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# RESEARCH MEMORANDUM

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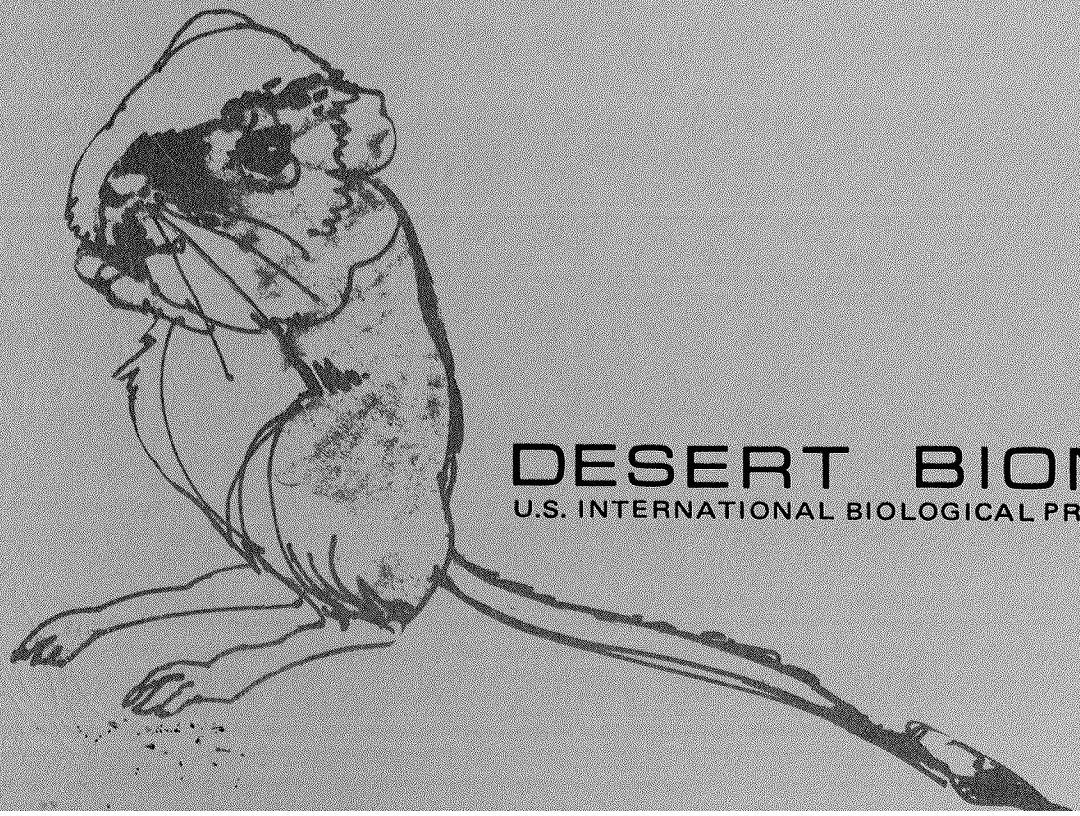

DESERT BIOME

U.S. INTERNATIONAL BIOLOGICAL PROGRAM

1971 PROGRESS REPORT

# BIOLOGY OF NEMATODES IN DESERT ECOSYSTEMS

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# ABSTRACT

Nematode extraction from soil was demonstrated to require several methods to accurately study the populations. Sugar flotation was the best general method and could be performed with 98-99% efficiency by recycling suspensions through sieves up to four times. For practical reasons a procedure with two wash cycles was adopted which consistently gave 75% recovery. During dry periods nematodes are under severe osmotic stress and the specimens recovered by sugar flotation are frequently in poor condition. Processing by Baermann funnel allows eggs to hatch and accentuates those groups with large dormant egg reservoirs in the soil such as the Cephalobidae, but it offers very poor extraction of Dorylaimidae. Good specimens for taxonomic purposes require the use of the laborious Cobb sieving-decanting technique.

Nematode biomass was derived from averaged counts/500cc soil of 20 spaced samples in a square hectare grid. These counts were multiplied by the calculated average wt./nematode in the population and adjusted to the soil volume in a hectare area 20 cm deep. Biomass estimations ranged from 0.93 to 2.2 kg/ha in desert soil during the dry season. Populations in the same area of a humid soil were not a great deal larger when measured by similar techniques.

Analysis of standard deviations of measurements of random nematodes from various size groups within a population indicated a random population of from 50-100 would be most representative of a given population.

Monthly sampling of sites in Deep Canyon Desert Research Center indicated great variability in nematode populations and strong seasonal influences related to precipitation. Adequate information on plant parasitic species could not be developed thus far due to extreme drought conditions in the areas studied.

Total nematodes and particularly plant-parasitic and predacious species were more concentrated around clumps of *Larrea* than in intervening nonvegetation areas in the Mohave Desert study site. The artificial addition of moisture had only short-term effects on the population. In a laboratory study nematodes reproduced rapidly in areas of decomposing organic matter and adequate moisture.

# INTRODUCTION

This study was a continuation from the 1971 project funded beginning April, 1971. Originally categorized as a methodological study, it has assumed many aspects of a process study as methods were developed to efficiently and quantitatively extract nematodes from desert soil. The size variation in the soil Nematoda is somewhat analagous to that of shrews and elephants in the Mammalia. Therefore, a combination of methods are required to study the entire nematode fauna to best advantage.

Studies in 1972 were limited almost entirely to areas in the Mohave and Colorado deserts, although some faunistic sampling was done outside these regions. These deserts were close at hand and more convenient than working on some of the validation sites, as originally proposed. They also represent areas with extreme arid conditions which were believed to focus the characteristics of the desert soil environment on the populations encountered there.

Nematodes represent an ubiquitous life form with remarkable adaptation to desiccation in many soil-inhabiting species. They are believed to be a major soil reservoir of cell carbon and nitrogen, particularly in desert soils. The majority of free-living arid soil forms feed on the primary decomposers, bacteria and fungi. This report includes estimations of nematode biomass obtained mainly from desert soils in a drought cycle, and explains methods which most accurately measure biomass.

# OBJECTIVES

The main objectives for 1972 were to complete studies initiated in 1971 on techniques best suited for quantitative analysis of desert soil-inhabiting nematodes, and to survey the nematode fauna of representative desert areas in order to best describe characteristics of these populations as to species, trophic groups, spatial relationships, and the influence of flora and soil type. The development of information on the role of various nematode species and/or trophic groups in desert soil biology, especially in the mineralization of organic matter and other important processes, was also a primary objective.

# METHODS

After additional studies and comparisons were conducted on various collection and extraction methods (see Data Set A3UMB32), the recovery of free-living nematodes from desert soils for studies during 1972 was accomplished mainly by two methods: 1) a modification of the Baermann funnel (BF) technique (Southey, 1970) in which 50 cc of soil is placed on a layer of tissue paper over a wire screen in a long-stemmed funnel for 48 hr; and 2) a modification of the sugar flotation (SF) method of Byrd, Nusbaum and Barker (1966) in which nematodes in a 500 cc soil sample are separated by mixing with a 1.0M sucrose solution and Separan 2610 (Dow Chemical Co., Midland, Michigan). A number of variations of the sugar flotation technique were investigated to overcome relatively poor recovery of nematodes from desert soils as compared to high recovery on typically agricultural soils. These variations included different concentrations of sucrose and Separan, the use of $MgSO_4$, different lengths of exposure to stirring in sucrose solutions, and repetitive processing of the same sample. To obtain nematodes in the best possible condition for fixation and taxonomic study, a modification of the Cobb sieving-decanting method (Southey, 1970) was used. Methods for processing nematodes to permanent mounts were the same as reported in 1971 (RM 72-12).

Nematode populations obtained from soil were usually fixed and then portions processed to permanent glycerine mounts. After sufficient study of specimens with oil immersion objectives at 1000x or 2000x magnification, living specimens could be recognized at lower magnifications (250x), which allowed more rapid counts of species and trophic groups. Examination and counting were on live populations since nematode behavior and activity could be utilized advantageously in recognizing species. This was done by placing aliquot suspensions in Hawksley nema counting slides (Southey, 1970), plankton counting slides, or in plastic grid dishes prepared in the laboratory.

Nematode biomass was derived from an estimate of the average number of nematodes per 500 cc soil sample in a square hectare to a depth of approximately 20 cm, and from an estimate of average nematode weight within the population by the formula:

$$\text{Biomass (kg/ha)} = \frac{\text{(av. wt per nema, mg) (av. no. per sample)} \times 100}{\text{(av. surface area per sample, cm)}}$$

Nematode numbers were determined by averaging numbers of nematodes extracted by either the sugar flotation (SF) technique and/or the Baermann funnel (BF) technique from each of 20 soil samples (500 cc each) of approximately 20 cm depth. The samples were taken at 10 m intervals along an east-west and a north-south transect within a sq ha (Fig. 1).

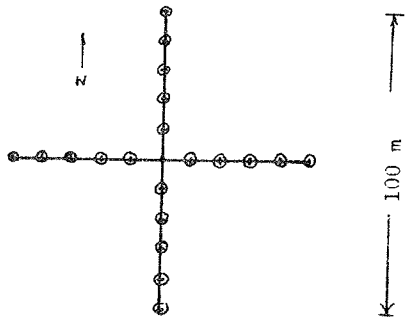


Figure 1. Pattern of sampling along 4 transects.

Average nematode weight was determined by using a microscope camera lucida at a magnification of 500x to measure the length and width of a minimum of 25 nematodes selected randomly from the pooled samples. Experiments were conducted to determine the number of nematode measurements required to be representative of the population at P = 0.05 by analyzing various replicate measurement sets for Standard Deviation from the Mean. Average wt/nematode was determined by a formula developed by Andrassy (1956):

$$G = \frac{w^2 \times L}{16 \times 100{,}000}$$

where w is the greatest body width (μ), L the body length (μ) and 16, an empirical value based on a geometric approximation of a nematode body. The factor 100,000 gives the weight (G) directly in mg.

On the Deep Canyon Desert Research Center, ten sites with different flora, elevation and topographic characteristics were selected for monthly study of the nematode fauna (Data Set A3UMB01). The sites constitute areas of about 1 sq m. The soil at all of the sites is extremely rocky and received a preliminary coarse screening at all samplings. Soil texture (particle size analysis) was measured by the hydrometer method (Day, 1965). Soil pH was measured by a standard glass electrode pH meter (Peech, 1965). The nematode population was extracted by the sugar flotation method and occasionally by supplementary methods mentioned above. The population was determined per 500 cc of soil and the species present categorized according to trophic groups, e.g., microbial feeders, fungal feeders, predators, and plant parasites. A study to establish species-volume relationships was undertaken near one site (#6) by taking five samples consisting of 1, 2, 4, 8, and 16 cores of soil, respectively, from a circular plot approximately 3 m in diameter in triplicate. Cores were approximately 6.5 cm diam. by 10 cm deep (approx. 300 cc). Samples were extracted separately by the sieving-decanting method. Material from the #100 and #325 mesh sieves was placed on filter paper over water and the nematodes collected after 24 hrs.

An experiment to determine the effect on the nematode fauna of irrigating or moistening desert soil was set up at a site in the Mohave Desert (Victor Valley) near Adelanto, California (Data Set A3UMB34). Eight steel cylinders about 60 cm diameter and 32 cm deep were set a few inches into the soil so that four cylinders encircled small clumps of creosote bushes and four were on soil without permanent plants. The area within the cylinders received water equivalent to about 1 in. rainfall in a period prior to the onset of normal winter rainfall. Similar areas near the cylinders served as controls.

Victor Valley has a 45 yr average annual rainfall of 5.25 in. During the latter half of 1972 the area received the following rainfall: Aug. - 0.15 in., Sept. - 0.05 in., and Oct. - 1.03 in. The soil at the test site is a sandy loam with an average of 14% clay, 74% sand and 11% silt. Soil pH is 8.1 in creosote bush clumps and 8.5 in bare ground sites.

For a preliminary experiment on the relationship of nematodes to decomposing organic matter or litter in desert soils, a collection of soil was obtained from Joshua Tree National Monument and a portion washed and sieved (Data Set A3UMB35). Organic matter collected on #20, 100 and 325 standard mesh screens was removed and dried. The organic matter from the soil was added to 3 X 20 cm glass tubes in a ratio of 2 cm litter to 12 cm of soil in four treatments of five replicates each. Treatment A had the 2 cm of organic material at the top of the column, treatment B at the center of the column, and treatment C at the bottom. Treatment D was an unamended control series. The columns were moistened by capillary action, covered with aluminum foil, and incubated at room temperature with occasional moistening to prevent drying. After 30 days, equal volumes of soil were pushed from the bottom of all columns and the nematodes were extracted by SF. Because of variability and irregularities in soil compactness in the columns, each portion of removed soil was weighed and the nematodes recovered were calculated to numbers per 100 g.

# RESULTS

## Collection methods

A number of methods involving moistening soil samples in the field during collection were attempted on the assumption that the disturbance involved in the collection of the sample disrupted the delicate moisture relationships in the pore

spaces of extremely dry desert soils. However, results indicated that field moistening or even moistening in the lab for short periods prior to processing produced no greater recovery of nematodes than returning samples to the laboratory in plastic bags, with care to prevent overheating, and then holding in a cold room until processed. Soil samples were never stored for longer than a few days.

Extraction methods

Comparisons between the Cobb sieving-decanting method, the sugar flotation method and the Baermann funnel method indicated that the largest numbers of nematodes were obtained by the latter method while the two former methods were roughly comparable. The sieving-decanting method, however, is very time consuming and subject to variability according to the skill of the operator. The SF method is rapid, simple and easily reproducible. Each method has certain disadvantages. Larger nematodes and certain species are usually not recovered well by BF, whereas eggs present in the soil frequently hatch and large numbers of small larvae are obtained. The sugar flotation method, when utilized on desert soils, frequently results in nematodes which are in extremely poor condition, due apparently to osmotic shock by the sucrose solution on nematodes which are probably already under severe osmotic stress. Nematodes extracted from normally moist soils are in much better condition when extracted by the sugar flotation method. The method also recovers dead and inactive nematodes in the soil.

In a test to determine efficiency of extraction with SF by four consecutive washings of each of ten replicate samples from both a desert soil and local field soils, it was observed that 75% of the nematodes present in a 500 cc soil sample were consistently extracted in the first 2 washings (Table 1). Additional information from experimental variation of the method resulted in the adoption of a standard practice for this method in which a 500 cc soil sample was processed twice in a 1.0M sucrose solution (242 g/liter) plus "Separan", stirred for 20 sec during each processing and the material collected on a #325 mesh (44μ) sieve.

Table 1. Percent recovery of total nematodes by the sugar flotation method in four consecutive washings of ten 500 cc samples of a desert soil and two field soils

| | 1st Wash | 2nd Wash | 3rd Wash | 4th Wash |
|---|---|---|---|---|
| Colorado Desert Soil | 42.2 | 34.4 | 16.4 | 7.0 |
| Mohave Desert Soil | 31.3 | 40.5 | 18.7 | 8.2 |
| Field Soil A | 44.3 | 31.2 | 15.6 | 8.6 |
| Field Soil B | 42.1 | 32.1 | 16.0 | 9.6 |

Deep Canyon

The nematode fauna study in Deep Canyon was carried out mainly at the ten individual sites within the area of the Center. Physical characteristics of the soil at these sites are given in Table 2 and the dominant vegetation in Table 3. Air and soil temperatures typical of summer extremes were recorded for the area during a 1-week period in August. The ambient air temperature peaks (low-25 C, high-44 C) occurred 3 hr prior to those of soil temperature at 10 cm, which were lowest (27.5 C) at 6-9 p.m. and highest (42.5 C) at 4-6 p.m. Soil at 30 cm and 60 cm had a narrow range of 34-37 C. Deep Canyon has a mean maximum air temperature (July) of 41 C and a mean minimum temperature (January) of 3.9 C. Maximum recorded temperature was 51.5 C.

Table 2. Physical characteristics of sample sites in Deep Canyon Desert Research Center

| Site # | pH | % clay | % sand | % silt | texture classification |
|---|---|---|---|---|---|
| 1 | 7.7 | 11 | 65.2 | 23.8 | sandy loam |
| 2 | 8.3 | 11 | 70.4 | 18.6 | sandy loam |
| 3 | 8.2 | 11 | 76.5 | 12.5 | sandy loam |
| 4 | 8.0 | 11 | 76.5 | 12.5 | sandy loam |
| 5 | 8.5 | 12.5 | 84.6 | 2.9 | loamy sand |
| 6 | 8.1 | 12.5 | 84.2 | 3.3 | loamy sand |
| 7 | 8.4 | 12.5 | 88.5 | 0 | loamy sand |
| 8 | 8.3 | 11 | 73.2 | 15.8 | sandy loam |
| 9 | 8.0 | 17.6 | 63.4 | 19.0 | sandy loam |
| 10 | 8.3 | 14.2 | 47.5 | 38.3 | loam |

Table 3. Dominant vegetation at ten Deep Canyon test sites

| Site Number | Vegetation |
|---|---|
| 1 | *Opuntia bigelovii* |
| 2. | *Encelia farinosa, Ambrosia dumosa, Echinocactus acanthodes* |
| 3 | *Larrea divaricata, A. dumosa, Opuntia rammosissima* |
| 4. | *Larrea divaricata* |
| 5 | *Acacia gregii* |
| 6. | *Dalea spinosa* |
| 7. | *Cercidium floridum* |
| 8. | *Fouquieria splendens* |
| 9. | *Acacia gregii* |
| 10 | *Chilopsis linearis* |

Monthly sampling of nematode populations at the ten $m^2$ sites in Deep Canyon began in January, 1972, during a season of exceedingly low precipitation. The total nematode counts at each site were highly variable and related to the microenvironment at each site. The composition of each population was of major interest and data gathered about the species present were organized to reflect numbers present in various trophic groups. Figure 2 illustrates seasonal distribution of the three major trophic groups at each site, based on the percentage of the total number of nematodes in each of the trophic groups observed at that site during the entire series of samples. The area had only one substantial rainfall of 11.2 mm during the 1971 season, which fell in Dec. (Table 4). That precipitation apparently accounted for the increased numbers of microbial- and fungal-feeding nematodes observed in January, 1972, but enough moisture may not have been present to activate the larger predacious nematodes. Deep Canyon has an average annual rainfall of 82.5 mm (3.25 in) but annual rainfall in 1971 amounted to only 34.8 mm (1.37 in). Precipitation in June, 1972, was reflected in a slight population increase while very low numbers occurred during the hot and desiccated late summer. The onset of early rains in late August and September brought a substantial increase in all groups in the October sampling. Site #6 was disturbed by flash flooding in October and relatively few nematodes were recovered at that time.

Table 4. Deep Canyon monthly precipitation, in inches

| | 1971 | 1972 |
|---|---|---|
| Jan. | 0.12 | --- |
| Feb. | 0.07 | --- |
| Mar. | 0.07 | --- |
| Apr. | 0.03 | 0.02 |
| May | 0.14 | --- |
| Jun. | --- | 0.35 |
| Jul. | 0.28 | --- |
| Aug. | 0.14 | 0.52 |
| Sept. | --- | 0.71 |
| Oct. | 0.08 | 0.39 |
| Nov. | --- | 1.06 |
| Dec. | 0.44 | (0.37) through 12/22 |

The population categorized as microbial feeders are all species in the Cephalobidae. At least one species in the Rhabditidae is known to occur in these populations but it has been observed only upon incubation of organic soil detritus in the laboratory and

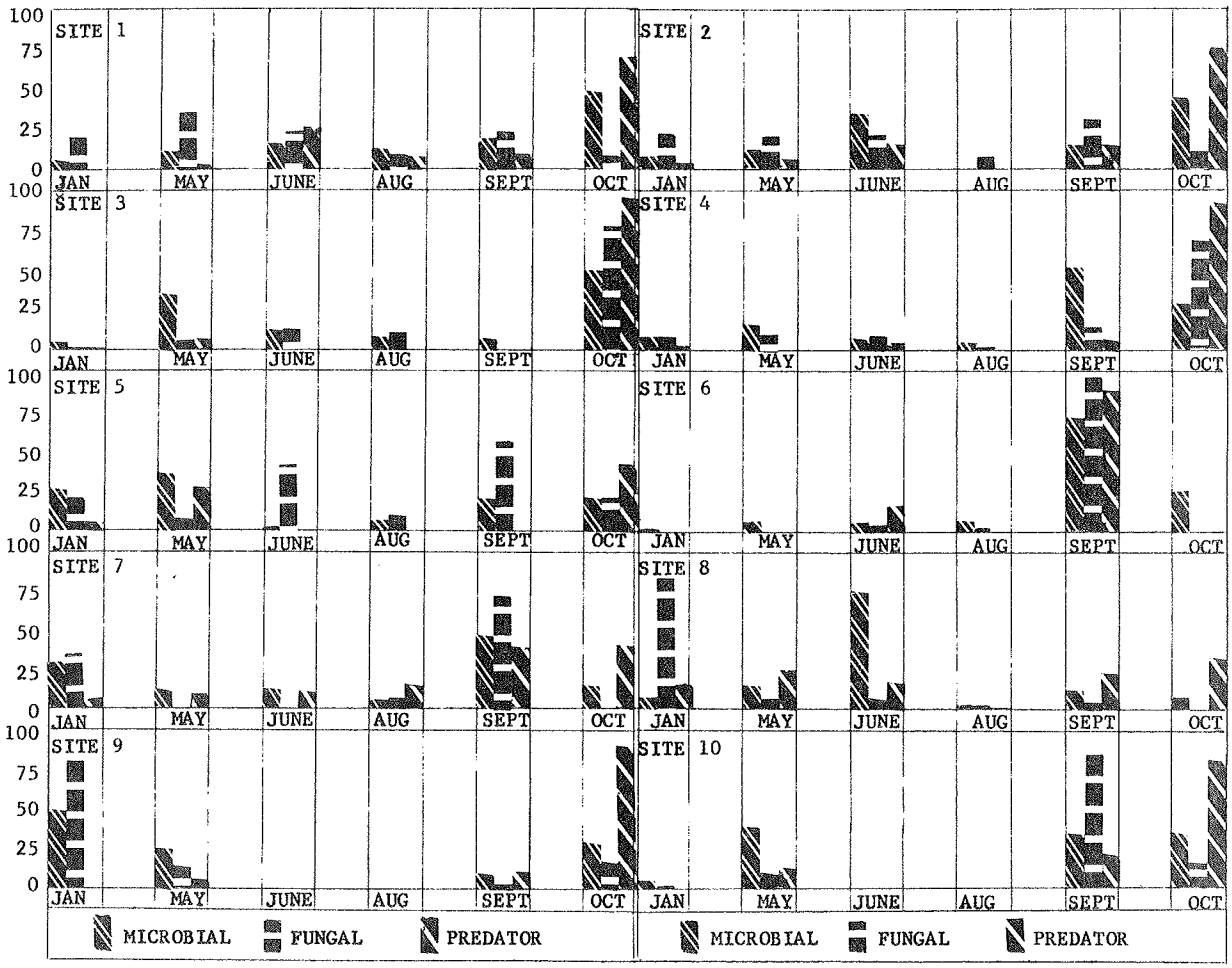


Figure 2. Seasonal distribution of nematodes in three major trophic groups at the ten study sites in Deep Canyon. Bars represent the percentage of the total number of nematodes in each of the trophic groups observed at that site during the entire series of samples.

not in populations in the field. *Ditylenchus* sp., *Aphelenchus avenae* and a pathogenic species of Aphelenchoides are the major elements of the fungus-feeding group, while *Eudorylaimus monohystera* and *E. diminitivus*, one of the smallest Dorylaiminae

known, are the major forms in the predacious group. Both of the latter species may have a somewhat omnivorous habit since they have been observed to feed on hyphae of soil fungi as well as nematodes in agar plates in the laboratory.

Plant parasitic nematodes are extremely sparse in the samples and have occurred only at certain sites. *Tylenchorhynchus acutus* is the most abundant species observed thus far, but *Paratylenchus* sp. and *Meloidogyne* sp. also occur in the area and are expected to become more abundant during the winter rains. *Meloidogyne* sp. has been observed to reproduce on *Larrea divaricata* in the greenhouse, but this association has not been confirmed at Deep Canyon.

At a sampling made in October, 1972, soil samples from each of the ten sites were processed by both BF and SF and the population counted as to nematode species present in each method. Table 5 lists the counts of the most numerous and consistently present species. The data indicate the variability which occurs between sites and that, on an average basis, BF favors recovery of Cephalobidae, SF recovers Dorylaiminae, which are not even recorded by the BF method, and both methods have similar results with *Aphelenchoides* sp.

An estimate of nematode biomass at the end of the dry season at this location was conducted purposely in an area representing an extreme desert niche (a dry wash with little vegetation) located near test site #6. Only the standard SF extraction was used (75% efficiency). The nematode population was made up almost entirely of a few species in the Cephalobidae and the fungus feeders, *Aphelenchoides* sp. and *Ditylenchus* sp. Other types were extremely sparse. The biomass was recorded as 0.7 kg/ha (Table 6), which corrected for efficiency of extraction represents a probable active biomass of about 0.9 - 1.0 kg/ha. The data are compared with other biomass estimations in Table 9.

Species-volume sampling was also conducted near site #6. Extraction by SF of three replicate 500 cc aliquots from each of the soil samples containing 1, 2, 4, 8, and 16 cores respectively, indicated that soil samples made up of at least 16 individual cores were more efficient than those with fewer cores (Table 7).

Observations on the number of individual species in samples of different numbers of individual cores were made by the Cobb sieving-decanting method due to the necessity of obtaining perfect living nematode specimens to facilitate identification of species. Results indicated (Table 7) that the 16-core samples were also more representative of the total nematode population than smaller numbers of cores/sample. A 24-core sample (combination of an 8-core and 16-core sample) did not increase the number of species observed.

Table 5. Percentage of dominant nematode types extracted by Baermann funnel and sugar flotation methods from Deep Canyon soils at ten test sites

| Site # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Avg. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Cephalobidae | | | | | | |
| Baermann funnel | 66 | 67 | 85 | 62 | 100 | 95 | 95 | 63 | 63 | 69 | 76% |
| sugar flotation | 59 | 72 | 32 | 27 | 66 | 100 | 83 | 10 | 37 | 78 | 56% |
| | | | | | Dorylaiminae | | | | | | |
| Baermann funnel | -- | -- | -- | 8 | -- | -- | 5 | 5 | 19 | 19 | 5% |
| sugar flotation | 32 | 13 | 49 | 51 | 16 | -- | 16 | 50 | 45 | 1 | 27% |
| | | | | | *Aphelenchoides* sp. | | | | | | |
| Baermann funnel | 11 | 24 | 15 | 27 | -- | -- | -- | 33 | 13 | 22 | 14% |
| sugar flotation | 3 | 8 | 9 | 19 | 16 | -- | -- | 40 | 14 | 2 | 11% |

Table 6. Nematode numbers/500 cc sample, Colorado Desert, Deep Canyon Desert Research Center, derived from 4 transects

| Transect | Nematode numbers by SF extraction Sample Number | | | | | Transect Mean |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| N | 4020 | 320 | 60 | 120 | 380 | 980 |
| S | 140 | 100 | 160 | 40 | 140 | 116 |
| E | 2860 | 120 | 500 | 40 | 420 | 788 |
| W | 240 | 500 | 120 | 60 | 120 | 208 |

Transect Center 400

Avg. no nemas/500 cc = 450

Estimated avg. wt./nema = $3.75 \times 10^{-4}$mg (n=50)

Biomass = 0.7 kg/hectare

Table 7. Total numbers of nematodes/500 cc soil in samples of different numbers of cores

| No. of Cores | 1 | 2 | 4 | 8 | 16 |
|---|---|---|---|---|---|
| Rep. 1 | 60 | 200 | 180 | 260 | 580 |
| 2 | 60 | 140 | 80 | 280 | 480 |
| 3 | 120 | 300 | 180 | 360 | 500 |
| Avg. | 80 | 213 | 136 | 300 | 520 |

Average number of nematode species observed/500 cc

| No. of Cores | 4 | 8 | 16 | 24 |
|---|---|---|---|---|
| | 1-3 | 3-4 | 5-6 | 5-6 |

An additional biomass sampling was made on the north slope of Seven Palms Valley in the Colorado Desert during drought conditions (Table 8). Both BF and SF methods were compared and the data clearly indicated the typically smaller average nematode size with increased total numbers obtained with the former method; while with SF, average nematode size was larger but total numbers were reduced. Biomass was approximately the same by either method. A few individual locations accounted for the high average total numbers with the BF method (e.g. N-2, N-4, E-3 and W-1), which may be due to large numbers of unhatched eggs in a very spotty distribution.

Victor Valley, Mohave Desert

The Victor Valley study site in the Mohave Desert is typical of the high desert region with uniform creosote bush - burro bush vegetation and a scattering of Joshua trees. The soil layer is thin, not exceeding 20 cm over most of the area. Nematode biomass was estimated toward the end of the dry season by two extraction methods (Table 10). Observed biomass was 1.7 kg/ha with the BF technique while with the SF method a biomass of 0.93 kg/ha was observed (Data Set A3UMB33). The latter technique extracts 75% of the nematodes present at the time of extraction (Table 1), therefore the actual biomass can be calculated to be approximately 1.2 kg/ha. The BF technique allows dormant eggs to hatch and possibly revives some forms with larvae tolerant to desiccation. Large numbers of recently-hatched first stage larvae are present in the counts with this method, whereas some larger species, particularly in the Dorylaiminae, are not efficiently extracted by this method. Large Dorylaiminae are not abundant at this site and their absence from data on total numbers is unimportant.

Table 8. Nematode numbers/500 cc soil, Colorado Desert: north slope, Seven Palms Valley site (Accession #573), transect samples, no. nematodes/500 cc soil

A. Baermann Funnel Extraction

| Transect | Sample Number 1 | 2 | 3 | 4 | 5 | Transect Mean |
|---|---|---|---|---|---|---|
| N | 1870 | 3760 | 1800 | 5000 | 2760 | 3038 |
| S | 1310 | 1800 | 280 | 1580 | 450 | 1084 |
| E | 2440 | 2030 | 5520 | 1490 | 2220 | 2740 |
| W | 3490 | 740 | 490 | 2610 | 1720 | 1810 |

Avg. no. nemas/500 cc = 2168

Estimated avg. wt./nema = $1.035 \times 10^{-4}$mg (n = 38)

Biomass = 0.9 kg/hectare

B. Sugar Flotation Extraction

| Transect | Sample Number 1 | 2 | 3 | 4 | 5 | Transect Mean |
|---|---|---|---|---|---|---|
| N | 1780 | 1940 | 1840 | 1820 | 1400 | 1756 |
| S | 1040 | 800 | 220 | 560 | 340 | 592 |
| E | 1780 | 1080 | 2580 | 840 | 1340 | 1524 |
| W | 2500 | 660 | 360 | 540 | 1820 | 1176 |

Avg. no. nemas/500 cc = 1262

Estimated avg. wt./nema = $1.835 \times 10^{-4}$ (n = 38)

Biomass = 0.93 kg/hectare

Table 9. Comparisons of nematode numbers, average weight, and biomass per hectare (to 20 cm depth) between desert sites and an irrigated agricultural field (DSCODES A3UMB33, A3UMB34)

| | Baermann Funnel Extraction | | | Sugar Flotation Extraction | | |
|---|---|---|---|---|---|---|
| | A. Nemas/500cc | B. Avg. Wt. per nema (mg) | C. Biomass (kg/ha) | A. Nemas/500cc | B. Avg. wt. per nema (mg) | C. Biomass (kg/ha) |
| Mohave Desert Site | 2820 | $1.5 \times 10^{-4}$ | 1.7 | 902 | $2.6 \times 10^{-4}$ | 0.9 |
| Colorado Desert site | 2168 | $1.0 \times 10^{-4}$ | 0.9 | 1262 | $1.8 \times 10^{-4}$ | 0.9 |
| Deep Canyon R. C. | -- | -- | -- | 450 | $3.75 \times 10^{-4}$ | 0.7 |
| Irrig. agric. field | 4174 | $0.9 \times 10^{-4}$ | 1.5 | 2124 | $2.0 \times 10^{-4}$ | 1.6 |

The difference in biomass observed by the two methods indicates a substantial reservoir of nematodes which survive the highly desiccated soil of the dry season as dormant eggs.

The total nematode fauna of this site was very carefully analyzed and found to contain the following species (see Data Set A3UMB34):

Aphelenchoidea

*Aphelenchoides* sp.
*Aphelenchus avenae*

Rhabditoidea

*Acrobeles* sp.
*Acrobeloides* sp.
*Cervidellus* sp.
*Chiloplacus* sp.
*Elaphonema* (sp. nov.)
*Zeldia* sp.

Tylenchoidea

*Ditylenchus* sp.
*Meloidogyne* sp.
*Pratylenchus* sp.
*Tylenchorhynchus* sp.
*Tylenchorhynchus cylindricus*

Dorylaimoidea

*Eudorylaimus* sp.
*Eudorylaimus monohystera*

The nematode population from the Victor Valley site in the Mohave desert was used in tests to determine the smallest number of nematode measurements (length and width) required for a reasonably accurate estimate of biomass. Mean measurements of five groups of 10 nematodes were compared with the mean length and width of 50 nematodes and were found to vary considerably beyond the Standard Deviation of the population means (n = 50) at P = 0.05. Mean measurements of five random groups of 25 nematodes as compared to measurements of a population of 125 nematodes showed four groups with mean lengths with the Standard Deviation (n = 125) at P = 0.05, and one group with a mean length not representative of the larger population (Figure 3). It is clear that groups larger than 25 random nematodes must be measured for accurate biomass determination.

The calculated difference between the high and low range of the Standard Deviation from the population mean length and width at P = 0.05 for a population of 50 random nematodes converted to biomass was 0.4 kg/ha, while the difference for a random population of 125 nematodes was 0.36 kg/ha. Measurement of from 50 to 100 random nematodes should therefore provide reasonably reliable estimates of biomass in desert populations. Further verification of these results is required.

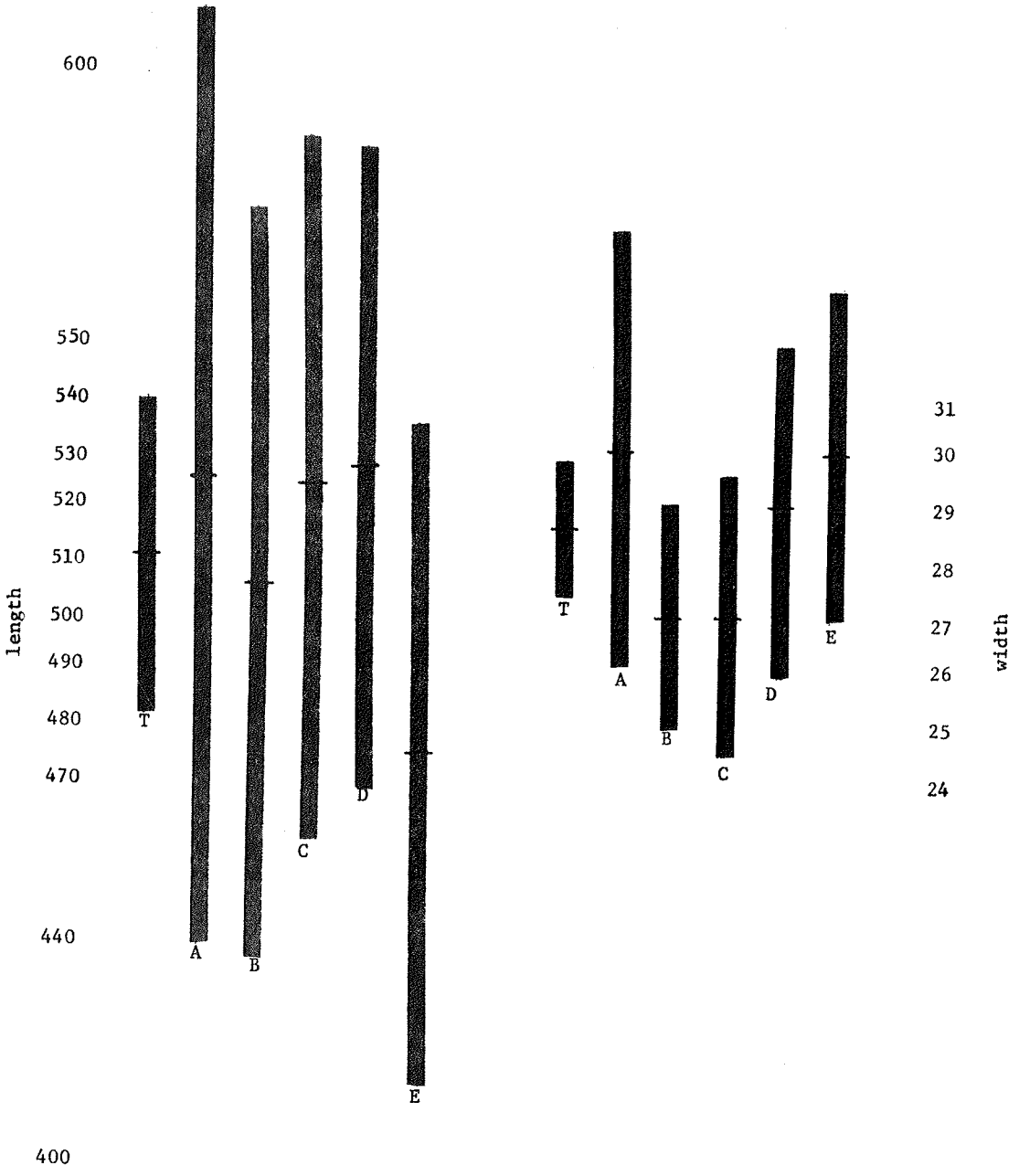


Figure 3. Comparison of Standard Deviations from mean length ($\mu$) at P = 0.05 between five groups of 25 random nematodes (A-E) and a group of 125 random nematodes from the same population.

Table 10. Nematode numbers/500 cc soil, Mohave Desert, upper Victor Valley (Accession #572)

A. Baermann Funnel Extraction

| Transect | Sample Number 1 | 2 | 3 | 4 | 5 | Transect Mean |
|---|---|---|---|---|---|---|
| N | 3490 | 2680 | 5820 | 2960 | 4550 | 3900 |
| S | 3190 | 1890 | 4140 | 4600 | 740 | 2910 |
| E | 3560 | 1330 | 1170 | 4400 | 1880 | 2460 |
| W | 2340 | 1050 | 3600 | 2590 | 1990 | 2310 |

sample 00 = 1170
Avg. no. nemas/500 cc = 2820
Estimated avg. wt./nema = $1.5 \times 10^{-4}$mg
Biomass = 1.7 kg/hectare

B. Sugar Flotation Extraction

| Transect | Sample Number 1 | 2 | 3 | 4 | 5 | Transect Mean |
|---|---|---|---|---|---|---|
| N | 9200 | 700 | 1340 | 1000 | 840 | 960 |
| S | 1060 | 580 | 480 | 1340 | 340 | 760 |
| E | 1480 | 260 | 400 | 1360 | 700 | 840 |
| W | 560 | 1240 | 1380 | 1300 | 380 | 972 |

sample 00 = 1300
Avg. no. nemas/500 cc = 902
Estimated avg. wt./nema = $2.6 \times 10^{-4}$mg
Biomass = 0.93 kg/hectare

The experiment on the effect of simulated rainfall on the nematode population in soil around the bases of *Larrea divaricata* and intervening non-vegetation areas compared with untreated check areas indicated that nematode numbers increase for at least three weeks after water is added, and then decline somewhat (Table 11). Nematodes are much more abundant around the bases of *Larrea* than in the non-vegetation areas on both control and moistened sites.

Table 11. Total nematodes/500 cc in artificially moistened microplots in soil around creosote bushes and in non-vegetation areas, compared with untreated check areas

| Treatment | Days after 1" simulated rainfall | | |
|---|---|---|---|
| | 6 | 21 | 45 |
| 1. *Larrea*-irrigated | 1530 | 3755 | 1165 |
| 2. *Larrea* check | 920 | 3495 | 1815 |
| 3. Non-vegetation irrig. | 790 | 2370 | 545 |
| 4. Non-vegetation check | n.d. | 1260 | 990 |

Analysis of populations according to trophic groups at 6, 21 and 45 days after addition of water, indicated that the fungal-feeding nematodes were not affected by the addition of moisture. Plant parasitic nematodes, which in this population consisted almost entirely of two species of *Tylenchorhynchus*, gradually increased in percentage over 45 days where water was added to *Larrea* clumps but remained relatively constant around *Larrea* without water. The *Tylenchorhynchus* spp. appear to be active throughout the dry season and very likely have an intimate association with the creosote bush. Two other plant-parasitic species, *Pratylenchus* sp. and *Meloidogyne* sp. were observed sporadically in very small numbers. These species apparently become active only during the winter rainfall season and may be associated with annuals. Microbial-feeding species in the Cephalobidae are consistently the largest group in the population. A new species of *Elaphonema*, heretofore a monospecific genus reported only from South Africa, was discovered in this population. A substantial rainfall about 20 days after the initiation of the test may have diminished differences in numbers due to the treatment. A comparison of trophic groups extracted by the BF and SF techniques 45 days after addition of water demonstrated the accentuation of microbial- and fungal-feeding species in the population by the former method (Table 12). The SF data showed that predacious Dorylaiminae and plant-parasitic forms were much more abundant in the root zones of *Larrea*. The marked differences due to the addition of water which remained at this date were a decrease in fungus feeders and predators in the population around *Larrea* and a rise in the proportion of plant feeders. Many of the miscellaneous group were probably young larvae of plant parasites which were difficult to identify accurately.

The preliminary experiment on the relationship of nematodes to decomposition of plant detritus in soil indicated a very distinct association with decomposing organic matter. In soil columns simulating a shallow soil profile with natural

litter added at the top of the column in treatment A, the center in treatment B, and at the bottom in treatment C, a several-fold increase in nematode population was observed over nonamended control columns (D) after 30 days. The average numbers of nematodes recovered from soil (adjusted to 100 g) at the bottom of the five columns in each treatment were as follows:

| Treatment: | A | B | C | D |
|---|---|---|---|---|
| | 381 | 244 | 414 | 101 |

Table 12. Percentage of total nematodes in various trophic groups 45 days after addition of water

| | Microbial Feeders | Fungal Feeders | Predators | Plant Parasites | Misc. |
|---|---|---|---|---|---|
| Sugar Flotation | | | | | |
| 1. *Larrea* irrigated | 41.9 | 11.5 | 11.6 | 20.0 | 15.0 |
| 2. *Larrea* check | 36.3 | 21.3 | 16.4 | 15.0 | 11.0 |
| 3. Non-vegetation irrigated | 62.5 | 17.5 | 2.5 | 5.0 | 12.5 |
| 4. Non-vegetation check | 55 | 16 | 9.2 | 13.8 | 6.0 |
| Baermann funnel | | | | | |
| 1. *Larrea* irrigated | 71.4 | 17.3 | 0.8 | 9.0 | 1.5 |
| 2. *Larrea* check | 47.8 | 43.3 | 3.0 | 5.9 | 0 |
| 3. Non-vegetation irrigated | 62.2 | 18.9 | 5.4 | 5.4 | 8.1 |
| 4. Non-vegetation check | 72.0 | 19.8 | 3.3 | 3.2 | 1.7 |

The largest number were recovered from the column with the litter at the bottom (actual litter zone). Treatment means for A and C were significantly different from controls at $P = 0.05$. In treatment B the litter may have been too moist, developing a somewhat anaerobic condition at the center of the column in the litter zone which depressed nematode and microbial reproduction.

The nematodes recovered from the five replicates were pooled for each treatment and examined for species present. The percentages of various types of nematodes in the population were the same as the nonamended control soil but with substantial increases in number. The major difference observed was in treatment A, where apparently all *Aphelenchoides* sp. (fungus feeders) were at the top of the column in the litter zone and were not observed in the population from that treatment

extracted at the bottom of the column. A large number of *Eudorylaimus diminutivus* were also present (44% of the total) in treatment A. The percentage of Dorylaiminae (predators) increased in all the amended columns.

## DISCUSSION

All of the sampling measurements for biomass estimation were carried out in exceedingly dry soils typical of the summer season. Rainfall in the previous season had been considerably below average and undoubtedly affected the nematode population as it had most plant life. Colorado Desert soils, particularly, were in a drought cycle and this apparently was reflected in the observation that biomass was the same by either the Baermann funnel (BF) method or by the sugar flotation (SF) method at the site selected (Table 8). The former method (BF) usually allows dormant eggs to hatch, increasing larval numbers in the population, and also accentuates actively motile nematodes. At the Victor Valley site in the Mohave Desert, which had a more regular rainfall season, the difference between the two methods was more noticeable. Additional desert biomass data during the normal rainfall season are required to assess nematode population potentials.

Almost no data are available on nematode biomass in soils except for total numbers per given area.

In our observations, the biomass in desert soils is only somewhat less than that found in some agricultural soils (Table 9). On the basis of populations per ha to a depth of 20 cm, there are surprisingly large populations of nematodes. Soils in humid areas, however, have considerable nematode populations in deeper soil strata, whereas desert soils are frequently very shallow. Cores cannot be taken deeper than 20 cm in most of the areas sampled in this study.

The desert soils studied have considerably fewer nematode species than humid soils. Among the microbial feeders, Rhabditidae were seldom observed in field samples but occur occasionally when soil crumbs are incubated on agar in petri dishes in the laboratory. The Cephalobidae are represented by many more species than occur in any other trophic group. Tylenchidae are far less represented in desert soils than in other soils. Dorylaimidae are usually not abundant but they are a relatively prominent component of desert nematode fauna. Here also, the number of species is relatively small; but the slower reproductive rate, larger size and distribution of the dorylaims, may place some species below the level of detection by common sampling methods.

Nematodes are essentially aquatic animals and must exist in a water film. The presence of active nematodes in apparently "powder-dry" soil is remarkable. The sugar flotation extraction method utilizes a solution of sucrose which retains nematodes in suspension while heavier soil particles are separated out by centrifugation or sedimentation. The sucrose solution places an osmotic stress on the nematodes for the brief period they are subjected to the separation process. Normally, the sucrose solution is rinsed rapidly from extracted nematodes and populations from humid soils are very little affected by the process. Nematodes from dry desert soils, however, are in very poor condition from the same process. It seems apparent that many of the populations we examined by this method were already under severe osmotic stress in the soil. The additional stress during processing apparently caused many nematodes to dehydrate to a lethal level. Comparison of tolerance to osmotic stress between nematode species from desert soils and species from humid soils would be useful. The eggs and possibly other stages of many desert species probably tolerate complete drying for certain periods. Since the SF method removes inactive and even dead nematodes, the poor condition of extracted nematodes often encountered may accurately reflect the condition of the nematodes in nature.

Barker and Nusbaum (1971) have reviewed studies which indicate that the SF technique is generally very efficient in recovery of most plant-parasitic nematodes. Seasonal differences may occasionally give better results by BF (Barker et al., 1969). Kimpinski and Welch (1971) indicated that SF extracted 42% of the total nematode population from clay and 38% from sand, while the BF method extracted 25% from both soils. Our data show 75% of the entire population can be extracted when the sample is run through the sieves twice. Two additional sievings of the sample suspension removed about 98-99% of the nematodes present but the additional operations are impractical in terms of time and material. A 75% extraction level is satisfactory for most studies. The advantage of using more than one method to study a population has been presented in studies relating only to plant-parasitic species in soil nematode populations (Barker et al., 1969, and Ayala et al., 1963).

The data collected on trophic groups indicate fungus-feeding species, particularly *Aphelenchoides* sp., appear to be uniformly distributed in the desert soils sampled and not related to clumps of vegetation. This has also been observed in non-desert soils (unpublished data). The Dorylaiminae and plant-parasitic forms, however, have a definite association with vegetation distribution. Microbial feeders are also more abundant in root zones. More information is needed on the biology of plant-parasitic forms than has been collected to date. The implication

of observing only a few individual larvae of obligate plant-parasites such as *Meloidogyne* sp. and *Pratylenchus* sp. is unclear. These species may be abundant only during periods of root growth after rains or may be associated with annuals.

An important factor in nematode population dynamics observed during this study and not indicated by any of the data is the presence of nematode parasites and predators. Parasitized individuals have been noticed and some of the population fluctuations observed may be due to parasitic fungi, protozoa or predacious fungi. All of these organisms were observed at various times. In one instance, black, surface-dried, plant debris from around the base of *Acacia gregii* was brought into the laboratory, moistened and incubated in a container for a few days. Populations of a rhabditid nematode and *Aphelenchoides* sp. reproduced rapidly. Both species declined in a relatively short period. When some of the incubated material was plated on agar a predacious fungus, *Dactylaria brochopaga*, appeared; another parasitic fungus attacked the rhabditid species and a parasitic protozoan attacked *Aphelenchoides* sp. To an unknown extent, carbon, nitrogen and other organic compounds are continuously cycled through nematode biomass in this manner in soil and decaying organic matter.

## EXPECTATIONS

The continuation of these studies into the coming year will develop data in the following areas:

Biomass

1. Improvement and combination of techniques which estimate biomass to provide data on percentage of population present as eggs, larvae, adults or dormant stages.
2. Comparison of wet- and dry-season populations.

Organic matter decomposition

1. Significance of nematode biomass in nitrogen and carbon cycling in desert soils.
2. Effect of desert soil organic matter levels (quantity and type) on composition of the nematode fauna.

Note: A supplementary report of studies on the relationship of organic matter decomposition to nematode population dynamics can be submitted early in 1973 as current studies are completed.

2.2.1.3.-22

Plant-nematode relationships

1. Identification of hosts or plants parasitized by phytophagous nematodes.
2. Association of plant-parasitic species with desert annuals.
3. Composition of rhizosphere vs. non-rhizosphere populations.
4. Mist-chamber extraction of nematodes from roots of dormant flora.

Bionomics

1. Nematode community structure by similarity of species (cluster analysis) relative to sites, soil type, vegetation and climatic factors.

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