae
4	12.3	0.2
7	27.9	10.4
∴ at end of week 1 = 1st rate of production	27.9	$(\frac{12.3 \times 93.7}{100}) + 0.2 + 10.4$ = 22.1
11	18.2	21.2
14	917	5.5
∴ at end of week 2 = 2nd rate of production	9.7	$(\frac{18.2 \times 93.7}{100}) + 2.12 + 5.5$ = 43.8
18	3.4	0.6
21	0.3	0.5
∴ at end of week 3 = 3rd rate of production	0.3	$(\frac{3.4 \times 93.7}{100}) + 0.6 + 0.5$ = 4.3

Table XXVII

Calculation of 'ideal' population potential of *A. avenae*

Time inoculum	Numbers Present			
	Female	Eggs	Larvae	Total
Week 1	1 (1st age group)	27.9 (1st rate*)	22.1	-
Total Week 1	1	27.9	22.1	51.0
Week 2	1 (1st age group) $\frac{22.1 \times 91.6}{100} = 20.2$ (2nd age group)	9.7 (2nd rate*) 27.9x20.2 = 563.6 (1st rate)	43.8 $\frac{27.9 \times 93.7}{100} = 26.1$ 22.1x20.2 = 446.4	-
Total Week 2	21.2	573.3	516.3	1110.8
Week 3	1 (1st age group) 20.2 (2nd age group) $\frac{516.3 \times 91.6}{100}$ = 472.9 (3rd age group)	0.3 (3rd rate*) 9.7x20.2 = 195.9 (2nd rate*) 27.9x472.9 = 13193.9 (1st rate*)	4.3 43.8x20.2 = 884.8 $\frac{573.3 \times 93.7}{100} = 537.2$ 22.1x472.9 = 10451.1	-
Total Week 3	494.1	13390.1	11877.4	25761.6
Week 4	20.2 (2nd age group) 472.9 (3rd age group) $\frac{11877.4 \times 91.6}{100}$ = 10879.7 (4th age group)	0.3x20.2 = 6.1 (3rd rate) 9.7x472.9 = 4587.3 (2nd rate*) 27.9x10879.7 = 303,543.6 (1st rate*)	4.3x20.2 = 86.9 43.8x472.9 = 20713.0 $\frac{13390.1 \times 93.7}{100}$ = 12546.5 22.1x10879.7 = 240,441.4	

*rate of production (Table XXVI)

contd./-

Table XXVII contd./-

Total Week 4	11372.8	308,137.0	261,241.3	580,751.1

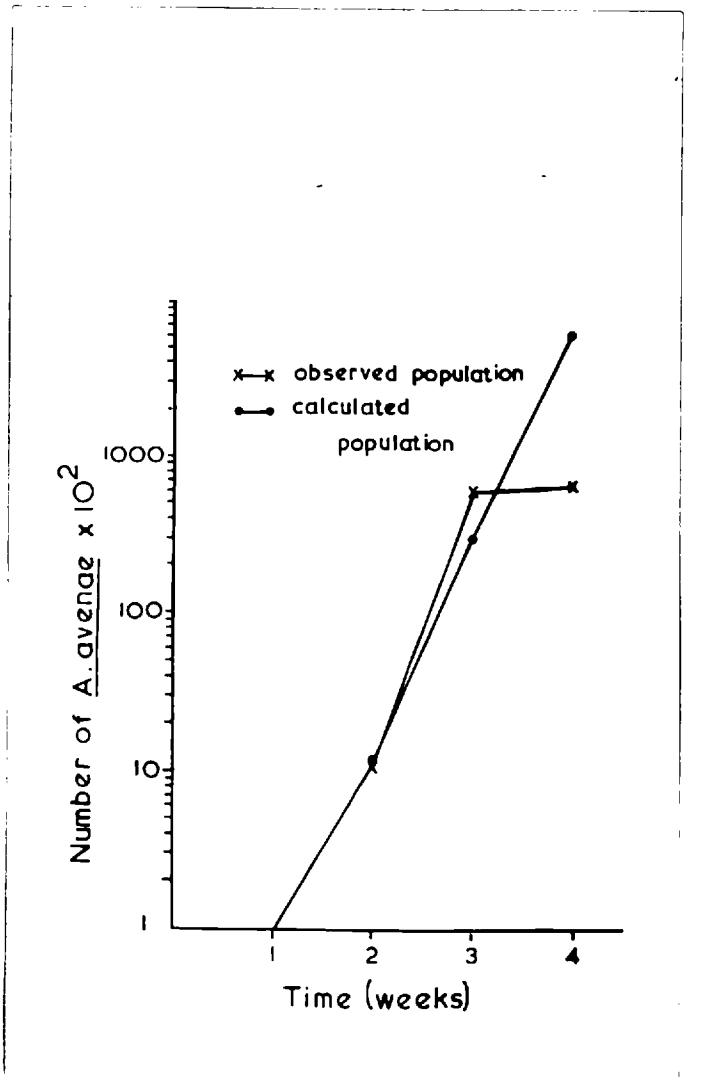
produced at the end of the first week 91.6% (mortality rate of larvae = 8.4%; Part II, Section II) should be females on the second week and they should have given rise to 27.9 x 20.2 eggs and 22.1 x 20.2 larvae (at the 1st rate of production) at the end of the second week. Of the 27.9 eggs present at the end of week 1, 93.7% should have developed to larvae at the end of week 2 (Table XXVII). Similarly by applying the appropriate rate of production to the groups of females formed and the respective mortality rates of the eggs and larvae, the total females, eggs and larvae present at the end of week 1, 2, 3 and 4 is calculated in Table XXVII. The females were aged into groups consisting of females varying in age but produced within the same week. But since the rate of egg production for any week of age of the female was observed and averaged over the whole of that week (Table XXVI) this discrepency would not cause much error. The mortality rate was not applied to the originally inoculated fourth-stage larva, for simplicity of calculation, especially since the error caused is negligible. The adult life of A. avenae is approximately three weeks (Part I, Section II) and therefore the 1st age group female is not present in week 4.

The calculated population changes and the observed population changes (from Fig. XIV) are plotted in Fig. XVII. The calculated (or 'ideal') populations are less than the observed populations at the end of the third week. At the end of the fourth week the calculated populations are far in excess of the numbers observed. Fisher (1969) found that A. avenae produced more eggs per female when four females were added per petri-dish than when only one was added. In my experiments on 'ideal' egg production each female was incubated in a separate culture with only short contact with her progeny (Part I, Section II). In the experiments where population growth was studied, the conditions were such

Fig. XVII.

OBSERVED AND CALCULATED POPULATION INCREASE OF

OF A. AVENAE OVER FOUR WEEKS.



that there was an increasing effect of A. avenae numbers on each other. In the first and second weeks the numbers produced by calculation and observation are similar probably because conditions as regards food are similar. The greater population observed, than calculated in week three may be explained by Fisher's (1969) observation. In the cultures where population increase was studied the B. cinerea was seen to be destroyed at the end of the third week (Part 11, Section 1). It is probable therefore that the 'real' plpulation was not increasing at the rate of the 'ideal calculated population, because of the lack of food and perhaps other adverse conditions caused by the numbers present, after week three.

The 'ideal conditions of my experiments on average egg production per female although ideal as regards food, and perhaps lack of accumulated excretory products of fungus and nematodes is not ideal in stimulating egg production.

PART III

COMPARATIVE NEMATODE ENERGETICS

Section I

COMPARATIVE STUDIES

A. Introduction

The calorific value of an organism is dependent on its chemical composition which is itself related to its life-history (age and stage) and environmental conditions. The calorific values of nematodes from various habitats and at various stages were compared. The energetics of storage (Ditylenchus dipsaci wool), tanning (Heterodera trijoli cysts), and starvation on egg laying (Aphelenchus avenae) were studied.

The chemical composition of the various structures of Ascaris lumbricoide has been widely studied (Fairbairn, 1955; Bird, 1956, 1957; Lee, 1965; and others). The relationship of the calorific value of these structures may be related to the proportion of various substances and this was tested.

B. Survey of calorific values of nematodes.

Nematodes parasitic on animals and plants or feeding on bacteria and fungi were collected or cultured to obtain large enough numbers for calorimetry. Table XXVIII gives the calorific values of various nematodes, their age and sex, and the food or habitat of the nematodes. The calorific values of a nematode parasitic in the lungs of frogs, a copepod in the gill cavity of Angler fish and an Acanthocephalan in the intestine of frogs and toads is given in comparison.

C. The energetics of storage and tanning; and starvation on viable egg out-put.

1. Storage of Ditylenchus dipsaci wool

D. dipsaci wool collected from bulbs of Narcissus sp were stored for 40 days at 10°C, 15°C and 26°C. The calorific values of the stored wool and wool freshly removed from bulbs was determined by bomb

Table XXVIII

Comparative calorific value of selected nematodes.

Species	Composition of sample	no. of samples	Mean calorific value kcal/g. dry wt.	food or habit
Pangrellus redivivius	eggs, larvae & adults	5	5.19	Bacteria
Ditylenchus myceliophagus	eggs, larvae & adults	4	4.94	Fungus
Aphelenchus avenae	eggs, larvae & adults	3	5.45	Fungus - Botrytis cinerea
Deladenus siricidicola	eggs, larvae & adults	2	5.21	Fungus - Amylosterium sp
Meloidogyne incognita	egg sacs	1	5.05	Roots of tomato
Heterodera avenae	larvae (L_2)	1	4.11	Roots of cereals
Heterodera trifolii	females	2	6.17	Roots of clover
Ditylenchus dipsaci	larvae (L_4) & adults	4	6.78	Bulbs of Narcissus sp
Ditylenchus dipsaci	wool (L_4)	3	6.85	Bulbs of Narcissus sp
Proleptus sp	females	5	4.27	Dog fish intestine
Proleptus sp	males	3	3.86	Dog fish intestine
Ascaroidea	males & females	2	4.76	Cod intestine
Anisakis sp	larvae	5	4.50	Herring coelom
Oxyurids	females	1	5.36	Frog - intestine
Aspiculuris tetraptra	males & females	1	5.17	Mouse large intestine
Litomosoides sp	adults	1	5.36	Cotton rat pleural cavity
Ascaris lumbricoides	males	3	4.15	pig intestine
Ascaris lumbricoides	females	4	4.53	pig intestine

contd./-

Table XXVIII contd./-

Haplometra sp	adults	2	5.22	Frog - lungs
Chondrocanthus gibbosus	adults	3	5.08	Angler fish - gill cavity
Acanthocephala	females	1	5.71	Frog & toad - intestine.

calorimetry. Table XXIX shows the calorific value of the various sample. The calorific value of freshly picked samples is higher than the stored samples. But there is no difference in the energy content of wool stored at the three given temperatures.

2. Tanning of Heterodera trifolii females.

Females of H. trifolii from a clover plant grown in a pot were sampled at the beginning of October, November and December. The females were picked individually off the roots of the plant with the least damage to the plant as possible. After removal of a quantity of females the plant was placed back in the pot until the next month. Random sampling was not attempted, but all cysts noticed were picked. In October most cysts were a golden yellow in colour and in November and December most cysts had turned dark brown. Table XXX shows that the calorific value of H. trifolii cysts before the onset of tanning is higher than the tanned cysts.

3. Egg laying and viability of eggs laid by starving A. avenae.

The eggs laid when a nematode is not feeding must be produced from energy already contained within the female. If sufficient energy is not available egg production may cease and/or non viable eggs be produced.

A. avenae females, 5 per watch glass with three-fold replication were incubated in tap water at 8°C, 15°C and 26°C. At the end of each 24 hour period, eggs (laid) were counted and the females were transferred into fresh water. The time taken for the eggs to hatch was noted. Hatching of eggs laid by progressively starved females was noted by incubating eggs laid per period for a further period. Fig. XVIII shows that the number of eggs produced per female declined after the 2nd day of starvation at 26°C and after the first day at 15°C and 8°C.

Table XXIX

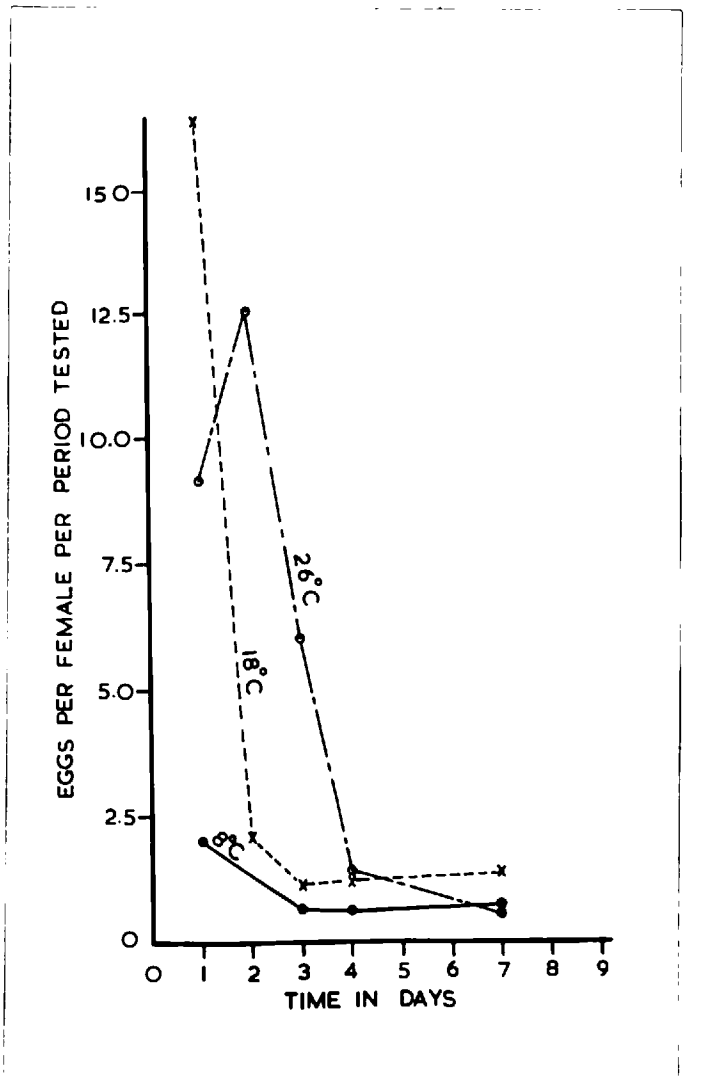
Calorific value of D. dipsaci in kcal/g.				
Sample no.	Freshly removed	Stored at 8°C	Stored at 15°C	Stored at 26°C
1	7.1271	6.1337	6.3589	6.4781
2	6.5218	6.1327	6.1601	6.1155
3	6.8903	6.3246	-	6.4116
Mean	6.8464	6.1970	6.2595	6.3351

Table XXX

Sample no.	Calorific value of H. trifolii cysts in kcal/g.		
	October	November	December
1	6.07	5.61	5.73
2	6.27	5.42	5.32
Mean	6.17	5.51	5.52

Fig. XVIII.

EGG LAYING BY STARVING FEMALES AT DIFFERENT TEMPERATURES.



Egg production continued at a very slow rate upto the day 7. Many dead females were observed on the day 7 of starvation, at 15°C and 26°C. Fig. XIX and XX show that there is no loss in the viability of eggs laid by progressively starved females. No hatch was obtained at 8°C and therefore these eggs were incubated at 26°C for a further 3 days when the majority hatched. The rate of hatch was slower at 15°C than at 26°C but similar percentage hatch was obtained after 6 days at 26°C and at 15°C.

D. The calorific values of various tissues of Ascaris lumbricoides.

A. lumbricoides was collected from pigs and the various tissues separated. Cuticle from males and females, body wall muscles of females, gut from males and females, and eggs. Finally whole males and females were prepared for calorimetry. Eggs were obtained by dissecting the distal 1 cm. portion of the uterus and dissolving the uterine wall by immersion in 10% KOH solution for 30 minutes. This procedure also removed the albuminous layer of the eggs which enabled drying which is essential for bomb calorimetry.

Pellets of dried males and females were made by first powdering the dry nematodes and then pelleting portions of the homogenate so that all tissues would be included in the pellet.

Table XXXI shows the calorific value of the various samples.

Fig. XIX.

PERCENTAGE HATCH OF EGGS LAID BY PROGRESSIVELY.

. STARVED FEMALES AT 26° C.

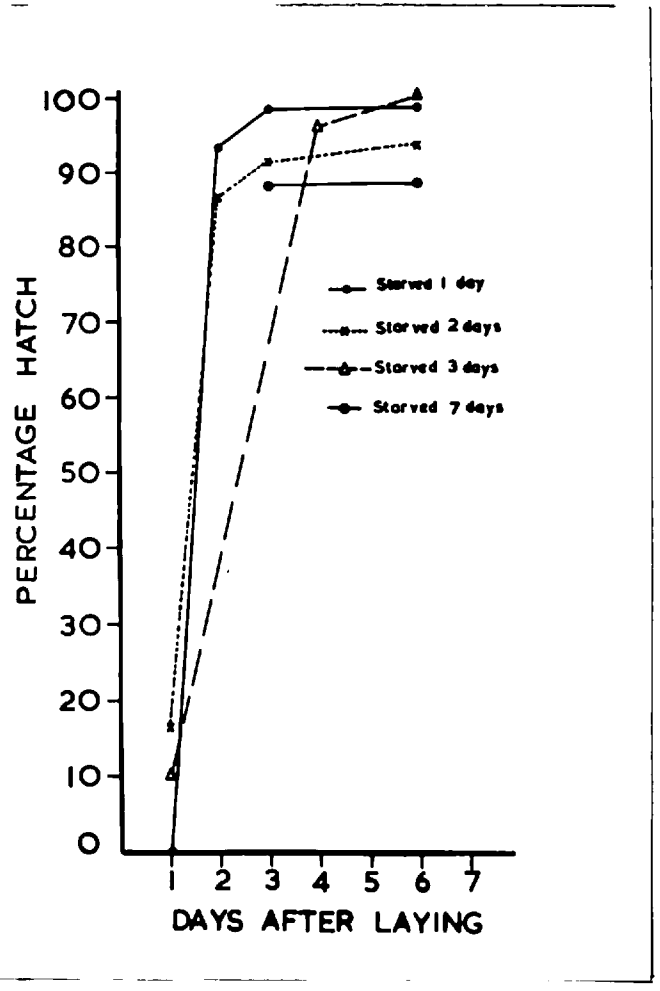


Fig. XX

PERCENTAGE HATCH OF EGGS LAID BY PROGRESSIVELY

STARVED FEMALES AT 26°C.

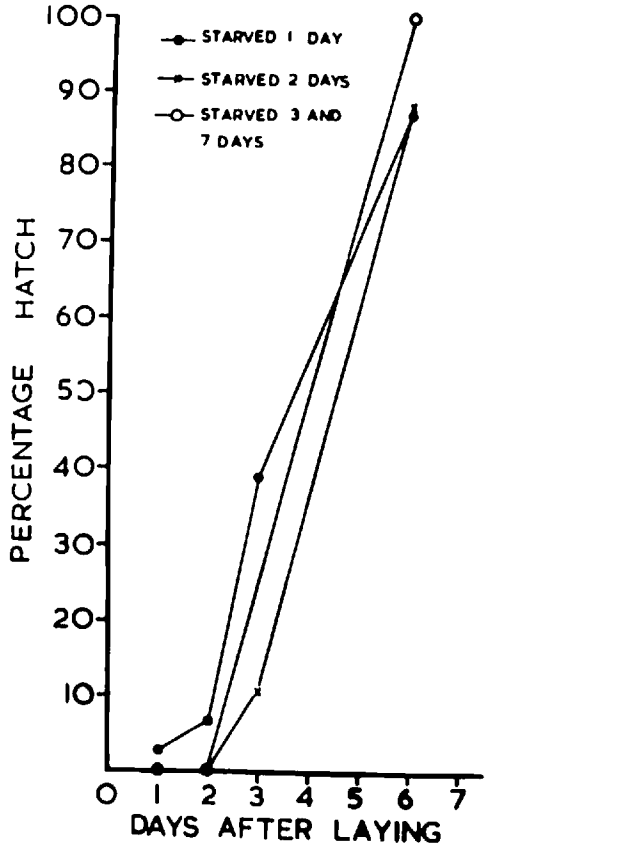


Table XXXI

Calorific value of tissues of A. lumbricoides

Composition of sample	sample no.	Calorific value kcal/g.	Mean calorific value kcal/g.
Cuticle (from males & females)	1 2	4.41 4.49	4.45
Body wall muscle (from females)	1 2 3	4.42 4.49 4.34	4.41
Gut (from males & females)	1 2	5.28 4.83	5.06
Eggs (without albuminous layer)	1 2	5.27 4.99	5.13
Males (homogenate of whole nematode)	1 2 3	4.54 4.24 3.68	4.15
Females (homogenate of whole nematode)	1 2 3 4	4.44 4.78 4.22 4.66	4.53

Section 11.

DISCUSSION, COMPARATIVE ENERGETICS.

the calorific values of nematodes from various habitats varied from 3.86 to 6.85 kcal./ gram. The lowest value of 3.86 kcal./ gram was of Proleptus sp males. The females of Proleptus sp had a higher calorific value (4.27 kcal. / gram). The calorific value of A. lumbricoides females is not significantly higher than that of the males.

The highest calorific value was obtained for Ditylenchus dipsaci wool. This is in agreement with Slabodkin's (1961) hypothesis that the non- feeding stages have a higher calorific content in accordance with the energy required for maintenance during the quiescent period. The calorific values of cysts of Heterodera trifolii was also high, probably becouse of the eggs contained within.

Other than for these exceptional values, the calorific values of plant and animal parasitic and free living nematodes was not very variable. The calorific values of parasites from three Phyla (Table XXVIII) are also in the same range as that of nematodes, and other animals as established by Golley (1960), Slobodkin & Richman (1961), and Comita & Schindler (1963).

The calorific value of D. dipsaci wool stored over 40 days at three different temperatures was similar. The energy used in maintenance may be very low even at 26°C, and therefore the energy content is not greatly effected.

The calorific value of cysts of H. trifolii is lower than the calorific value of younger females. The tanning process and also the maturation of eggs probably decreased the energy per unit weight of cysts.

The A. avenae females used in the egg-laying experiments were

cultured at 26°C before starvation and therefore the lower egg out-put per female at 15°C and 8°C may be due to the change in temperature as well as to the incubation temperature itself. At all three temperatures the females continued to lay eggs upto the 7th day when dead and dying females (with larvae developed within them) were noticed at 26°C and 15°C. The females at 8°C were all still alive on the 7th day. Egg production was very low through-out the time of incubation at 8°C. It is probable that at this temperature energy is conserved and therefore the adult is able to maintain itself without food for a longer period.

The viability of the eggs laid was not effected by the nutritional condition of the female. Eggs laid by females starved for 7 days still had a similar percentage hatch as eggs laid by females which were only starved for one day (Fig. XIX and XX). The development of the eggs to hatching age was much slower at 15°C than at 26°C, but by the 6th day of being laid 88% to 100% of the eggs hatched. At 8°C the eggs did not hatch even after 6 days of being laid but hatched when removed to 26°C.

Of the tissues of A. lumbricoides tested for their calorific value, the gut and the fertilised eggs had the highest calorific value. Fairbairn (1957) found that 17.5% of the wet weight of fertilised eggs was lipid. The high lipid content probably contributed to give the eggs a higher calorific value.

GENERAL DISCUSSION

Measurement of energy provides a unifying means of relating biological processes and interactions. The flow of energy through a host organism and the points at which a parasite may disturb this flow can be found and quantified by the construction of energy budgets for a host and its parasites. Here lies a means of assessing pathogenicity, efficiency of parasitism (of any particular host parasite system) and the efficiency of a parasite. Although it may be an over simplification of the host-parasite complex, energy flow studies provide a mechanism for quantifying efficiency and reveal factors which require further research.

Pathogenicity may be defined as the net loss in production to the host caused by a parasite. It is calculated by the difference in gross production efficiencies $(\frac{P}{I})$ of uninfected and infected hosts.

i.e. $\frac{Ph}{Ih} - \frac{Pi}{Ii}$

where Ph and Ih = net production and ingestion respectively of uninfected host and Pi and Ii = net production and ingestion of infected host. Pathogenicity may be expressed in calories or as a percentage when comparing pathogenicity of different infections or levels of infection. Quantitative assessment of pathogenicity may be particularly useful in the study of plants parasitised by nematodes, where a precise statement on the yield of the plant may be balanced against economy by the farmer.

If the most efficient host parasite system is that in which the total loss in gross production to the host is equal to the energy ingested by the parasite (i.e. 100% efficiency), efficiency of parasitism may be expressed as:-

$$\frac{Ip}{\frac{Ah}{Ih} - \frac{Ai}{Ii}} \times 100 = \text{percentage gross efficiency of parasitism.}$$

where Ip = parasite ingestion, Ah and Ih = assimilation and ingestion respectively of uninfected host and Ai and Ii = assimilation and ingestion respectively of infected host.

If the parasite causes the host to have a higher maintenance requirement, (i.e. respiration) it would be shown by the calculation of net efficiency of parasitism :-

$$\frac{Ip}{\frac{Ph}{Ih} - \frac{Pi}{Ii}} \times 100 = \text{percentage net efficiency of parasitism.}$$

The more efficiently the parasite converts the ingested energy to production and the more efficiently the parasite obtains its energy from the substrate it metabolises, the less the demand it makes on the host. Therefore, calculation of gross production efficiency ($\frac{P}{I}$) and net production efficiency ($\frac{P}{A}$), (Ivlev, 1939 a and b) of a parasite may yield some information on the virulence of a particular parasite if allowance is made for size of infection and parasite. However, this will be counteracted if the host is able to utilize the end-products of the parasite's metabolism.

My studies were made on a model of a host- parasite system. The techniques and methods of analysis may be useful in the study of a real host-parasite relationship.

The efficiency of A. avenae as a converter of energy was established for the length of its life. This was high throughout life, with a depression on about day eight, corresponding with a cessation of growth between day seven and eight and the on-set of egg laying. The female continued to feed when both growth and egg production had ceased. It is probable then, that the cost to the host was maintained even though the nematode was not productive. However, the experiments were done

in the laboratory where natural predators and other hazards were absent from the environment. In nature the lenth of life of the female may not be as long and the unproductive period in life in the cultures, may not be natural.

A. avenae is not known to cause much damage to plants, although it is known to feed on them (Chin & Estey, 1966). It appears from preliminary observations on ingestion that the efficiency of assimilation $(\frac{A}{I})$ of A. avenae is high although this cannot be established until more accurate values of ingestion are obtained. The energy required for life processes of a parasite only give rise to the minimum pathogenicity to the host. The variety of hosts of A. avenae and its migrant manner of feeding as well as its efficient assimilation, probably explains its low demand on plants.

When 'ideal' population increase was calculated from studies of egg laying by isolated females and compared with populations obtained in cultures, it was found that the increase in population was greater in the presence of many nematodes. Fisher (1969) suggested that the increase in the average egg production when four nematodes are contained in a petri-dish may be related to the attraction of A. avenae to a damaged food source, rather than a direct effect of one nematode on another. But, I observed that A. avenae moved from one feeding site to another very frequently, attacking cells in areas which were not previously damaged. It is possible that there are other effects of A. avenae on each other, that are favourable to population increase. In my study the greater populations observed than calculated, may not only be caused by a higher egg production by the females but also a lower mortality rate of all the stages.

The survey of calorific values of nematodes from various habitats

showed that the range in values may be dependent on the habitat, stage and sex of the nematode.

The gut and the fertilized eggs of A. lumbricoides had the highest calorific value. But the values obtained were lower than was expected from their chemical composition. This is also true of the calorific values of the muscle layer.

A. avenae was used in this study because of the difficulties envisaged in applying the priciples of energetics to nematode - host systems, at the beginning of this work. Although the study was not on a true host - parasite system it enabled a detailed study of the nematode, and the application of energetics to a real host - parasite system to be initiated.

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APPENDIX

Table 1

The size of the medium bulb of different ages of A. avenae

Worm no.	Day 1		Day 4		Day 5	
	Width	Length	Width	Length	Width	Length
1	9.5	12.7	19.0	20.6	19.0	19.0
2	9.5	12.7	19.0	19.0	20.6	20.6
3	9.5	11.0	19.0	20.6	22.2	22.2
4	9.5	11.0	19.0	20.6	22.2	22.2
5	9.5	12.7	15.9	16.7	22.2	22.2
6	-	-	-	-	15.9	15.9
7	-	-	-	-	15.9	15.9
8	-	-	-	-	23.0	23.0
	Day 6		Day 7		Day 10	
1	19.0	22.2	22.2	22.2	22.2	25.4
2	22.2	23.8	22.2	22.2	22.2	22.2
3	19.0	22.2	22.2	22.2	22.2	22.2
4	24.6	25.4	22.2	20.6	22.2	24.6
5	22.2	22.2	22.9	25.4	24.6	22.2
6	20.6	22.2	19.0	22.2	22.2	22.2
7	25.4	23.8	19.0	20.6	20.6	22.2
8	22.2	22.2	22.2	20.6	25.4	23.7

contd./-

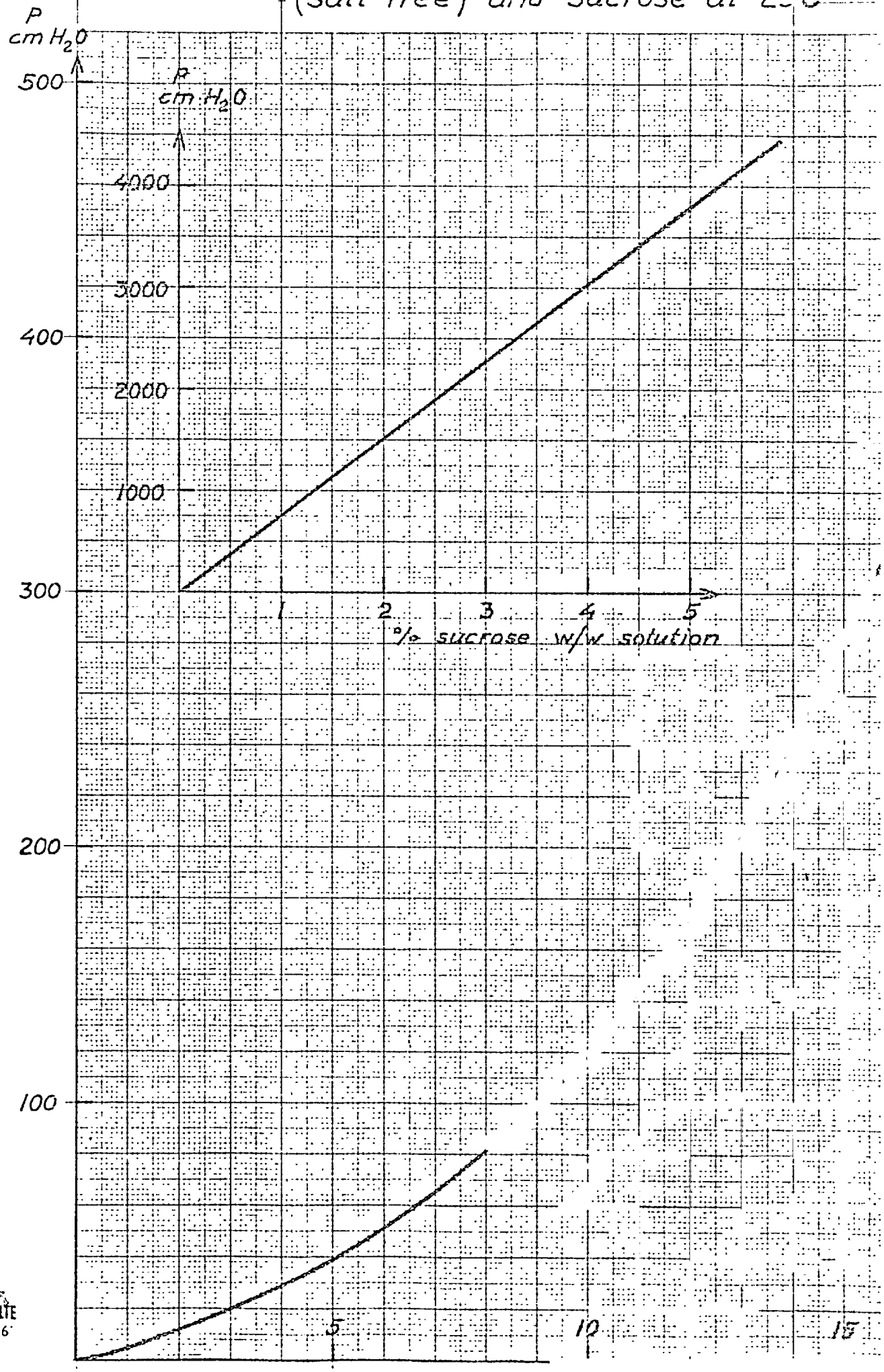
Table 1 contd./-

Worm no.	Day 16		Day 24	
	Width	Length	Width	Length
1	22.2	22.2	19.0	22.2
2	22.2	23.8	22.2	22.2
3	22.2	23.8	22.2	23.0
4	20.6	22.2	22.2	22.2
5	20.6	23.0	22.2	23.0
6	22.2	25.4	19.0	22.2
7	19.0	22.2	22.2	22.2
8	19.0	22.2	22.2	23.0

Appendix

Fig. I

Osmotic pressure of Ficoll (salt free) and sucrose at 25°C



J325 01 - 523 A4 - 1x1 mm
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APPENDIX
Table II

Calculation of mean calorific value of Aphelenchus avenae eggs

Egg no.	Dimensions of rectangle: long axis (a) μ	Dimensions of rectangle: short axis (b) μ	Volume rectangle axbxb (μ^3)	Volume square bxbxb (μ^3)	Volume sphere $4/3\pi r^3$ (μ^3)	Volume sq. -volume sphere (μ^3)	Volume egg vol. rect. -(vol. sq. vol. sph.) (μ^3)	Weight of egg *1 $m=d \times \forall$ (μg)	Dry *2 weight of egg (μg)	Calories *3/egg
1	51.15	25.00	35718.75	15625.00	8183.61	7441.39	28277.36	0.02969122	0.00587292	0.3203^{-4}
2	62.51	26.79	44863.43	19227.18	10081.56	9145.62	35717.81	0.03750370	0.00741823	0.4045^{-4}
3	66.08	25.00	41300.00	15625.00	8183.61	7441.39	33858.61	0.03555154	0.00703209	0.3836^{-4}
4	67.87	25.00	42418.75	15625.00	8183.61	7441.39	34325.14	0.03594690	0.00701576	0.3826^{-4}
5	64.30	25.90	43133.08	17373.98	9099.46	8274.52	34858.56	0.03660149	0.00723977	0.3948^{-4}
6	61.17	25.90	41033.45	17373.98	9099.46	8274.52	32758.93	0.03439688	0.00680370	0.3710^{-4}
7	53.58	25.00	33487.50	15625.00	8183.61	7441.39	26046.11	0.02734842	0.00540952	0.2950^{-4}
8	65.19	26.79	46786.86	19227.18	10081.56	9145.62	37641.24	0.03952330	0.00781771	0.4263^{-4}
9	61.62	25.00	38512.50	15625.00	8183.61	7441.39	31071.11	0.03262467	0.00645316	0.3519^{-4}
10	61.62	23.21	33194.69	12503.23	6556.97	5946.26	27248.43	0.02861085	0.00565923	0.3086^{-4}

contd/-

11	70.83	23.61	39482.77	13160.92	6990.68	6170.24	33312.53	0.03497816	0.00691868	0.3773^{-4}
12	70.23	25.00	45143.75	15625.00	8184.54	7440.46	37703.29	0.03958845	0.00783060	0.4270^{-4}
13	63.89	22.22	31544.41	10970.68	5746.44	5224.24	26320.17	0.02763618	0.00546643	0.2981^{-4}
14	65.28	25.00	40800.00	15625.00	8184.54	7440.46	33359.54	0.03502752	0.00692844	0.3778^{-4}
15	65.28	25.00	40800.00	15625.00	8184.54	7440.46	33359.54	0.03502752	0.00692844	0.3778^{-4}
16	69.45	26.39	48374.01	18378.79	9616.30	8762.49	39611.52	0.04159209	0.00783516	0.4273^{-4}
17	68.00	23.00	35972.00	12167.00	6373.21	5793.79	30178.21	0.03168712	0.00626771	0.3418^{-4}
18	62.00	24.00	35712.00	13824.00	7241.14	6582.86	29129.14	0.03058560	0.00604983	0.3299^{-4}
19	76.00	24.00	43776.00	13824.00	7241.14	6582.86	37193.14	0.03905279	0.00772464	0.4213^{-4}
20	64.00	25.00	40000.00	15625.00	8184.54	7440.46	32559.54	0.03418751	0.00676228	0.3688^{-4}
21	64.00	24.00	36864.00	13824.00	7241.14	6582.86	30281.14	0.03179519	0.00628908	0.3430^{-4}
22	69.00	26.00	46644.00	17576.00	9206.48	8369.52	38274.48	0.04018820	0.00794922	0.4335^{-4}
23	65.00	22.00	31460.60	10648.00	5577.52	5070.48	26390.12	0.02770962	0.00548096	0.2989^{-4}
24	69.00	24.00	39744.00	13824.00	7241.14	6582.86	33161.14	0.03481919	0.00688723	0.3756^{-4}
25	64.00	26.00	43264.00	17576.00	9206.48	8369.52	34894.48	0.03663920	0.00724723	0.3952^{-4}
26	70.00	25.00	43750.00	15625.00	8184.54	7440.46	36309.54	0.03812501	0.00754113	0.4112^{-4}

contd./-

27	64.00	26.00	43264.00	17576.00	9206.48	8369.52	34894.48	0.03663920	0.00724723	0.3952^{-4}

*1 Density = 1.05 g/ml.

*2 Dry weight = 19.78% wet weight

*3 micro gram (μg) = 0.005453 calories

$\Sigma x = 0.00100381$

$N = 27$

$\bar{x} = 0.3718^{-4}$

S.E. $= 0.0734^{-4}$

APPENDIX

Table III

Density g/ml of aqueous Ficoll solutions

Density (g/ml)	% conc. of Ficoll soln (w/v)
1.06	17.8
1.05	15.0
1.04	12.0
1.03	9.3
1.02	6.4
1.01	3.4

APPENDIX

Table IV

Growth of A. avenae at 26°C, from photographs

No. of Nematodes	Day 1		Day 2	
	Length/u	Width/u	Length/u	Width/u
1	186.08	9.30	202.56	9.28
2	191.80	9.30	212.81	8.97
3	181.14	9.30	266.66	14.10
4	174.45	11.63	261.53	15.38
5	168.64	8.14	205.12	8.97
6	176.78	9.30	216.66	10.25
7	216.32	10.48	203.84	12.82
8	-	-	203.84	12.82
9	-	-	221.79	11.54
10	-	-	215.38	10.25
11	-	-	-	-
12	-	-	208.97	12.82
13	-	-	182.04	11.54
14	-	-	194.86	12.82
15	-	-	238.45	16.67
16	-	-	217.94	11.54
17	-	-	261.53	17.95
Total	1122.59	67.45	3513.98	197.67
Mean	160.37	9.63	219.62	12.35

contd./-

Table IV contd./-

No. of Nematodes	Day 3		Day 4	
	Length/u	Width/u	Length/u	Width/u
1	165.00	10.75	249.15	16.50
2	187.50	8.75	260.07	19.80
3	375.00	20.00	425.70	24.75
4	362.50	20.00	432.15	22.58
5	190.00	10.00	179.85	13.20
6	188.75	8.75	201.30	14.85
7	265.00	13.75	531.13	28.05
8	205.00	10.00	308.55	23.10
9	202.50	8.75	178.20	11.50
10	205.00	11.25	238.65	21.45
11	-	-	-	-
12	258.75	16.25	-	-
13	256.25	15.00	-	-
14	302.50	17.50	-	-
15	370.00	16.25	-	-
16	188.75	11.25	-	-
17	397.50	18.75	-	-
Total	4120.00	217.00	3064.75	195.78
Mean	257.50	13.56	300.48	19.58

contd./-

Table IV contd./-

No. of Nematodes	Day 5		Day 6	
	Length/u	Width/u	Length/u	Width/u
1	390.50	22.00	-	-
2	-	-	-	-
3	737.10	31.59	980.10	42.00
4	652.86	31.59	875.90	46.10
5	168.94	13.7	-	-
6	203.28	13.7	-	-
7	195.02	27.47	663.84	27.24
8	482.63	28.88	783.70	34.05
9	-	-	-	-
10	473.85	24.75	811.36	34.05
11	800.28	33.00	866.68	32.92
12	552.83	26.13	-	-
13	600.21	27.47	-	-
14	663.39	31.39	922.00	32.92
15	-	-	958.88	38.50
16	-	-	424.12	22.70
17	-	-	673.06	40.86
Total	6520.89	3118.7	7117.84	351.34
Mean	543.41	251.56	711.78	35.13

APPENDIX

Table V

Growth of A. avenae at 26°C

No. of Nematodes	Day 1			
	Length /u	Width /u	Wet weight /ug	Calories*
1	244.83	13.46	0.027723	0.2990^{-4}
2	236.17	13.46	0.026742	0.2885^{-4}
3	234.25	12.50	0.022876	0.2468^{-4}
4	225.60	13.46	0.025545	0.2755^{-4}
5	223.17	12.96	0.023427	0.2567^{-4}
6	238.91	14.82	0.032795	0.3537^{-4}
7	227.80	12.04	0.020639	0.2226^{-4}
8	206.50	14.82	0.028346	0.3058^{-4}
9	227.52	12.50	0.022219	0.2397^{-4}
10	215.98	11.54	0.017976	0.1939^{-4}
11	225.60	13.46	0.025545	0.2755^{-4}
12	225.60	14.42	0.029319	0.3162^{-4}
13	226.56	13.46	0.025654	0.2767^{-4}
14	215.98	13.46	0.024456	0.2638^{-4}
15	221.75	12.50	0.021655	0.2336^{-4}
16	245.79	12.50	0.024003	0.2589^{-4}
17	236.17	12.50	0.030640	0.2488^{-4}
18	234.25	12.50	0.022880	0.2467^{-4}
19	227.58	11.54	0.018942	0.2043^{-4}
20	218.87	13.46	0.024783	0.2673^{-4}
21	217.91	11.54	0.018137	0.1956^{-4}
Mean	227.47	12.99	0.024130	0.2603^{-4}
S.E.	2.15	0.21	0.000807	0.0087^{-4}

*Calories per unit dry weight = Wet weight x 0.1972 x 0.005453

contd./-

Table V contd./-

No. of Nematodes	Day 2			
	Length /u	Width /u	Wet weight /ug	Calories
1	348.83	16.35	0.058281	0.6286^{-4}
2	220.18	11.54	C.018326	0.1977^{-4}
3	259.60	16.35	0.043373	0.4678^{-4}
4	224.99	12.50	0.021972	0.2370^{-4}
5	291.33	15.38	0.043070	0.4646^{-4}
6	267.30	14.42	0.034738	0.3747^{-4}
7	269.22	16.35	0.04459	0.4852^{-4}
8	325.95	16.35	0.054459	0.5874^{-4}
9	275.95	13.46	0.031246	0.3370^{-4}
10	254.80	17.31	0.047717	0.5147^{-4}
11	294.22	18.27	0.061380	0.6621^{-4}
Mean	275.67	15.30	0.041777	0.4506^{-4}
S.E.	11.65	0.63	0.004232	0.0456^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 3			
	Length /u	Width /u	Wet weight /ug	Calories
1	419.00	22.00	0.126747	1.3671^{-4}
2	314.00	16.00	0.050240	0.5419^{-4}
3	239.00	13.00	0.025244	0.2723^{-4}
4	243.00	14.00	0.029767	0.3210^{-4}
5	248.00	14.00	0.030380	0.3277^{-4}
6	471.00	21.00	0.129819	1.4003^{-4}
7	449.00	20.00	0.112250	1.2108^{-4}
8	260.00	16.0	0.041600	0.4487^{-4}
9	419.93	23.53	0.145312	1.5674^{-4}
10	318.94	18.63	0.069185	0.7463^{-4}
11	303.25	18.63	0.065782	0.7095^{-4}
12	478.00	22.00	0.144595	1.5596^{-4}
13	397.00	20.00	0.099250	1.0705^{-4}
14	504.00	28.00	0.246960	2.6638^{-4}
15	425.81	22.55	0.135328	1.4597^{-4}
16	310.11	17.65	0.060379	0.6513^{-4}
Mean	362.50	19.19	0.094553	1.0199^{-4}
S.E.	23.09	1.01	0.014876	0.1604^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 4			
	Length μ	Width μ	Wet weight μg	Calories
1	754.13	14.0	0.092381	0.9965^{-4}
2	755.30	29.0	0.397005	4.2823^{-4}
3	762.46	25.0	0.297836	3.2126^{-4}
4	687.47	26.0	0.290456	3.1330^{-4}
5	489.83	23.0	0.161950	1.7469^{-4}
6	800.06	35.42	0.627335	6.7667^{-4}
7	577.12	29.17	0.306916	2.9663^{-4}
8	577.12	29.17	0.306916	3.3105^{-4}
9	658.38	29.17	0.350130	3.7767^{-4}
10	572.96	39.59	0.561275	6.0541^{-4}
11	520.87	27.09	0.238906	2.5769^{-4}
Mean	650.52	27.87	0.330100	3.5293^{-4}
S.E.	32.68	1.98	0.047045	0.5099^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 5			
	Length μ	Width μ	Wet weight μg	Calories
1	758.30	35.42	0.594591	6.4135^{-4}
2	795.80	41.67	0.863636	9.3156^{-4}
3	758.30	39.59	0.742835	8.0125^{-4}
4	804.13	37.50	0.706755	7.6234^{-4}
5	704.14	33.34	0.489182	5.2765^{-4}
6	741.63	37.50	0.654607	7.0609^{-4}
7	662.47	37.50	0.582249	6.2804^{-4}
8	862.56	41.67	0.936087	10.0970^{-4}
9	654.21	35.42	0.512973	5.5331^{-4}
10	887.47	37.50	0.780003	8.4135^{-4}
11	737.47	41.67	0.800334	8.6327^{-4}
12	887.47	41.67	0.963121	10.3886^{-4}
13	887.47	35.42	0.695874	7.5060^{-4}
14	770.80	37.50	0.677461	7.3074^{-4}
15	770.80	45.84	1.012300	10.9191^{-4}
16	664.35	33.34	0.461678	4.9798^{-4}
17	408.36	25.00	0.159516	1.7206^{-4}
Mean	750.35	37.51	0.684306	7.3812^{-4}
S.E.	28.35	1.13	0.051426	0.5547^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 6			
	Length μ	Width μ	Wet weight μg	Calories
1	580.83	28.00	0.284607	3.0699^{-4}
2	654.83	34.00	0.473115	5.1032^{-4}
3	672.83	36.00	0.544992	5.8785^{-4}
4	991.62	50.00	1.54941	16.7126^{-4}
5	741.63	37.50	0.651828	7.0309^{-4}
6	741.63	41.67	0.804849	8.6814^{-4}
7	879.13	50.00	1.37364	14.8167^{-4}
8	783.30	35.42	0.614194	7.7947^{-4}
9	824.96	39.59	0.808135	8.7166^{-4}
10	1179.12	37.50	1.03634	11.1784^{-4}
11	987.46	41.67	1.07163	11.5591^{-4}
12	941.63	41.67	1.02190	11.0226^{-4}
13	1045.79	54.17	1.91797	20.6881^{-4}
Mean	848.06	40.55	0.934815	10.1733^{-4}
S.E.	48.39	2.02	0.128533	1.3705^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 7			
	Length /u	Width /u	Wet weight /ug	Calories
1	1024.64	46.15	1.36394	14.7120^{-4}
2	1024.48	46.15	1.36905	14.7672^{-4}
3	982.33	40.38	1.00108	10.7981^{-4}
4	916.95	40.08	0.920621	9.9302^{-4}
5	995.79	47.92	1.42916	15.4156^{-4}
6	1020.79	47.92	1.46504	15.8026^{-4}
7	754.13	35.42	0.591321	6.3782^{-4}
8	945.79	41.67	1.02641	11.0713^{-4}
9	1024.96	43.75	1.22615	13.2258^{-4}
10	1033.29	45.84	1.35704	14.6376^{-4}
11	1037.46	50.00	1.62103	17.4851^{-4}
12	1087.45	52.09	1.84416	19.8919^{-4}
13	941.63	54.17	1.72694	18.6275^{-4}
14	1037.46	47.92	1.48897	16.0606^{-4}
15	1120.79	50.00	1.75123	18.8896^{-4}
16	1120.79	56.25	2.21641	23.9071^{-4}
Mean	1004.55	46.61	1.39991	15.1000^{-4}
S.E.	22.07	1.37	0.09943	1.0745^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 8			
	Length μ	Width μ	Wet weight μg	Calories
1	1028.05	50.00	1.60633	17.3265^{-4}
2	1001.18	48.33	1.46159	15.7653^{-4}
3	1041.18	50.00	1.62684	17.5478^{-4}
4	1018.05	48.33	1.48622	16.0310^{-4}
5	954.71	36.66	0.80193	8.6445^{-4}
6	1174.72	51.66	1.95940	21.1349^{-4}
7	1044.71	48.33	1.52514	16.4508^{-4}
8	988.05	48.33	1.44242	15.5586^{-4}
9	881.37	41.66	0.95604	10.3123^{-4}
10	1011.71	45.00	1.28045	13.8114^{-4}
11	888.04	40.00	0.88804	9.4965^{-4}
12	1088.05	50.00	1.70008	18.3373^{-4}
13	961.38	43.33	1.12811	12.1683^{-4}
Mean	1006.25	46.28	1.37405	14.8142^{-4}
S.E.	21.63	1.27	0.09548	1.0389^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 9			
	Length μ	Width μ	Wet weight μg	Calories
1	1146.72	54.99	2.16723	23.3767^{-4}
2	1106.72	53.33	1.96726	21.2197^{-4}
3	1136.06	54.99	2.14708	23.1594^{-4}
4	1133.39	53.33	2.01466	21.7310^{-4}
5	1086.72	50.00	1.69800	18.3154^{-4}
6	1063.39	53.33	1.89023	20.3889^{-4}
7	1033.38	56.66	2.07345	22.3651^{-4}
8	1030.05	50.00	1.60945	17.3603^{-4}
9	1110.05	48.33	1.62053	17.4797^{-4}
10	1083.39	50.00	1.69280	18.2592^{-4}
11	1030.05	48.33	1.50374	16.2200^{-4}
12	1030.05	48.33	1.50374	16.2200^{-4}
13	1000.45	46.66	1.36133	14.6839^{-4}
14	993.38	43.33	1.16566	12.5733^{-4}
15	1036.72	50.00	1.61987	17.4727^{-4}
16	1286.73	58.33	2.73622	29.5141^{-4}
17	1000.05	48.33	1.45994	15.7476^{-4}
18	1080.05	50.00	1.68758	18.2029^{-4}
19	1040.05	46.66	1.41522	15.2652^{-4}
20	1050.05	50.00	1.64070	17.6973^{-4}
Mean	1073.57	50.75	1.74874	18.8626^{-4}
S.E.	18.20	0.83	0.07999	0.8628^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 10			
	Length μ	Width μ	Wet weight μg	Calories
1	1142.88	58.92	2.47974	26.7475^{-4}
2	1107.17	53.57	1.98581	21.4198^{-4}
3	1092.88	53.57	1.96018	21.1433^{-4}
4	1260.75	50.00	1.96992	21.2484^{-4}
5	1107.17	51.00	1.79984	19.4139^{-4}
6	1071.45	53.57	1.92174	20.7287^{-4}
7	1032.16	48.21	1.49934	16.1726^{-4}
8	1035.74	50.00	1.61834	17.4562^{-4}
9	1157.17	53.57	2.07549	22.3871^{-4}
10	1071.45	51.78	1.79546	19.3666^{-4}
11	1039.31	46.42	1.39970	15.0978^{-4}
12	1082.17	50.00	1.69089	18.2387^{-4}
13	1082.38	47.06	1.49818	16.1600^{-4}
14	1041.21	47.06	1.57961	17.0383^{-4}
15	1176.50	50.00	1.83828	19.8285^{-4}
16	1082.38	47.06	1.49818	16.1600^{-4}
17	1129.44	52.94	1.97839	21.3397^{-4}
18	1141.21	52.94	1.99900	21.5621^{-4}
19	1152.97	50.00	1.80152	19.4319^{-4}
20	1105.91	47.06	1.53075	16.5113^{-4}
21	1164.74	52.94	2.04022	22.0067^{-4}
Mean	1113.19	50.84	1.80765	19.4981^{-4}
S.E.	11.91	0.68	0.05711	0.6160^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 12			
	Length /u	Width /u	Wet weight /ug	Calories
1	1085.74	46.42	1.46223	15.7723^{-4}
2	1185.74	50.00	1.85272	19.9842^{-4}
3	1064.31	48.21	1.54605	16.6763^{-4}
4	1032.16	46.42	1.39007	14.9939^{-4}
5	1229.15	50.00	1.92055	20.7158^{-4}
6	1050.02	44.64	1.30775	14.1060^{-4}
7	985.74	44.64	1.22770	13.2425^{-4}
8	1107.17	48.21	1.60831	17.3479^{-4}
9	1028.59	49.99	1.60653	17.3287^{-4}
10	1092.88	53.57	1.96018	21.1433^{-4}
11	1135.74	46.42	1.52957	16.4986^{-4}
12	1121.45	50.00	1.75227	18.9007^{-4}
13	1235.74	53.57	2.21641	23.9072^{-4}
14	1125.02	53.57	2.01783	21.7651^{-4}
15	1164.31	51.78	1.95107	21.0451^{-4}
16	1153.60	57.14	2.35405	25.3918^{-4}
17	1171.45	53.57	2.10110	22.6634^{-4}
18	1185.74	53.57	2.12673	22.9398^{-4}
Mean	1119.70	50.09	1.77395	19.1346^{-4}
S.E.	16.58	0.85	0.07764	0.8375^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 16			
	Length μ	Width μ	Wet weight μg	Calories
1	1188.88	52.78	2.06994	22.3272^{-4}
2	1183.32	58.34	2.51718	27.1514^{-4}
3	1166.66	58.34	2.48175	26.7692^{-4}
4	1199.99	52.78	2.08928	22.5359^{-4}
5	1166.66	50.00	1.82291	19.6626^{-4}
6	1233.32	55.56	2.37947	25.6660^{-4}
7	1166.66	52.78	2.03125	21.9099^{-4}
8	1222.21	52.78	2.12797	22.9532^{-4}
9	1255.54	50.00	1.96178	21.1606^{-4}
10	1211.10	52.78	2.10862	22.7445^{-4}
11	1211.10	50.00	1.89234	20.4116^{-4}
12	1225.02	57.14	2.49979	26.9638^{-4}
13	1028.59	50.00	1.60717	17.3356^{-4}
14	1085.74	50.00	1.69647	18.2988^{-4}
15	1050.02	53.57	1.88331	20.3141^{-4}
16	1007.16	50.00	1.57369	16.9745^{-4}
17	987.59	41.07	1.03164	11.1278^{-4}
18	1057.16	50.00	1.65181	17.8172^{-4}
19	914.30	42.85	1.04923	11.3174^{-4}
Mean	1134.32	51.62	1.91977	20.7074^{-4}
S.E.	22.97	1.02	0.09766	1.0535^{-4}

contd./-

Table V contd./-

No. of Nematodes	Day 24			
	Length μ	Width μ	Wet weight μg	Calories
1	1144.43	50.00	1.78817	19.2880^{-4}
2	1111.10	44.45	1.37207	14.7998^{-4}
3	1133.32	52.78	1.97320	21.2838^{-4}
4	1133.32	50.00	1.77081	19.1007^{-4}
5	1133.32	50.00	1.77081	19.1007^{-4}
6	1144.43	52.78	1.99254	21.4924^{-4}
7	1233.32	55.56	2.37947	25.6660^{-4}
8	1155.54	50.00	1.80553	19.4752^{-4}
9	1055.55	44.45	1.30347	14.0598^{-4}
10	1122.21	47.23	1.56455	16.8759^{-4}
Mean	1136.65	49.73	1.77206	19.1142^{-4}
S.E.	13.86	1.13	0.09899	1.0678^{-4}

APPENDIX

Table VI

Growth of A. avenae at 15°C

No. of Nematodes	Day 4			
	Length μ	Width μ	Wet weight μg	Calories
1	352.82	21.27	0.098640	1.0639^{-4}
2	358.61	18.21	0.074813	0.8070^{-4}
3	345.11	19.23	0.079762	0.8603^{-4}
4	362.46	20.19	0.092345	0.9961^{-4}
5	323.90	18.27	0.067572	0.7289^{-4}
6	318.12	19.23	0.073524	0.7930^{-4}
7	337.40	17.31	0.063186	0.6815^{-4}
8	374.03	19.23	0.086446	0.9324^{-4}
9	335.47	19.23	0.077534	0.8363^{-4}
10	350.90	21.15	0.098103	1.0582^{-4}
Mean	345.88	19.33	0.081193	0.8758^{-4}
S.E.	5.51	0.39	0.003897	0.0421^{-4}
	Day 8			
1	514.30	25.00	0.200898	2.1670^{-4}
2	514.30	28.57	0.262372	2.8301^{-4}
3	635.73	25.00	0.248332	2.6786^{-4}
4	542.87	25.00	0.212059	2.2874^{-4}
5	535.73	28.57	0.273304	2.9480^{-4}
6	607.16	25.00	0.237172	2.5582^{-4}
7	564.30	25.00	0.220430	2.3777^{-4}
8	514.30	26.78	0.230525	2.4865^{-4}
9	675.01	25.00	0.263676	2.8441^{-4}
Mean	567.08	25.99	0.238752	2.5753^{-4}
S.E.	19.69	0.52	0.008347	0.0900^{-4}

contd./-

Table VI contd./-

No. of Nematodes	Day 12			
	Length /u	Width /u	Wet weight /ug	Calories
1	978.59	39.28	0.943678	10.1789^{-4}
2	935.73	39.71	0.922211	9.9474^{-4}
3	900.02	39.28	0.867911	9.3617^{-4}
4	778.59	32.14	0.502667	5.4220^{-4}
5	935.73	32.14	0.604119	6.5163^{-4}
6	807.19	39.71	0.795499	8.5806^{-4}
7	757.16	32.14	0.488832	5.2728^{-4}
8	807.16	32.14	0.521112	5.6209^{-4}
9	1161.49	38.46	1.073780	11.5822^{-4}
10	1061.49	38.46	0.981329	10.5850^{-4}
11	807.66	34.61	0.604661	6.5221^{-4}
Mean	902.80	36.19	0.755072	8.1445^{-4}
S.E.	38.61	1.05	0.065044	0.7016^{-4}

contd./-

Table VI contd./-

No. of Nematodes	Day 24			
	Length/u	Width/u	Wet weight/ug	Calories
1	1091.62	37.50	0.959432	10.3488^{-4}
2	974.96	37.50	0.856898	9.2429^{-4}
3	1091.62	41.67	1.184670	12.7784^{-4}
4	1104.12	41.67	1.198240	12.9247^{-4}
5	1117.88	39.28	1.078000	11.6278^{-4}
6	1178.60	49.99	1.840830	19.8559^{-4}
7	1128.59	39.28	1.088330	11.7392^{-4}
8	1096.45	42.85	1.258260	13.5721^{-4}
9	1157.17	42.85	1.327940	14.3237^{-4}
10	1114.30	42.85	1.278740	13.7931^{-4}
11	1342.88	42.85	1.541060	16.6225^{-4}
12	1264.31	42.85	1.450890	15.6500^{-4}
13	1278.60	49.99	1.997010	21.5406^{-4}
14	1217.88	49.99	1.902180	20.5177^{-4}
Mean	1154.21	42.94	1.354460	14.6098^{-4}
S.E.	25.38	1.14	0.094513	1.0156^{-4}

APPENDIX

Table VII

Growth of A. avenae at 8°C

No. of Nematodes	Day 4			
	Length/u	Width/u	Wet weight/ug	Calories
1	240.59	14.15	0.030107	0.3247^{-4}
2	235.88	15.10	0.033614	0.3626^{-4}
3	237.76	13.21	0.025931	0.2797^{-4}
4	249.08	17.00	0.044990	0.4753^{-4}
5	283.05	15.10	0.040336	0.4351^{-4}
6	256.63	13.21	0.027989	0.3019^{-4}
7	273.62	13.21	0.029842	0.3219^{-4}
8	228.33	13.21	0.024903	0.2686^{-4}
9	218.89	11.32	0.017531	0.1891^{-4}
10	277.39	13.21	0.030254	0.3263^{-4}
Mean	250.12	13.87	0.030550	0.3295^{-4}
S.E.	6.93	0.49	0.002458	0.0265^{-4}

contd./-

Table VII contd./-

No. of Nematodes	Day 8			
	Length /u	Width /u	Wet weight /ug	Calories
1	317.22	17.24	0.058927	0.6356^{-4}
2	296.53	17.24	0.055087	0.5942^{-4}
3	256.88	15.52	0.038672	0.4171^{-4}
4	334.46	18.96	0.075145	0.8105^{-4}
5	212.53	14.29	0.027125	0.2926^{-4}
6	239.32	17.86	0.047711	0.5146^{-4}
7	260.68	14.29	0.033270	0.3589^{-4}
8	239.26	16.07	0.038617	0.4165^{-4}
9	299.96	16.07	0.048415	0.5222^{-4}
10	278.54	16.07	0.044957	0.4849^{-4}
Mean	273.54	16.36	0.046792	0.5047^{-4}
S.E.	12.19	0.47	0.004383	0.0473^{-4}

Nc. of Nematodes	Day 24			
	Length /u	Width /u	Wet weight /ug	Calories
1	482.63	23.91	0.172446	1.8601^{-4}
2	473.93	21.74	0.139995	1.5101^{-4}
3	404.36	19.57	0.967899	1.0440^{-4}
4	445.87	20.84	0.121027	1.3055^{-4}
5	462.54	22.92	0.151865	1.6381^{-4}
6	404.20	18.75	0.888135	0.9580^{-4}
7	412.53	20.84	0.111978	1.2078^{-4}
8	507.08	21.43	0.145546	1.5699^{-4}
9	474.94	19.65	0.114616	1.2363^{-4}
10	435.66	19.64	0.105029	1.1329^{-4}
Mean	450.37	20.93	0.124811	1.3463^{-4}
S.E.	11.29	0.51	0.008444	0.0911^{-4}

APPENDIX

Table VIII

Population change over 4 weeks of A. avenae on B. cinerea on PDA at 26°C.

Week 1 - Whole extract of 3 replicate plates counted.

Replicate	Eggs	L_2	L_3	L_4	Female	Total
1	3	3	3	3	5	17
2	1	3	2	3	6	15
3	0	3	12	2	1	18
Total	4	9	17	8	12	50
Mean/plate	1.3	3.0	5.7	2.7	4.0	16.7

Week 2 - Whole extract of 3 replicate plates counted.

Replicate	Eggs	L_2	L_3	L_4	Female	Total
1	435	60	95	80	78	748
2	457	243	183	201	94	1178
3	399	399	200	84	135	1217
Total	1291	702	478	365	307	3143
Mean/plate	430.3	234.0	159.3	121.7	102.7	1047.7

Week 3 - 5 ml. of 250 ml. of extract counted
3 counts/replicate plate

Replicate 1

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	157	261	229	276	221	1144
2	143	345	330	289	191	1298
3	155	388	366	252	269	1430
Total	455	994	925	817	681	3872
∴ total in plate	7583.3	16566.6	15416.6	13616.6	11350.0	64533.3

contd./-

Table VIII contd./-

Week 3 Replicate 2

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	435	152	90	66	113	856
2	364	172	129	87	105	857
3	454	166	96	78	102	898
Total	1253	490	315	231	322	2611
∴ total in plate	20883.3	8166.6	5250.0	3850.0	5366.6	43516.6

Week 3 Replicate 3

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	518	261	204	146	167	1296
2	462	268	234	146	199	1309
3	469	217	192	124	202	1204
Total	1449	746	630	416	568	3809
∴ total in plate	24150.0	12433.3	10500.00	6933.3	9466.6	63483.3

	Eggs	L_2	L_3	L_4	Female	Total
∴ total in 3 plates	52616.6	37166.7	31166.7	24400.0	26183.4	171533.2
Mean	17538.9	12388.9	10388.9	8133.3	8727.8	57177.7

Table VIII contd./-

Week 4. 5 ml. of 250 ml. of extract counted
3 counts/replicate place.

Replicate 1

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	45	515	221	150	243	1174
2	42	450	238	135	241	1106
3	54	553	249	149	236	1241
Total	141	1518	708	434	720	3521
∴ total in plate	2350.0	25300.0	11800.0	7233.3	1200.0	58683.3

Replicate 2

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	127	711	366	282	209	1695
2	144	646	351	227	177	1545
3	131	595	399	219	176	1520
Total	402	1952	1116	728	562	4760
∴ total in plate	6700.0	32533.3	18600.0	12133.3	9366.6	79333.3

Replicate 3

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	437	208	214	94	108	1061
2	514	208	231	95	86	1134
3	435	169	214	74	89	981
Total	1386	585	659	263	283	3176
∴ total in plate	23100.0	9750.0	10983.3	4383.3	4716.6	52933.3

contd./-

Table VIII contd./-

	Eggs	L_2	L_3	L_4	Female	Total
∴ total in plates	32150.0	58808.3	41383.3	14043.3	18590.0	190949.9
∴ Mean	10716.7	19602.8	13794.4	4681.1	6196.7	63647.7

APPENDIX

Table IX

Population fluctuation over 6 weeks of A. avenae on B. cinerea on sand and czapek dox liquid at 26°C. A. avenae inoculum (added one week after B. cinerea inoculum).
10 ml. of A. avenae inoculum diluted with 90 ml. of water. 3 samples of 0.15 ml. of even suspension counted. 1 ml. of original suspension inoculated into each flask.

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	112	0	12	32	34	34
2	109	0	15	33	21	40
3	123	0	23	44	31	25
Total in 0.15 x 3	344	0	50	109	86	99
∴ Total in 1 ml. of original inoculum*	7644	0	1111	2422	1911	2200

* calculated to the nearest whole number.

contd./-

Table IX contd./-

Week 1 0.015 ml. of 600 ml. counted. 3 counts/replicate plate

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	38	24	13	0	0	1
2	45	28	16	1	0	0
3	45	28	14	1	1	1
4	35	20	15	0	0	0
5	39	18	17	1	2	1
6	36	16	15	3	0	2
Total in 0.15 x 6	238	134	90	6	3	5
Total in 600 ml.	158666	89333	60000	4000	2000	3333

Replicate 2

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	21	15	6	0	0	0
2	26	16	7	0	1	2
3	36	19	11	1	2	3
4	34	18	15	1	0	0
5	31	20	5	3	2	1
6	28	18	9	1	0	0
Total in 0.15 x 6	176	106	53	6	5	6
Total in 600 ml.	117333	70666	35333	4000	3333	4000

contd./-

Table IX contd./-

Replicate 3

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	46	8	35	0	0	3
2	59	14	38	3	0	4
3	47	9	30	2	2	4
4	53	12	31	6	1	3
5	49	12	30	3	2	2
6	35	3	27	3	0	2
Total in 0.15 x 6	289	58	191	17	5	18
Total in 600 ml.	192666	38666	127333	11333	3333	12000

Replicate 4

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	60	13	40	4	3	0
2	51	11	30	6	1	3
3	61	15	37	4	5	0
4	52	13	31	5	3	0
5	81	23	47	7	1	3
6	86	27	44	10	5	0
Total in 0.15 x 6	391	102	229	36	18	6
Total in 600 ml.	260666	68000	152666	24000	12000	4000

contd./-

Table IX contd.

Week 2

Replicate 1

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	37	10	24	2	0	1
2	50	15	31	4	0	0
3	30	7	22	1	0	0
4	45	16	26	0	1	0
5	56	25	26	3	0	2
6	55	22	32	0	0	1
Total in 0.15 x 6	273	95	163	10	1	4
Total in 600 ml.	182000	63333	108666	6666	666	2666

Replicate 2

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	26	6	15	1	0	4
2	23	3	20	0	0	0
3	18	7	10	0	1	0
4	20	4	16	0	0	0
5	18	5	11	0	2	0
6	32	10	15	3	1	3
Total in 0.15 x 6	137	35	87	4	4	7
Total in 600 ml.	91333	23333	58000	2666	2666	4666

contd./-

Table IX contd.

Replicate 3

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	28	12	10	2	1	3
2	33	13	12	3	2	3
3	25	10	7	4	1	3
4	35	15	11	5	2	2
5	33	11	15	1	2	4
6	35	16	9	3	3	4
Total in 0.15 x 6	189	77	64	18	11	19
Total in 600 ml.	126000	51333	42666	12000	7333	12666

Replicate 4

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	29	14	13	1	0	1
2	33	17	16	0	0	0
3	28	13	14	0	0	1
4	37	15	18	1	2	1
5	26	9	17	0	0	0
6	30	16	11	1	1	1
Total in 0.15 x 6	183	84	89	3	3	4
Total in 600 ml.	122000	56000	59333	2000	2000	2666

contd./-

Table IX contd./-

Week 3

Replicate 1

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	26	8	16	0	0	2
2	21	20	0	0	0	1
3	11	8	3	0	0	0
4	23	4	13	2	2	2
5	19	7	8	2	0	2
6	35	14	19	0	0	2
Total in 0.15 x 6	135	61	59	4	2	9
Total in 600 ml.	90000	40666	39333	2666	1333	6000

Replicate 2

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	46	15	28	0	2	1
2	31	14	15	0	2	0
3	20	5	13	2	0	0
4	15	9	5	1	0	0
5	29	9	17	0	1	2
6	29	13	14	1	0	1
Total in 0.15 x 6	170	65	92	4	5	4
Total in 600 ml.	113333	43333	61333	2666	2333	2666

contd./-

Table IX contd./-

Replicate 3

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	20	13	1	3	1	2
2	9	6	3	0	0	0
3	8	2	4	2	0	0
4	4	2	2	0	0	0
5	7	3	2	1	1	0
6	4	2	0	2	0	0
Total in 0.15 x 6	52	28	12	8	2	2
Total in 600 ml.	34666	18666	8000	5333	1333	1333

Replicate 4

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	18	5	10	3	0	0
2	19	17	1	1	0	0
3	12	8	4	0	0	0
4	11	4	5	1	0	1
5	9	4	5	0	0	0
6	6	3	3	0	0	0
Total in 0.15 x 6	75	41	28	5	0	1
Total in 600 ml.	50000	27333	18666	3333	0	666

contd./-

Table IX contd./-

Week 4

Replicate 1

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	53	25	22	4	1	1
2	43	23	14	5	0	1
3	15	7	6	2	0	0
4	26	7	16	1	1	1
5	14	7	6	1	0	0
6	14	6	6	1	0	1
Total in 0.15 x 6	165	75	70	14	2	4
Total in 600 ml.	110000	50000	46666	93333	1333	2666

Replicate 2

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	26	10	15	1	0	0
2	20	9	10	0	1	0
3	11	4	6	0	1	0
4	21	7	14	0	0	0
5	29	12	15	2	0	0
6	16	9	7	0	0	0
Total in 0.15 x 6	123	51	67	3	2	0
Total in 600 ml.	82000	34000	44666	2000	1333	0

contd./-

Table IX contd./-

Replicate 3

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	25	13	8	4	0	0
2	21	5	13	3	0	0
3	12	1	8	2	0	1
4	15	8	4	2	0	1
5	10	3	7	0	0	0
6	14	4	7	2	0	1
Total in 0.15 x 6	97	34	47	13	0	3
Total in 600 ml.	64666	22666	31333	8666	0	2000

Replicate 4

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	15	5	8	0	0	2
2	21	15	6	0	0	0
3	30	13	15	2	0	0
4	31	14	14	3	0	0
5	28	11	15	2	0	0
6	27	13	13	1	0	0
Total in 0.15 x 6	152	71	71	8	0	2
Total in 600 ml.	101333	47333	47333	53333	0	1333

contd./-

Table IX contd./-

Week 5

Replicate 1

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	4	0	3	0	0	1
2	10	5	4	0	1	0
3	13	5	4	2	1	1
4	6	2	1	1	1	1
5	8	5	0	0	1	2
6	14	8	4	2	0	0
Total in 0.15 x 6	55	25	16	5	4	5
Total in 600 ml.	36666	16666	10666	3333	2666	3333

Replicate 2

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	9	4	5	0	0	0
2	13	9	4	0	0	0
3	18	12	6	0	0	0
4	14	13	1	0	0	0
5	15	4	4	4	2	1
6	28	20	6	0	0	2
Total in 0.15 x 6	97	62	26	4	2	3
Total in 600 ml.	64666	41333	17333	2666	1333	2000

contd./-

Table IX contd./-

Replicate 3

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	15	11	2	2	0	0
2	8	4	3	0	0	1
3	20	12	7	0	0	1
4	16	9	7	0	0	0
5	12	8	0	2	0	2
6	20	12	4	3	0	1
Total in 0.15 x 6	91	56	23	7	0	5
Total in 600 ml.	60666	37333	15333	4666	0	3333

Replicate 4

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	6	4	0	0	0	2
2	5	1	4	0	0	0
3	17	9	7	1	0	0
4	16	10	4	2	0	0
5	16	7	8	1	0	0
6	14	8	5	1	0	0
Total in 0.15 x 6	74	39	28	5	0	2
Total in 600 ml.	49333	26000	18666	3333	0	1333

contd./-

Table 1X contd./-

Week 6

Replicate 1

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	12	10	1	0	0	1
2	23	13	6	4	0	0
3	25	9	6	6	1	3
4	15	8	4	1	0	2
5	28	16	7	2	0	3
6	15	9	1	1	4	0
Total in 0.15 x 6	118	65	25	14	5	9
Total in 600 ml.	78666	43333	16666	9333	3333	6000

Replicate 2

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	34	20	5	0	4	5
2	32	17	11	0	0	4
3	26	8	13	0	0	5
4	20	5	11	0	1	3
5	27	17	4	2	1	3
6	16	5	5	1	2	3
Total in 0.15 x 6	155	72	49	3	8	23
Total in 600 ml.	103333	48000	32666	2000	5333	15333

contd./-

Table IX contd./-

Replicate 3

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	11	11	0	0	0	0
2	16	15	1	0	0	0
3	14	12	1	1	0	0
4	14	12	2	0	0	0
5	26	20	4	1	0	1
6	18	16	1	0	0	1
Total in 0.15 x 6	99	86	9	2	0	2
Total in 600 ml.	66000	57333	6000	1333	0	1333

Replicate 4

Count no.	Total	Eggs	L_2	L_3	L_4	Female
1	38	6	31	1	0	0
2	38	14	23	1	0	0
3	36	16	19	0	1	0
4	31	16	12	3	0	0
5	46	6	34	5	0	1
6	25	8	15	2	0	0
Total in 0.15 x 6	214	66	134	12	1	1
Total in 600 ml.	142666	44000	89333	8000	666	666

APPENDIX

Table X

Population fluctuation over 4 weeks of *A. avenae* on *B. cinerea* on PDB at 26°C. One fourth stage larva inoculated 2 weeks after *B. cinerea* inoculum. First reading taken 2 weeks after *A. avenae* inoculum.

Week 2 - Whole extract of 3 replicate plates counted.

Replicate	Eggs	L_2	L_3	L_4	Female	Totals
1	463	640	146	92	38	1379
2	324	630	138	111	89	1292
3	402	683	129	89	44	1347
Total	1189	1953	413	292	171	4018
Mean/plate	396.3	651.0	137.7	97.3	57.0	1339.3

Week 3 - 5 ml. of 150 ml. of extract counted.
3 counts/replicate plate.

Plate 1

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	27	110	49	78	51	-
2	27	112	56	58	52	-
3	17	139	38	60	62	-
Total count	71	361	143	196	165	-
∴ total in plate	710	3610	1430	1960	1650	9360

contd./-

Table X contd./-

Plate 2

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	116	127	36	27	9	-
2	98	111	30	22	7	-
3	85	97	35	15	7	-
Total count	299	335	101	64	23	-
∴ total in plate	2990	3350	1010	640	230	8220

Plate 3

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	6	64	2	1	1	-
2	2	39	3	2	0	-
3	7	11	3	3	2	-
Total count	15	114	8	6	3	-
∴ total in plate	150	1140	80	60	30	1460

	Eggs	L_2	L_3	L_4	Female	Total
∴ total in 3 plates	3850	8100	2520	2660	1910	19040
∴ Mean	1283.3	2700.0	840.0	886.7	636.7	6346.7

contd./-

Table X contd./-

Week 4 - 5 ml. of 150 ml. of extract counted.
3 counts/replicate plates.

Plate 1

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	18	157	13	15	8	-
2	29	227	24	19	13	-
3	28	285	11	29	13	-
Total count	75	669	48	63	34	-
∴ total in plate	750	6690	480	630	340	8890

Plate 2

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	9	286	5	8	9	-
2	10	300	12	8	1	-
3	4	299	21	14	8	-
Total count	23	885	38	30	18	-
∴ total in plate	230	8850	380	300	180	9940

Plate 3

Count no.	Eggs	L_2	L_3	L_4	Female	Total
1	6	299	136	94	52	-
2	28	180	125	66	24	-
3	13	158	112	47	34	-
Total count	47	637	373	207	110	-
∴ total in plate	470	6370	3730	2070	1100	13740

contd./-

Table X contd./-

	Eggs	L_2	L_3	L_4	Female	Total
∴ total in 3 plates	1450	21910	4590	3000	1620	32570
∴ Mean	483.3	7303.3	1530.0	1000.0	540.0	10856.7