THE ECOLOGY OF *POLLENIA RUDIS* AND ITS HOST EARTHWORMS

"Words fail to describe their general depravity; it is beyond expression. If you wish to be happy, be sure you don't introduce cluster flies into your family."

Dall; 1882.

THE ECOLOGY OF *POLLENIA RUDIS* (DIPTERA:CALLIPHORIDAE) AND ITS HOST EARTHWORMS (LUMBRICIDAE), WITH SPECIAL REFERENCE TO THE HOST-PARASITE RELATIONSHIP BETWEEN *P. RUDIS* AND *EISENIA ROSEA*

by

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SCOPE AND CONTENTS:

The distribution of Eisenia rosea, the main host of P. rudis, is influenced mainly by soil moisture and bulk density. Cluster-fly larvae locate hosts by random locomotion through the soil pores, whereupon penetration is induced by a substance present in the worm slime. Penetration occurs mainly on the upper surface of the worm, with fewer penetrations occurring towards either end of the worm. Several species of earthworm, including some which do not normally act as hosts, were infected in the laboratory.

The encystment, surface casting, burrowing and autotomising behaviour of E. rosea varies with soil moisture and bulk density. The penetration rate of P. rudis larvae into E. rosea is affected by this behaviour, as well as by the soil pore morphology. The success of maintenance of infected worms is also affected by the worm behaviour, which in turn depends on the soil conditions.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

General

Pollenia rudis (Fabricius), the cluster fly, is a member of the family Calliphoridae or blow-fly family. The larvae of this family are generally saprophagous or are parasites of vertebrates. P. rudis larvae are unusual in that they are parasites of earthworms.

Adult cluster flies have long been familiar to entomologists in Europe and Asia, as well as in North America. Although it is a common fly, few investigations of its life cycle and ecology have been carried out. Most reports refer to the enormous numbers of adults which enter buildings in the fall to hibernate, forming, in dark parts of the building such as attics and closets, the clusters which give this species its common name (Robineau-Desvoidy, 1863; Dall, 1882).

Although the adults feed mainly on exudates from plants, they have also been reported feeding on fecal material, carrion and refuse (Seguy, 1950). This feeding behaviour, combined with the habit of hibernating in buildings, make these flies potential carriers of disease, in the same manner as reported for other Calliphoridae (Yao, Yuan and Huie, 1929; Pipkin, 1942; Russo, 1931; Hall, 1947). When the buildings entered are hospitals, this aspect of cluster-fly behaviour becomes especially

important, as no effective method of control has yet been found.

There are two main reasons for this failure:

(1) Lack of knowledge of the cluster-fly life cycle.

(2) Lack of mass-rearing methods for obtaining flies for experimental purposes.

An understanding of the factors affecting cluster-fly ecology, especially the host-parasite relationship, would provide a firm basis for studies aimed at developing a mass-rearing method, and for any attempts at designing a successful method of control. A prerequisite of the study of any host-parasite relationship, however, is an understanding of the host's ecology.

The aim of the research for this thesis was to study the effect of soil factors on the ecology of the main host earthworm species, and to elucidate the factors affecting cluster-fly ecology, especially the host-parasite relationship. The thesis can thus be divided into three distinct sections:

(1) The ecology of the main host earthworm species.

(2) Host-location behaviour and penetration behaviour by the first instar larvae of P. rudis.

(3) Factors affecting the host-parasite relationship.

Soil factors influencing the distribution of the host earthworms

The ecology of earthworms can be discussed on a large and a small scale: the large scale would be at the level of soil map units, while the small scale would be on a

micro-topological level. Small scale aggregations have been shown by Svendsen (1957) to be larger in size than $1m^2$.

1) Large-scale distribution:

The large-scale distribution, and the related topic of overall numbers in an area depends on the reproductive and mortality rates of the worm species. Differences between areas, in terms of presence or absence, is due to the reproductive rate being too low or the mortality rate too high to allow for maintenance of a population. Alternatively, one may assume that the species has never been introduced into the area.

The reproductive rates of various species have been studied by Evans and Guild (1948), in terms of cocoon production per worm under different soil conditions. Their results are summarised as follows:

(a) Lumbricus rubellus (Hoffmeister) has a high rate of cocoon production (circa 100 per worm per annum); Allolobophora caliginosa (Savigny) and A. chlorotica (Sav.) have an intermediate rate (25 p.a.) while Eisenia rosea (Sav.) has a low cocoon production rate (8 p.a.). These figures represent a maximum under optimal experimental soil conditions.

(b) The rate of cocoon production increases with soil temperature.

(c) Each species has an optimal soil moisture content for maximum cocoon production, the rate falling sharply on

either side of the optimum. It is not the moisture *per se* which is important but the capillary potential or pF; that is, the force with which it is held in the soil, thus the optimum varies with soil type, pF values being lower in coarser than in finer soil at any percentage moisture content.

(d) The nature of the organic matter is important, the reproductive rate being higher in certain types of organic matter. Barley (1961) indicates the probability that worms get most of their mineral nutrients from organic matter; thus the effects of organic matter may be influenced by the mineral nutrient status of the plant material from which the organic matter was derived.

(e) All the *Allolobophora* species, plus *E. rosea*, have a diapause during the winter months, and also when the soil dries out in the summer. Thus for several months there may be no reproduction at all.

(f) The cocoons of *E. rosea* contain only one young worm and take an average of 17 1/2 weeks for incubation (compared with the cocoons of *E. foetida* (Sav.) which may contain up to 8).

With regard to mortality, Gerard (1967) has shown that the highest level occurs among freshly hatched worms due to fluctuations of surface soil moisture content.

From the work of Evans and Guild (1948) and Gerard (1967), it can be seen that vegetation, climate and soil type will exert a strong influence on the numbers of worms

in a given area. Some effects of these factors are indicated in publications pertaining to E. rosea.

Aleininikova and Izosimov (1958) studied the effects of surface vegetation. They found that under agricultural crops a range of 1 to 8 specimens of E. rosea were found. Under perennial grass the numbers were 3 - 4 times higher, and under field - protecting forest belts the numbers were highest. These results more probably reflect agricultural practices than the influence of vegetation. Zicsi (1958) found that all stages of E. rosea occur in the upper layers of the soil and are thus destroyed in great numbers by cultivation. This conclusion is supported by the work of Dzangaliev (1969).

Whether or not soil pH is important in determining the distribution of earthworms has been a controversial subject for many years. Bodenheimer (1935), using solutions of varying pH found that each species has a pH optimum for survival; he did not mention how he varied the pH, and as he found 100% mortality within a few weeks of starting the experiment, the value of his results is uncertain. The conclusion of Petrov (1946) appears more in keeping with the general view; namely that pH does not affect earthworm distribution except where it is so extreme that it excludes earthworms from the area. Murchie (1954) found E. rosea in soils of pH down to 4.8, but the absolute limits have not been established.

The effect of soil texture on E. rosea is uncertain. Guild (1948) found maximum numbers in light loam, with high numbers also in clay. Allee et al (1930) found maximum numbers in sandy loam, and Murchie (1954) noted that they were absent from soils with less than 6% sand. It can be seen that there is no consensus of opinion on the most suitable texture.

There are many chemical and physical factors operating in the soil, many of them interacting to various degrees, and it seems probable that it is the effect of a number of these interacting factors which determines the large-scale distribution and abundance of E. rosea. This would explain the lack of agreement between the results of the various researchers, especially with regard to soil texture.

The only study involving a number of factors was made by Murchie (1954), but his method of sampling ("number of worms per shovelful,") was somewhat inexact. There is no statistical treatment of the data and his conclusions, apart from upper and lower limit definition, are not really apparent from his data. His distribution records, however, did indicate that E. rosea was generally a streambank species.

2) Small-scale distribution:

Within an area, the small scale distribution of earthworms will be controlled by the small-scale variations in soil physical factors. This follows from the work of Svendsen (1957) who reported that earthworms in general

are sufficiently active to develop aggregations as a result of slower movement through favourable localities.

Using soil moisture gradients constructed in the laboratory, Hower (1965) concluded that high soil moisture alone could explain the occurrence of large numbers of E. rosea. However, in this study, large populations of active E. rosea have been found where soil moisture values are low; thus it appears that both the small-scale and the large-scale distributions are determined by factors other than soil moisture alone.

Murchie (1958) attempted to study the effects of several factors on the small-scale distribution. He studied the ecology of E. rosea in a woodland site, although in an earlier paper he had stated it to be a streambank species, with the result that many of his sample sites had no specimens, and the maximum density found was only $25/m^2$, although he has reported a maximum of $250/m^2$ from streambank sites (Murchie, 1954). The area in which he worked had soil conditions almost uniform throughout; thus there were no real soil factor changes to relate to worm distributional changes. His experimental design was generally unsuited to the problem, for the following reasons:

(i) A number of transects were studied, with sampling sites about 5m apart. Grieg-Smith (1952) and Kershaw (1957) give a method by which the use of transects can give an indication of aggregations, using an analysis of variance technique.

The method can also be used with covariance analysis to relate aggregations to ecological factors. However, unless certain conditions are rigorously adhered to, the method cannot be used. Murchie's technique did not allow the application of this method.

Apart from the application of Grieg-Smith and of Kershaw the main use of transects is to indicate ecological trends, but the almost uniform nature of the site in Murchie's study negated this advantage. The whole study might have been better based on the use of random samples, although more than the 38 sites used would be required for proper statistical analysis.

(ii) The main statistical treatments which could be applied to the results obtained are correlation and regression analysis. Correlation analysis is unsuitable because (as has already been indicated) earthworms are probably influenced by several factors simultaneously. Multiple regression methods fail when applied to highly correlated variables; thus, even if it had been used, its usefulness is severely reduced, due to the nature of soil factor interrelationships.

(iii) No indication was given as to whether the worms found were mature or immature. Satchell (1955a) has indicated that the different stages have different distribution, aggregations of young worms often occurring as a result of reproduction.

In view of this critique, that Murchie (1958) found no relationship between soil factors and worm distribution is not surprising.

It can be seen that no well-substantiated information about the ecology of E. rosea exists; thus it was necessary to investigate this subject to provide a basis for the study of the host-parasite relationship.

The numbers of worms in an area, i.e. the large-scale distribution, will be important to the cluster-fly larva, by influencing the probability of host location. However, under the more or less random oviposition behaviour (which will be discussed in the next section), it is the local conditions that will affect the host-parasite relationship (i.e. the factors affecting the small-scale host distribution). The study was therefore limited to the soil factors influencing the small-scale distribution of E. rosea.

Care was taken to avoid incurring the type of criticisms applied to the earlier works, and methods of statistical analyses were used that were not previously available, or have not been previously applied to the study of earthworm ecology.

Parasitism by the cluster fly, Pollenia rudis

Parasitism by P. rudis on earthworms was first recorded by Hoffmeister, in 1845, but details of the relationship remained unknown until reported by Keilin (1911, 1915) working in Paris, France. Apart from a paper by Barnes (1924) in England, all other research has been done on the life cycle in North America, which appears to be quite different from that in Europe. These differences

are indicated in Table A. The details of the life cycle in Europe are drawn from the works of Keilin (1911, 1915) and Barnes (1924), whereas those in North America are from Webb and Hutchison (1916), Garrison (1924), De Coursey (1927, 1932, 1951), Pimentel and Epstein (1960) and Hower (1965). As is indicated in Table A, all the researchers in North America agree on the main details of the life cycle. However, there is some disagreement on several points, mainly with regard to host range and feeding habits. Some of these points are shown in Table B.

From Table B it can be seen that the only worm infected at all attempts is E. rosea. With the other reported host species, infection has been found by some workers but not by others. The results of Pimentel and Epstein (1960) on wounded and crushed earthworms indicate that many species have the potential to act as hosts, although infection has so far been reported only in four species of worms, E. rosea, A. chlorotica, A. caliginosa and L. terrestris (Linnaeus).

One point of disagreement not indicated by Table A is whether or not the cluster-fly larvae are continuously parasitic during their development. The larva must locate and pentrate a host earthworm if it is to survive. Attempts to rear the larvae on other materials have all failed (De Coursey, 1927; Tao 1927). De Coursey (1927) found that the larvae frequently emerge from the wound and crawl about

TABLE A

Differences in Cluster-Fly Life Cycle

in Europe and North America

	Europe	North America
Copulation of adults	Fall	Spring
Storage of sperm over winter	Yes (Barnes) No (Keilin)	No
Oviposition	Spring (Barnes) Fall (Keilin)	Spring
Main overwintering stage	Adult (Barnes) Larva (Keilin)	Adult
Dormancy of larva	Present (Keilin)	Absent
Penetration site	Male genital pore (Keilin)	General body surface
Possible method of host location (nothing proven)	Seminal vesicle secretion (Keilin)	Slime (Pimentel, pers. comm.)
Presence of worms in the soil required for oviposition	Yes (Barnes)	No
Total development time (egg to adult)	10 - 12 months	27 - 39 days
Generations per year	1 (possibly 2) (Keilin)	3 - 4

TABLE B

Earthworm Hosts of the Cluster Fly, (*Pollenia rudis*)

Author	Hosts Investigated			
	E. rosea	*A. chlorotica*	*A. caliginosa*	Other Species
Keilin (1911)	-	I	-	-
(1915)	I	I	-	-
Webb and Hutchison (1916)	-	I	-	-
Barnes (1924)	-	-	-	*Lumbricus herculeus* N
Garrison (1924)	-	I	-	-
De Coursey (1927)	I	N	-	-
(1932)	-	-	I	-
Pimentel and Epstein (1960)	I	-	N	reactions to crushed worms see text.
Krivosheina (1961) (In Russia)	-	-	-	*Lumbricus terrestris* I

I = Infected

N = Not infected

- = Not tested

over the worm. However Pimentel (pers. comm., 1969) believes that the larvae are obligate parasites and must remain within the worm.

All authors working in North America indicate that the presence of earthworms in the soil is not necessary for oviposition by the cluster fly. However, for the survival of the species, it is essential that the fly lays its eggs in the vicinity of host earthworms. If the worm itself does not induce oviposition, then it must be some factor of the host's environment which triggers oviposition.

There are two clues as to what this factor might be. The first (De Coursey, 1951) is that female cluster flies, when ovipositing on turf, laid their eggs in the loose soil at the mouth of worm holes, implying that soil compactness or soil particle aggregate size or even the presence of a worm hole may be important. The second clue (Hower, 1965) is that the distribution of E. rosea, the main host species, is determined to some extent by the moisture content of the soil. Miss Hower also found that cluster flies were most abundant in the moist areas where E. rosea was found, suggesting that moisture is also a possible factor involved.

The observations of De Coursey (1951) also indicate a possible method of host location by the larvae. If the larva hatches at the mouth of an earthworm burrow, it would merely have to follow the burrow down to find the worm. It could do this by reacting to light, gravity, humidity and

temperature among possible suggestions or it could follow a product of the worm. On the other hand, worms occur in such large numbers in many areas, that the larvae, which are very active, would be able to locate a host by burrowing randomly through the soil.

All authors agree as to the mode of oviposition. The female cluster flies lay their eggs singly or in small batches of up to 7 eggs. After each batch is laid, the fly crawls or flies some distance away before laying another batch, thus distributing the eggs over a wide area. Each female probably lays 100-130 eggs, although definite counts have not been made, owing to the fact that the flies could not be reared in the laboratory.

Little research has been performed on the adults, except for some experiments by De Coursey (1927) on the reactions of the hibernating adults to light and temperature, and to contact. He found that at temperatures above 50°F, the adults showed a positive phototaxis and negative thigmotaxis. At temperatures below 50°F, the opposite was observed.

METHODS

I - Relating the distribution of E. rosea to soil factors

Choice of experimental areas

Murchie (1956) lists the earthworm, E. rosea, as a streambank species. Aleininikova and Izosimov (1958) indicate that the highest numbers are found in wooded sites on the borders of cultivated land. Earthworm counts in this study were made in a series of samples in several localities which combined both the above characteristics. On the basis of this initial survey, two areas were then chosen for examination, one in Ancaster and one in Dundas, both in Wentworth County, Ontario.

The areas were chosen on the bases of two characteristics:

a) Both contained a large population of E. rosea.

b) Both exhibited marked changes in soil characteristics within short distances. These differences were in those characteristics which could be estimated by field testing, e. g. texture, moisture.

Sampling procedure

Within each area, 100 sample sites were laid out, on a 10 x 10 grid, 1.5 meters apart. At each sample site, a soil sample was taken for the estimation of the population of E. rosea.

Svendsen (1955) found that hand-sorting of soil samples gave better estimates of earthworm populations than chemical extraction procedures. Zicsi (1962) found that the minimum sample size for estimating earthworm numbers was 1/16 m^2. It has already been mentioned that E. rosea is a surface dwelling species. Thus at each sample site, a sample 25 x 25 x 10 cm was taken and hand-sorted to obtain the estimate of the earthworm population. Mature (adult) and immature (juvenile) worms were listed separately.

Samples were also taken for the measurement of the following soil characteristics:

1) Soil moisture - % by weight and % by volume.
2) pH - The pH of a 1:2.5 soil: water mixture was measured using a Beckman Zeromatic SS-3 pH meter.
3) Porosity and gas-filled porosity.
4) Bulk density.
5) Organic carbon and loss of ignition.
6) Texture - % sand, % silt and % clay.

At each site, the soil temperature at a depth of 5 cm was taken at three points. Three measurements of soil compactness were also taken, using a Soiltest Penetrometer (Soil Test Inc., Chicago). The mean temperature and compactness for each site is given in the results.

Methods of Soil Analysis

1) Soil moisture and bulk density:

These are included together as both are measured from

the same sample taken in a bulk-density ring of volume 144 cm^3. [The bulk-density ring was cut from a cylindrical steel pipe, one edge being sharpened for ease of penetration.] Soil moisture by weight and by volume, and bulk density (BD) are calculated from the following measurements:

w_1 = wet weight of soil

w_2 = oven dry weight of soil

w_3 = moisture present = $(w_1 - w_2)$

V = volume of soil (i.e. volume of BD ring)

Thus:-

Moisture (% by weight) = $(w_3 / w_2) \times 100$

Moisture (% by volume) = $(w_3 / V) \times 100$ (assuming the volume of 1 g water is 1 cm^3)

Bulk density = w_2 / V

The oven dry weight (w_2) was obtained by drying the samples at 105°C for 24 hr.

2) Porosity and Gas-filled porosity:

The total volume (V) of soil can be divided into three parts:

(1) Volume of solids (V_1)

(2) Volume of liquids (V_2)

(3) Volume of air-filled spaces (V_3) = Gas-filled porosity.

The total volume (V) is the volume of the BD ring, and V_2 is obtained from the moisture content (% by volume). V_1 is calculated from the dry weight of the sample and the particle density. V_3 is calculated by subtraction:

$$V_3 = V - (V_1 + V_2)$$

Particle density was measured by the displacement method. For each sample, 20 g of finely-ground oven-dried soil were added to 25 ml distilled water in a measuring cylinder and the change in volume noted. Particle density is calculated from the following measurements:

w_1 = weight of soil = 20 g

v_1 = initial volume = 25 ml

v_2 = final volume

v_3 = volume of soil = $(v_2 - v_1)$

Thus:

Particle density (PD) = w_1 / v_3

Thus: $V_1 = \frac{BD \times V}{PD}$

Porosity is the volume not taken up by soil, i.e. the total available pore space.

Porosity = $V - V_1$

3) Loss on Ignition (LOI):

Five grams of oven-dried soil which had been ground and passed through a 2 mm mesh sieve were placed in a crucible, heated for 30 min in a muffle furnace at 700°C and then placed in a desiccator for 20-30 min to cool. LOI is calculated from the following measurements:

w_1 = initial weight of soil = 5 g

w_2 = weight of soil after ignition

Thus: $LOI = \frac{(w_1 - w_2)}{w_1} \times 100$

The figure of LOI is taken as a rough measure of organic carbon present in carbonate-free soils.

4) Soil organic carbon:

In view of the many samples to be analysed, a more rapid procedure than the standard volumetric method of carbon-content determination was desired. The use of rapid spectrophotometric methods for the determination of soil organic carbon is commonly regarded (Jackson, 1958; Jacobs and Reed, 1964) as being less accurate than volumetric methods, such as the method of Walkley (1947) or the modification of Tyurin's method described by Mebius (1960). This is probably because the first reports of spectrophotometric methods (Carolan, 1948; Graham, 1948) indicated that the method was useful only with soils of low organic matter content.

Graham (1948) compared values obtained by complete oxidation of the organic matter in a combustion train with results obtained when 1 g samples were carried through the wet combustion process (Walkley, 1947), but examined spectrophotometrically rather than volumetrically. He found that the spectrophotometric method was inaccurate for soils containing more than 4.5% organic matter. Carolan (1948), who introduced a minor modification (filtration) into Graham's method, also found that above 4.5% organic matter content the method was inaccurate.

It should be remembered, however, that the method is sensitive, not to percent organic matter, but to the

absolute amount of organic carbon in the sample used, and it is over this point that both the original users of spectrophotometry, and the critics of the method, have fallen into error. To illustrate the nature of the error, a series of experiments, using sucrose as a substrate of known carbon content, was carried out.

A 2.38% sucrose solution was used, giving a 1% carbon content. Aliquots of 0.5 ml to 20 ml were pipetted into 500 ml flasks and evaporated to dryness, giving substrates containing 5 mg to 200 mg carbon, each of which was digested with 10 ml 1N $K_2Cr_2O_7$ and 20 ml H_2SO_4 of a minimum concentration of 95.5%. After 30 minutes, 1 ml aliquots of the digest were diluted 1 : 5 and 1 : 10 and then filtered. Readings of optical density were taken at wavelength 550, 600, 620, 645, and 680 mμ of both diluted and undiluted digest. All experiments were conducted at 23°C. Calibration curves relating weight of organic carbon in the substrate to optical density were constructed. Of the wavelengths used, 620 mμ was found to be the best, followed by 645 mμ, the most commonly reported wavelength. Other wavelengths were much less suitable. The undiluted digest was unsuitable at all wavelengths for constructing calibration curves, as the readings were clustered at the top of the optical density scale, and could not be determined accurately. Both 1 : 5 and 1 : 10 dilutions were suitable for the construction of calibration curves. The calibration curves

for 645 mμ and 620 mμ are shown in fig. 1 for both 1 : 5 and 1 : 10 dilution.

In the present study, using sucrose as a substrate of known carbon content, it was found that a good correlation ($r = 0.999$) between optical density and substrate organic carbon existed only when the carbon content was less than 25 mg; thus it is obvious that the method will be inaccurate for large samples of high organic matter content.

For a 1 g soil sample, using the conventionally accepted soil carbon : organic matter conversion factor of 1.724 (Metson, 1961), the upper limit of accuracy will be 4.31% organic matter (2.5% organic carbon). It may also be noted that Howard (1966) has shown this conversion factor to be too low in most cases. It is apparent therefore, that the limiting factor of the earlier workers was sample size, as they worked with standard 1 g samples. Orlov and Grindel (1967) using soil samples of only 0.3 found a good relationship between spectrophotometric determinations and volumetric determinations by the Tyurin method, as described by Arinushkina (1961), in soils with organic matter contents as high as 10%, i.e., with a carbon content well within the range indicated by the sucrose experiments. This indicates that the spectrophotometric method is just as accurate as the volumetric or dry combustion methods, provided that the sample size is such that the total organic carbon present is within the range of the calibration curve. Metson (1961)

also found that by reducing the sample size, he obtained a linear relationship between spectrophotometric readings and soil organic carbon.

The spectrophotometric method is based on the wet combustion method of Walkley (1947), and it is this point that is the source of further controversy, as it has been found that the oxidation of organic matter is incomplete, the more inert forms of carbon being unaffected. However, Metson (1961) shows that the error is constant, thus by applying a conversion factor of 1.15, the total organic carbon can be determined.

It can thus be seen that the spectrophotometric method can be used, giving a rapid and accurate technique, provided the difficulties of sample size and incomplete combustion are taken into consideration.

For application to soil organic-carbon measurement, the results of the sucrose experiments indicated that readings should be made at 620 mμ, using a 1 : 5 dilution. The method could be applied to all levels of organic matter content, by adjusting the size of the soil sample so that there was never more than 25 mg organic carbon present. A guide to the size of sample required could be found in the loss on ignition figures.

5) Soil texture determination:

A 20 g soil sample was used. Standard sieves were used to separate particles of the following size intervals

(in microns): > 1000; 600-1000; 200-600; 53-200; 45-53; < 45. The sub - 45 μ fraction was examined using a Quantimet 720 Image Analysing Computer*, which gave a particle frequency distribution of the sizes (in microns) 30 - 45; 20 - 30; 15 - 20; 10 - 15; 5 - 10; 2 - 5; 1 - 2; 0.5 - 1. From these data, the texture could be expressed in % sand (50 - 2000 μ), % silt (2 - 50 μ), and % clay.

6) Pressure plate apparatus:

The moisture tension curves or pF data for three soil samples were obtained using a pressure plate apparatus. (Volumetric Pressure Plate Extractor, Soilmoisture Equipment Co., Santa Barbara, California.) One sample from the Dundas area, from the central region of the gradient indicated by the ordination approach, and two samples from the Ancaster area, from the two main groups of sample sites indicated by the ordination approach, were used.

Methods of data analysis

Four methods were used to analyse the data for relationships between the distribution of adult and juvenile *E. rosea* and soil factors.

(i) Simple correlation coefficient

(ii) Stepwise multiple linear regression

(iii) The isonome method

(iv) An ordination approach

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(i) Simple correlation coefficient (r) calculated by:

$$r = \frac{\Sigma x_1 . x_2 - \frac{\Sigma x_1 . \Sigma x_2}{n}}{\sqrt{\left(\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n}\right) \cdot \left(\Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n}\right)}}$$

(ii) Stepwise regression: Biomedical Computer Program BMD02R (Dixon, 1968) was used. Logarithmic and square-root tranformations of the data were used, as well as the raw data themselves.

(iii) Isonome method (Ashby and Pidgeon, 1942). In using isonomes, there is a problem in deciding which method of interval selection to choose. At least three methods of selecting intervals are possible.

a) Upper, lower and middle quartiles.

b) Equal intervals, e.g. upper, lower and middle thirds.

c) Natural groupings selected by a modified use of the clinographic curve (Monkhouse and Wilkinson, 1966). As used originally, this was a cartographic method of illustrating sudden changes in terrain. It is modified here in order to detect "plateaus" in the data. The observed values for a particular soil factor, for each site, are sorted into ascending order, then arranged graphically from lowest to highest. In this study there were 100 sample sites, thus the abscissa consists of the numbers 1 to 100, and the ordinate the values for the soil factor, or organism

population density. When the values are plotted, they fall on a rising curve in which there may be breaks or level areas. The isonomes should be chosen to include the level areas between them.

To select the best method, a correlation approach was developed. The actual data values are correlated with the values given by the isonomes. When the correlation coefficients for the three types of isonomes are compared, the highest value indicates the method which best represents the data.

(iv) The ordination approach. The theory and application of ordination methods in ecology is discussed by Orloci (1966), who also describes the similarity coefficient which is used in this analysis. Ordination methods in ecology have been restricted almost completely to the study of plant communities (Kershaw, 1968). A principle component analysis is used to determine the major axes of the ordination, and the relative positions of the sample sites along the axes. The values for the soil factors can be superimposed on these to determine if the major axes correspond to any environmental gradient. A computer program devised by Professor K. A. Kershaw of the Department of Biology, McMaster University, Hamilton, Ontario, was used. This included a subroutine BIGMAT for Principle Component Analysis, by M. Elson and R. E. Funderlic, Central Data Processing, Oak Ridge, Tennessee.

II-Behaviour of first instar P. rudis larvae

Method of obtaining larvae for experimental purposes

Mated cluster flies were collected by sweeping vegetation, especially in grassy areas. The following procedure for obtaining larvae was developed.

a) A moist paper towel is placed on the bottom of a 1-gallon glass jar, with a mesh screen cover. The moist towel keeps the humidity at 100% RH. Flies are introduced through a port in the cover. The jar of flies is kept in an illuminated constant-temperature room at 20°C. Flies were found to lay eggs readily under these conditions, but at lower temperatures (15°C) no oviposition was observed. At 27°C the flies would also lay eggs readily under these conditions, but at lower relative humidity (60%) very few eggs were laid at this temperature. At 21°C, good oviposition rates were obtained with relative humidity as low as 60%.

b) After about 24 hr the flies are removed from the jar, and the eggs washed from the paper and the sides of the jar with distilled water and then concentrated by filtration.

c) The portion of filter paper with the eggs adhering to it is placed in a small vial with a tightly fitting cap. The wet paper is allowed to adhere to the side of the vial, rather than being placed at the bottom.

d) A little distilled water is placed in the bottom of the vial which is then kept at 20°C. When larvae hatch, they become trapped in the water and can be removed by a pipette, in which they can be easily counted. It was found that a 12-hr immersion in distilled water had no apparent adverse effects on the larvae.

By using this procedure, a maximum number of eggs and larvae can be obtained which can be easily counted and manipulated.

The importance of keeping the eggs and larvae moist at all time was indicated by the following experiments:

1) Effect of temperature and humidity on hatching

As relative humidity can be controlled by H_2SO_4 (Buxton, 1931), 5 ml of each concentration of acid were placed in large test tubes. Eggs were placed in a small plastic container supported above the level of the acid. Each test tube was kept stoppered to maintain equilibrium within. Humidities of 100%, 90%, 75%, 50%, and 30% RH were used and temperatures of 15°C, 27°C and 35°C. Three lots of 10 eggs each were used under each set of conditions. A similar experiment was done with the eggs on moist filter paper, at each of the temperatures used. The cumulative number hatched was recorded each day for a five-day period.

2) Effect of humidity on larval survival

Humidities of 100%, 90% and 70% RH were used at 21°C. This lower temperature was selected since at 35°C and 75% RH the larvae were found dead on top of the eggs

from which they had hatched. Three sets of 10 larvae, hatched on moist filter paper (the optimum conditions found in the previous experiment) at 21°C, were used under each set of conditions, the larvae being placed on a platform raised above the surface of H_2SO_4 of the required strength (Buxton 1931). Three sets of 10 larvae were kept on moist filter paper as a control. The times for 50% and 100% mortality were found, and the average time of the three tests recorded.

Factors affecting the behaviour of the larvae

1) Moisture preference of larvae

The bottom of a petri dish was covered, half with moist filter paper and half with dry filter paper. Ten larvae were placed on the paper, in the centre of the petri dish. After 1 hr at 20°C, the number of larvae on the moist filter paper, and the number on the dry, were counted. This test was repeated three times.

2) Test for photokinesis

An arena was formed from a petri dish (14 cm in diameter) with a floor of moist filter paper. A larva was placed in the center of the arena. A light was arranged to produce a strip of light, 3 cm wide, down the center of the arena. The path of the moving larva was recorded for 15 min or until the larva contacted the edge of the arena, whichever was first. The experiment was conducted at 20°C and was repeated 18 times.

3) Effect of temperature on rate of locomotion

The arena described above was floated on the surface of water in a water bath. A larva was placed in the center of the arena and its tracks recorded. The tracks were marked at 1-min intervals so that the rate of locomotion in mm/min could be calculated. Temperatures of 15°C, 20°C and 25°C were used, with nine tests at 15°C and seven tests each at 20°C and 25°C.

4) Test for phototaxis

An open-ended box lined with moist black paper was constructed. A beam of light was shone down the length of the box on a horizontal plane, and a larva placed in the center of the floor. The track of the larva was recorded. The experiment was repeated 10 times at 20°C.

5) Reactions to gravity

A plexiglass runway was used, with a floor of moist filter paper. The runway, which was 20 x 1 x 0.3 cm was kept in a closed chamber at 100% RH and 20°C. The chamber was tilted to an angle of 60° to the horizontal and 12 larvae were placed in the center of the runway. After 10 min the numbers above and below the starting point were recorded.

6) Behaviour on encountering breaks in surface

a) A circle of moist filter paper, 2 cm in diameter, was supported above the surface of a petri dish. A larva

was placed in the center of the circle and its behaviour noted. The experiment was repeated five times.

b) The above experiment was repeated using a circle 7 cm in diameter.

c) In a dish of tightly compacted soil, a series of holes 2 mm in diameter, was made around the circumference of a circle, 15 mm in diameter. A larva was placed in the center of the circle of holes and its behaviour noted. The larva was then transferred to a similar situation in which the rims of the holes were rounded, not sharp. The experiment was repeated with five different larvae.

7) Response to a temperature gradient

Because the larvae were found to have no reaction to light and gravity, an apparatus had to be constructed in which the larvae would be unable to climb up the sides and out of the apparatus. The apparatus was designed to produce a circular temperature gradient, with low temperatures around the periphery restricting larvae to the central area.

The apparatus was constructed of 0.33 cm thick copper sheet, with a base plate 32 cm in diameter. A rim 1.5 cm wide was left for clamp attachment; then a channel 2 cm wide was constructed to conduct circulated chilled water, leaving a central area 25 cm diameter. A vertical copper rod was attached to the bottom of the plate centrally for heating. The rest of the underside of the base plate was covered with asbestos to insulate it from direct radiation by the heat source, a bunsen burner. Sockets fitted into the

underside of the base plate permitted the insertion of thermocouples. These sockets were at 2.5 cm intervals, in three rows radiating out from the center of the arena. The whole upper surface of the apparatus was painted with white enamel to nullify any adverse effects of the copper ions. The arena was lined with moist filter paper and covered with a sheet of glass, supported on the walls of the cooling trough which were 2.5 cm high.

When the central rod was heated and chilled water circulated round the outer channel, a circular temperature gradient was created. Measurement of the temperature at each socket allowed isotherms to be constructed. Larvae were placed around an isotherm and left for 30 min, then the number moving up the gradient (towards the center) and the number moving down (towards the periphery) were recorded. The experiment was conducted a number of times using different temperature ranges. These ranges are indicated in table 45. The experimental larvae were all obtained under the constant conditions indicated earlier.

Movement of larvae through soil

Undisturbed soil samples were taken using Kubiena boxes (Kubiena, 1953). The samples were frozen at -20°C overnight, then cut into 1/2" cubes, one face being the natural upper surface. The cubes were fitted into plastic mesh baskets to preserve their shape and to keep them undisturbed and were then allowed to thaw out to room

temperature. Thirty-five larvae were placed on each of two cubes, left for 30 min to penetrate into the soil, and then killed by freezing. The soil block was thawed again and placed over formaldehyde vapour for two days to preserve the larvae. The thick-sectioning method of Haarlov and Weis-Fogh (1935) was used. The cubes were placed in 2% agar for 24 hr at 50°C, for impregnation. The impregnated blocks were cooled and placed in absolute alcohol for at least one week to harden. The blocks were then removed from their baskets and sectioned with a razor blade. Serial 1 mm-thick sections were obtained, and examined by reflected light to determine the position of the larvae in relation to the soil pores and structures.

Feeding behaviour of first instar larvae

1) Reactions to crushed earthworms:

Pimentel and Epstein (1960) reported P. rudis larvae feeding on crushed earthworms of a number of species. This observation was tested with the earthworm species common in the Hamilton area. The following species of worms were used: A. caliginosa; A. chlorotica; L. terrestris; L. rubellus, E. rosea and Eiseniella tetraedra (Sav.).

The earthworms were crushed with a mortar and pestle and a larva placed on the remains. Five larvae were used to test each species.

2) Reactions of larvae to earthworms killed by freezing:

The six species mentioned above were used. They were

killed by placing them in the freezer of a refrigerator for 5 min. After freezing, the worms were allowed to thaw at room temperature before placing five larvae at different points along the dorsal surface of each worm.

3) Reactions of larvae to seminal vesicle homogenate:

Keilin (1911, 1915) had suggested that the seminal fluid of earthworms might be the attractant for the larva, so that tests were made with three species of worm A. caliginosa, A. chlorotica and E. rosea. The seminal vesicles were removed from four specimens of each species and homogenised. A spot of the homogenate was placed on a piece of moist filter paper and a larvae placed beside it. The reactions of the larva to the homogenate were noted. The test was repeated with five larvae for each species of worm.

4) Reactions of larvae to slime:

Earthworms of the six species mentioned above were used. When treated with CO_2 and allowed to revive, the worms produced slime copiously. By touching a fine glass road to the surface of the worm a globule of slime could be collected. The globule of slime was placed on moist filter paper, a larva placed beside it, and the larval behaviour noted. The test was repeated with five larvae for each species of worm. The experiments were repeated using dried slime, remoistened slime, and diluted slime. One drop of slime was mixed with 1, 4, and 9 drops of distilled water, giving dilutions of approximately 1:2, 1:5, and 1:10.

5) Reactions of larvae to coelomic fluid:

The six species mentioned above were used. A slit was made dorso-laterally in the body wall of the worm. The incision was dorso-lateral rather than dorsal to avoid cutting the dorsal blood vessel and getting blood mixed with the coelomic fluid. A drop of coelomic fluid was extracted with a capillary tube and placed on moist filter paper. A larva was placed beside the spot and its behaviour noted. The experiments were repeated five times.

6) Reactions of larvae to washed portions of the worm:

Pieces of each species of worm were washed in distilled water and dried by blotting with filter paper. A larva was placed on the piece of worm and its behaviour noted. The following parts of the worm were tested:

a) seminal vesicle

b) body wall (outer surface)

c) body wall (inner surface)

d) gut

Each experiment was repeated five times.

Attempts to develop an artificial culture medium

Five larvae were placed on the surface of each of the following culture media and their behaviour noted:

1) 2% agar in a small vial.

2) 2% agar, the surface of which had been smeared with slime from E. rosea.

3) Five E. rosea were homogenised and mixed with 10 ml of 2% agar, which was cooled and its surface coated with slime from E. rosea.

III The host-parasite relationship

Infection of earthworms

The infection of earthworms in the laboratory was studied:

(1) Earthworms were placed in small tightly-stoppered glass tubes, one worm to each tube. Four or five small pieces of filter paper were placed in each tube to prevent moisture and slime accumulating to the extent where the larvae would be trapped. Twelve to fifteen larvae were placed with each worm. The following day, each worm was examined for infection, and the site of infection recorded. The experiment was set up using 8 A. chlorotica, 16 E. rosea, 5 A. caliginosa, 5 L. terrestris, and 5 L. rubellus.

(2) The experiment was repeated with 50 E. rosea and 8 - 10 larvae per tube. The infected worms were transferred to individual tubes of soil and examined every day to determine the fate of the larva.

(3) 16 E. rosea were placed in a glass container 8.75 x 6.25 x 3.15 cm, with loose soil to a depth of 1.25 cm. One hundred and fifty larvae were added and left for 24 hr. The worms were then examined for infection and returned to the soil. The experiment was repeated with 20 A. caliginosa and 300 larvae.

For all the above experiments, control worms were kept in similar apparatus, without larvae, to ensure that any effects recorded, especially death of the host, were due to the infection by the larvae and not to the experimental conditions. Experiments (1) - (3) were conducted at room temperature (21-24°C).

(4) Effect of temperature and multiplicity of infection (MOI) on numbers of infections of E. rosea under standard soil conditions:

MOI is defined as the ratio of parasites to hosts. Glass trays, 8.75 x 6.25 x 3.15 cm, were used and loose soil added to a depth of 1.5 cm. Twenty E. rosea were added to each tray, and larvae added to give MOI's of 0.5 : 1, 1 : 1, 2 : 1, 3 : 1, 4 : 1, 5 : 1, 7.5 : 1, and 10 : 1. Temperatures of 13°C and 20°C were used as these represented the upper and lower limits found in the field. The number of tests at each MOI was as follows:

MOI:	0.5:1	1:1	2:1	3:1	4:1	5:1	7.5:1	10:1
13°C	1	3	3	8	2	5	1	1
21°C	-	3	2	-	-	2	-	1

The number of infected worms and any change in the general health of the worms was noted (i.e., whether or not the worms became unhealthy apart from the P. rudis infection).

(5) Effect of soil conditions on host location and penetration success of larvae:

The study of the ecology of E. rosea indicated that

two factors were of major importance to the worm, namely soil moisture and bulk density (BD) (see RESULTS).

The effects of these factors on the success of host location and penetration by the P. rudis larvae was studied using soil from the Ancaster experimental area. The soil of this area was of two types:

I - High bulk density, low moisture, low organic matter, high sand.

II - Low bulk density, high moisture, high organic matter, low sand.

The first set of experiments was conducted with Type - I soil. Soil was added to glass trays to give bulk densities of 1.25, 1.50 and 1.75 in a final volume of 87.5 cc. The moisture content (% by volume) was adjusted to 15%, 30% and 45%. The levels of the factors reflect the values found in the field. The moisture and BD levels were varied in combination according to a 2^3 factorial experimental design. Sixty larvae and 20 E. rosea (MOI = 3:1) were added to each tray. All experiments were at 15°C, as earlier results indicated that at 20°C there was a high mortality of worms, probably due to the onset of decay processes starting at the site of penetration (possibly even of attempted penetration) by the larvae.

a) Pilot experiment

Soil was adjusted to the correct moisture and BD levels, then the worms placed on the surface. In the driest

soil, especially at the higher bulk densities, the worms would not burrow; thus the soil had to be removed, the worms placed on the bottom of the tray, then the soil compacted back to the correct bulk density. The number of larvae which penetrated a worm, and the burrowing behaviour of the worm were noted.

b) Main experiments ((i) - (iii))

For these tests, the moisture was not adjusted immediately. The worms were allowed to burrow into wet soil of the correct BD; then the soil was dried out to the correct moisture content before adding the larvae. This avoided the burrowing difficulties encountered in the Pilot experiment. Because of observations made in the Pilot experiment, the worm encystment and casting behaviour were also recorded. The set of experiments was repeated three times.

c) Main experiments ((iv) - (vi))

The above experiment was repeated in soil Type - II. Initially three replicates were set up, but even more problems were found with regard to worm behaviour and survival, probably due to the increased moisture suction pressure in this soil, as indicated by the pressure plate apparatus (fig. 4). The experiment was therefore restricted mainly to observations of worm behaviour. Larvae had already been added to six of the nine trays of the first replicate, so success rates are recorded here, but not for the other tests. At the time the experiment was curtailed, only three trays of the third

replicate had been set up; thus the behavioural patterns in the third replicate are incomplete.

(6) Effects of soil conditions on the maintenance of the infection:

Soil of Type - II was used. The BD levels of the previous experiment were used, but at higher moisture levels in order to compensate for the higher moisture suction pressure in this soil. Moisture contents of 25%, 45% and 65% were used, in a 2^3 factorial experimental design as above. In this set of experiments, however, the soil moisture conditions had to be set before the infected worms were added. To facilitate burrowing, small holes were made in the soil, the rim moistened with a drop of distilled water, and each worm allowed to burrow in the hole provided. After the worm was completely covered, the mouth of the hole was sealed with soil. Five infected E. rosea were used in each test, and examined 10 days after the hatching date of the larvae, approximately one week from the time the worm was infected. Three replicates were made at 15°C and one at 20°C. The fate of the worms was recorded. Three replicate experiments using uninfected worms were used both as controls for the experiment with infected worms, and also as a test for the worm behaviour under the different sets of conditions.

(7) Survival of infected worms under optimal encystment conditions for soil type - II:

Results of previous experiments indicated that

infected worms survived better when encysted than when unencysted. A set of experiments was conducted to find the effect of drying conditions, as opposed to the effect of the constant conditions described above on encystment. The experiment was set up as a two-way classification experimental design. Only the two lower moisture levels of the above experiment were used (i.e. 25% and 45%) as behaviour at the upper moisture level was found to be uniform (i.e. no encystment took place). Bulk density levels of 1.75, 1.50, 1.25 and 1.00 were used. The soils of Ancaster type-II were initially wet, and were dried to their final level over a period of a week, when the worm behaviour was noted.

The optimal conditions for encystment were indicated to be 25% moisture for the soil type-II, the BD being unimportant with the drying conditions used. Five hundred worms were placed in jars of loose soil, 20 per jar, and 60 - 100 larvae added to each jar. After three days, the worms were divided between four trays, under optimal encystment conditions. Two of the trays were placed at 15°C and two at 20°C, and left undisturbed for three weeks, the time recorded in the literature as being required for the larvae to reach the pupal stage. Both infected and uninfected worms were added to the soil, as previous observations indicated that P. rudis larvae may parasitize several hosts in the course of their development. Although the actual number of infected worms used was not known exactly, it can be estimated from the results of previous experiments

(8) Survival of infected worms in media other than soil:

Nine infected E. rosea were placed in stoppered vials of distilled water. Five were placed in stoppered vials without water. Uninfected controls were used. In all cases the worms were transferred to fresh containers each day. All were kept at 15°C.

(9) Field data:

In the course of collecting earthworms for experimental purposes, and soil samples for analysis, a few infected worms were found. The species of worm infected and the instar of the larva were recorded.

Worm behaviour, soil moisture content and BD were also recorded in seven sites in the Ancaster region, on August 10, 1971. A 25 x 25 cm sample site was examined to a depth of 60 cm. In site # 2, sample (i) was from the upper horizon, and sample (ii) from the lower horizon at a depth of about 15 cm. Site # 6 was from soil Type - II of the Ancaster experimental area, and site # 7 from soil Type - I.

Effect of soil atmospheric conditions

It seemed possible that the success of the infection was limited by adverse soil atmospheric conditions, acting on the host, the parasite, or both. No information is available about the effects of different conditions on either organism. A modification of the method of Heydecker (1955) was used to produce varying atmospheric conditions. Cylinders

of nitrogen, oxygen and carbon dioxide were connected via flow meters to a mixing vessel, then to a series of flasks containing the experimental organisms. A flowmeter was placed at the outlet, and the time taken for correct flushing of the apparatus determined by testing the outflow using gas chromatography. Flowmeters fitted with tube # 600, were from Matheson of Canada Ltd., Whitby, Ontario. Before mixing, the CO_2 and N_2 were passed through Pyrogallol solution to remove any O_2 impurities which might affect the final level of O_2, as very low levels of O_2 were being used. The N_2 was also passed through distilled water to maintain a high humidity in the experimental apparatus. By varying the flow rates of the different gases, different atmospheric conditions could be produced.

The flow of gas was maintained throughout the experiment in order to prevent changes due to respiration. The apparatus was flushed with the gas mixture for 30 min before introducing the experimental organisms through ports in the flask stoppers. The positive pressure of the gas flow prevented any air from entering the apparatus. E. rosea from different localities were examined, as well as first-instar, cluster-fly larvae. Freshly collected (same day) worms were used in all tests, except for some tests on worms from an earthworm culture maintained in the laboratory. The relative amounts of CO_2, N_2, and O_2 in each test, the site and date of collection (i.e. date of experiment) of the worms,

and the number of worms used in each test are indicated in the results section along with the percent mortality after 24 hr. Worms were kept three to a flask during the experiments.

The larvae used in these tests were all freshly hatched, obtained using the procedure outlined previously.

RESULTS

I - The host - *Eisenia rosea*

Relation of worm distribution to soil factors

Results of soil analysis

Sketch maps of the two experimental areas indicate the positions of the sample sites (figs. 2-3). The actual values recorded for each soil factor are given in tables (2-31), the order of the data values reflecting the relative positions of the sample sites. For easy visual interpretation of the data, the maps produced by the isonome method are also included in tables 2 - 31.

The correlation coefficients for the three different methods of isonome interval selection are indicated in tables 39 - 40 along with r^2, the variation accounted for by each set of isonome intervals. For each soil factor, the method giving the highest correlation coefficient was used to map the factor.

In the Dundas area, it can be seen that there is a gradient from a region of high sand and low moisture to a region of low sand and high moisture content. A similar gradient exists in the Ancaster area, but here a region of high bulk density corresponds to the high sand, low moisture content region.

Correlation coefficients

The correlation coefficients between numbers of worms and soil factors, and also among the soil factors per se are indicated for the Dundas area in table 32, and for the Ancaster area in table 33. For easy reference, only the high values are indicated ($\geq$ 0.5). The code for the factors is given in table 1. For the Ancaster area, the distribution of adult and juvenile worms is not correlated with any soil factor at a level greater than r = 0.5, while for the Dundas area, the adult worms are positively correlated with silt and moisture content, and negatively correlated with sand.

Stepwise regression

The following results are included in tables 34 - 38:

a) The variable entered at each step by the regression model (using the code given in Table (1)).

b) The correlation coefficient (r) relating observed values to the estimate given by the variables entered by the regression coefficient.

c) The total variation accounted for by the variables entered (r^2).

d) The increase in r^2 resulting from the entry of each new variable.

Only the variables giving an increase in r^2 of 0.01 or more are indicated. Juvenile and adult worms are treated separately as the dependent variables. The two experimental

areas were treated separately. The following combinations were examined, for both juvenile and adult worms.

1) The regression of number of worms against the raw data of the soil factors (Table 34).
2) The regression of the square root transformation of number of worms against raw data (Table 35).
3) The regression of the logarithmic transformation of number of worms against raw data (Table 36).
4) The regression of number of worms against the square root transformation of the raw data (Table 37).
5) The regression of number of worms against the logarithmic transformation of the raw data (Table 38).

The results indicate that the distribution of juvenile worms is never as completely related to soil factors as is that of the adult worms. The number of worms per site in the Dundas area is related to silt or sand, depending on whether the transformation is applied to the worm numbers or to the soil data, and to organic carbon. Silt and sand have already shown to be highly negatively correlated. In the Ancaster area the number of worms per site is related to temperature, porosity and compactness regardless of the transformation used.

Ordination method

The Principal Components Analysis gives the relative position of each sample site along each of the principal axes. Only the first three principal axes are considered,

as they account for most of the variation (table 41). The co-ordinates of the sites along these three axes give the position of each site in 3-dimensional space. To represent this graphically, each site is projected onto three planes, bounded by axes one and two, one and three, and two and three; thus each site is represented by three points on the diagrams. The arrangement of the sites on the three planes is given for the Dundas area in fig. 7 and for the Ancaster area in fig. 8. Figs. 9-10 show the actual site reference numbers as indicated in figs. 2-3 for the two areas. From the reference numbers, the values for the soil factors for that site can be obtained and superimposed on the point scatter of figs. 7-8. The values for moisture content and number of adult worms are shown for the Dundas area (fig. 11-12) and for the Ancaster area (figs. 13 and 15). The Ancaster bulk density values are also shown (fig. 14). All the environmental gradients found are shown in figs. 5-6. The direction of the arrow indicates increasing values of the soil factor. The amount of variation accounted for by the first five principal axes are given for the two areas in table 41.

Moisture tension in soil samples

The moisture tension curves obtained using the pressure plate apparatus are given for three samples in fig. 4. The numbers of the samples relates to their reference number as indicated in figs. 2-3. The results

indicated that in the Dundas soil, a given moisture content was held with more tension than in the Ancaster area. In the Ancaster area, a difference was found in moisture holding properties between the low sand, low bulk density sample (#33) and the high sand, high bulk density sample (#85). A given moisture content was held with more tension in the low sand, low bulk density sample.

II - Behaviour of first-instar cluster-fly larvae

Obtaining experimental larvae

In the course of devising a method for obtaining and maintaining experimental larvae, the following two experiments were carried out:

1) Effect of atmospheric temperature and humidity on hatching.
2) Effect of atmospheric humidity on larval survival at 21°C.

It was found that the greatest hatch rate was obtained with the eggs in contact with a moisture film (moist filter-paper). Hatching was sharply curtailed at 90% RH and zero at 50% RH or less (table 42). In table 42 each figure in the triplet represents the number of eggs hatched in each set of ten eggs, the values for each day being cumulative. When the larvae emerged from the eggs, they died in less than 2 hr even at 100% RH, unless in contact with a moisture film (table 43).

Factors affecting the behaviour of the larvae

These are the results of the following seven experiments:

1) Moisture preference of the larvae - choice between moist and dry filter-paper.
2) Test for photokinesis.
3) Effect of temperature on rate of locomotion.
4) Test for phototaxis.
5) Reactions to gravity.
6) Behaviour on encountering breaks in the surface.
7) Response to a temperature gradient.

When presented with a choice between moist and dry filter-paper, of ten larvae used in each of three tests, eight, ten and nine chose moist respectively, after 1 hr at 20°C. That is, 90% of the larvae chose the moist filter-paper. On the basis of this experiment, all further experiments on larval behaviour were carried out on moist filter-paper. On moist filter-paper, the rate of locomotion of the larvae was found to be greatest at 20°C and least at 15°C, with that at 25°C being intermediate (table 44). No consistent trend of direction of movement was shown by first-instar cluster-fly larvae in temperature gradients, however (table 45).

The moving larvae exhibited no photokinesis or phototaxis. Of the twelve larvae used to test the reaction to gravity, six moved up the inclined plane and six moved down, which was interpreted to mean that the larvae show no reaction to gravity.

The behaviour of the larvae on encountering breaks in the surface varied with the angle of approach. On 2-cm

diameter filter-paper circles, the larvae did not have space to deviate greatly from an angle of approach at right angles to the edge of the circle, and always turned away from the edge. On 7-cm diameter filter-paper circles, if a larva approached the edge more or less at right angles, it turned away, but if a larva changed direction between the starting point and the edge, and approached from a shallower angle, it usually twisted over the edge and down the side of the support. When larvae were placed on compacted soil with holes with either sharp or rounded rims, the larvae went down the holes with the rounded rims, and turned away from those with sharp rims.

Movement of larvae through soil

When the soil cubes were sectioned, larvae could be seen in the soil pores, and in the gaps between grass stems and the surrounding soil (fig. 18-19). Larvae were never seen to produce tunnels of their own, but appeared to follow the naturally occurring pores.

Feeding behaviour of first-instar larvae

These are the results of the following six experiments:

1) Reactions of larvae to crushed earthworms.
2) Reactions to earthworms killed by freezing.
3) Reactions to seminal vesicle homogenate.
4) Reactions to slime.
5) Reactions to coelomic fluid.
6) Reactions to washed portions of worm.

First-instar larvae were observed to feed on crushed earthworms of the following species: E. rosea, A. caliginosa, A. chlorotica, L. terrestris, L. rubellus and Eiseniella tetraedra. Larvae placed on the surface of individuals of all these species killed by freezing behaved uniformly: they remained in one position and probed repeatedly at the surface of the worm with their mouth hooks (mandibles), rather than moving about over the surface of the worm. The same behaviour was evoked by contact with slime and coelomic fluid from the worms. When the slime was diluted, dried, or remoistened, however, it failed to evoke this response. Fresh slime, however, induced the larvae to remain in one position probing with their mandibles until they died a day or two later. Fresh coelomic fluid, on the other hand, lost its power to induce this behaviour within 24 hr. In fact, after 2 hr fewer than half the larvae remained on the test spot of coelomic fluid.

Neither washed parts of the six earthworm species, nor fresh seminal vesicles from A. chlorotica, A. caliginosa, and E. rosea evoked any response by the larvae.

Attempts to develop an artificial culture medium

On the surface of agar in a small vial, larvae moved over the surface and up the walls of the vial. When the agar was smeared with slime from E. rosea, however,

the larvae burrowed into the agar. If this agar contained homogenised E. rosea, the larvae penetrated the agar, but did not feed.

III - The host-parasite relationship

Infection of earthworms

When individual worms were placed in small glass tubes containing small pieces of moist filter paper, 12-15 larvae added to each tube, and the tubes place in darkness at room temperature (21-24°C), the following infection rates were observed (the numerator is the number of infections found, and the denominator the number of worms of that species tested): A. chlorotica 3/8; E. rosea 7/16; A. caliginosa 0/5; L. terrestris 1/5 and L. rubellus 1/5. The sites of these infections are given in table 46. When the experiment was repeated using 8-10 larvae per tube, with E. rosea, the rate was found to be 13/50. The sites of infection on these thirteen E. rosea are given in table 47. The larvae generally penetrated the upper surface of the worm, with fewer penetrations occurring towards either end of the host (figs. 16-17). Although the number of segments per worm varied, the worms were all approximately the same size (100-125 segments); thus the results are meaningful when considered together.

The progress of these thirteen infected worms was examined daily until the worm died, or until the larva

left the worm (fig. 20). Larvae usually left worms which had started to decompose. In a few cases the larva repenetrated a less decomposed portion of the worm.

When the infection rate was studied in loose soil, rather than in glass tubes, it was found that no worms were infected 24 hr after the start, but all worms had several larvae on the outside, making probing movements. The worms and larvae were returned to the soil for a further 24 hr after which time the worms had been infected. The following infection rates were found for loose soil: A. caliginosa 1/20 using 300 larvae; E. rosea 12/16 using 150 larvae.

The effect of varying the multiplicity of infection (the ratio of larvae to worms) on the penetration rate by the larvae intc E. rosea in loose soil was studied at 13°C and 20°C. It was found that at 20°C, many of the worms, both infected and uninfected, rapidly decayed (table 48). In control experiments with E. rosea in loose soil at 20°C, in the absence of larvae, no decay was observed. At 13°C few decayed experimental worms were found except at a multiplicity of infection of 10:1. The multiplicity of infection levels of 7.5:1 and less, at 13°C, were examined (table 49) to determine the effect of multiplicity of infection on the penetration rate in loose soil. It was found that the multiplicity of infection level had no effect on the penetration rate, a constant 14% of all larvae used penetrating successfully.

Since a constant penetration rate was found using standard conditions of loose soil, it was thought that by varying the soil conditions, varying penetration rates would be found, giving an insight into the effect of soil conditions on host location and penetration by the larvae. Bulk density and soil moisture content were shown to be of major importance to the earthworm host; thus the effects of these factors was studied in relation to larval penetration behaviour, under constant conditions of soil volume, multiplicity of infection, and temperature. Twenty E. rosea and 60 P. rudis larvae were used giving a multiplicity of infection of 3:1 in a soil volume of 87.5 cc at 15°C. The results of the study indicated that the penetration rate was much lower than in loose soil. In fact, in most instances, no penetrations at all were observed, in both soil type-I and type-II. The results of the pilot experiment indicated that the low success rate was partly due to the earthworm behaviour in the experimental soil mixtures, especially the surface casting and encystment behaviour, thus for this set of penetration experiments a record was kept of the earthworm behaviour (Appendix A). The worm behaviour was very unpredictable, but a few trends were apparent:

a) Surface casting occurs mostly in the high bulk density, high moisture soil, and is more prevalent in soil type-II.

b) Encystment is most frequent in soil type-II.

c) At the lowest moisture content (15%), in soil type-II, the worms died whether or not they were encysted.

d) Worms were often found to leave the soil; however, the conditions under which this behaviour was observed were very varied and could not be predicted.

In view of the large number of zero values for penetration (table 50), no statistical analysis of the 2^3 factorial experimental design was attempted.

The effects of bulk density and soil moisture content were also examined with respect to the maintenance of infected E. rosea. This was studied in soil type-II, with three replicates at 15°C and a fourth at 20°C (table 51). Most of the worms were dead by the end of one week, and the condition of the worms still living varied greatly from replicate to replicate. No difference in the fate of the infected worms was found between 15°C and 20°C. Considering the four replicates together, 43 of the original 180 worms were still alive after one week. Of the surviving worms 10 were encysted and 33 not encysted. Six of the 10 encysted worms were still infected (60%), whereas 14 of the 33 non-encysted worms were still infected (42%) after one week. Of the non-encysted worms which had lost the infection, 10 did so by autotomy of the infected segments (i.e. autotomy occurred in 30% of the non-encysted worms). Only one encysted worm lost the infection by autotomy

(i.e. autotomy occurred in only 10% of encysted infected worms). In view of the large number of deaths which occurred, no statistical analysis of the 2^3 factorial experimental design was performed.

Since survival of infected worms was best when the worms were encysted, experiments were conducted to determine the optimal encystment conditions. Using conditions as in the previous experiment (in which infected worms were added to soil of the required bulk density and moisture content), the behaviour of uninfected E. rosea at 15°C was studied (table 52). Encystment was restricted almost completely to the lowest moisture content, but at this moisture content, worms were also found to leave the soil, or to die without encysting. A different approach was used, therefore, in an attempt to increase the encystment rate. In this case, the experimental soil was initially moist, and was dried to the prescribed moisture level over a period of one week (table 53). When the results were analysed by a two-way classification experimental design, it was found that bulk density did not affect encystment, but that moisture content did. Using this drying-soil method, 61% of E. rosea encysted at 25% moisture content, and no worms left the soil.

When infected E. rosea were kept under these conditions, after a period of three weeks some worms were still infected, the larvae having reached the third instar (table 54).

Infected worms could be maintained for 6-10 days

in distilled water, the larvae remaining in the first instar. In vials without water, the infection was maintained successfully as far as the second larval instar, in two cases.

In the course of collecting soil samples for analysis, and worms for experimental purposes, a number of infected worms were found. Details of these field collections are given in table 55. Examples of earthworm behaviour in the field are given in table 56. At low moisture contents the worms encyst, although the size of the worms which encyst, and the depth of encystment, depend on the soil type. The data from site #2 indicate a preference for high bulk density under similar moisture conditions.

Effects of soil atmospheric conditions

It was thought that the low level of success found in maintaining infected worms in the laboratory might be due to adverse effects of the soil atmosphere. The effect of different gas mixtures on E. rosea and first-instar P. rudis larvae was therefore studied (table 57). Both worms and larvae were found to be able to tolerate gas mixtures much more adverse than occur in the field, under conditions more extreme than used in attempts to maintain infected worms in the laboratory, although the effect of the gas mixtures on the worms varied greatly, depending on the site and time of collection.

DISCUSSION

Relation of earthworm distribution to soil factors

From Tables 32-33 it can be seen that for the Ancaster area, the distribution of adult and juvenile *E. rosea* is correlated with no soil factor at a level greater than $r = 0.5$. For the Dundas area, the adult-worm distribution is positively correlated with silt, and negatively correlated with sand. Sand and silt themselves have a correlation coefficient of -0.97; thus it cannot be determined if the worms are reacting positively to the silt, or negatively to the sand. The adult-worm distribution is also positively correlated with volumetric soil moisture. Silt has a high positive correlation with moisture, while sand has a high negative correlation. In view of the studies by Hower (1965), it is certain that soil moisture is affecting the distribution, but whether or not soil texture is important cannot be determined from this analysis alone, as it is possible that the correlation between worm distribution and both sand and silt occurs as a result of the correlation between these factors and soil moisture. These factors do not completely account for the worm distribution in general, as no significant correlations are found in the Ancaster area.

It should also be noted that the distribution of juvenile worms is not correlated with the distribution of the adult worms.

The results of the stepwise regression (Tables 34-38) indicate that the distribution of juvenile worms for both sites is never as completely related to soil factors as is the distribution of the adult worms. This is almost certainly a reflection of the observation by Satchell (1955) that aggregations of young worms often occur as a result of reproduction. The effects of reproduction on the distribution of juvenile E. rosea cannot be separated from the effects of the soil factors at present, so only the factors affecting the distribution of the adult worms will be considered.

The adult-worm distribution of the Dundas site (according to the results of the regression analysis) is affected by sand and silt and by organic carbon. All of these factors are highly correlated with moisture. As with the correlation coefficient, it cannot be determined whether or not silt, sand and organic carbon are affecting worm distribution directly, or simply reflect the high correlation between these factors and moisture, which definitely does have an effect (Hower, 1965).

In the Ancaster area, the main factor to which the distribution of worms relates is temperature, with porosity or compactness of secondary importance. None of these

factors is important in the Dundas area. It should be noted, however, that each of these three factors is the resultant of a number of other factors, e.g. temperature is a function mainly of soil moisture and vegetation cover; compactness is a function mainly of bulk density (BD), porosity and moisture and possibly content of silt and clay; and porosity is very highly correlated with BD. Thus it cannot be determined if a factor itself, or a combination of these other factors, is determining the distribution of E. rosea.

Neither correlation nor regression therefore, gives a satisfactory insight into the soil factors which determine worm distribution. Although the distribution of adult worms within an individual area can be explained on the basis of factors extracted by the two analyses, when two areas are compared, it is found that different factors are important. Therefore, for more general application, an alternate explanation must be found.

To examine the distribution of worms and the soil factors within each area, and to compare the two areas, distributions were mapped, the limits of each isonome interval being chosen statistically (tables 2-31).

In the Dundas area (tables 2-16) there is a single environmental gradient going from a region of high sand and low moisture content to a region of low sand and high moisture. All other factors are correlated with one or other

of these two variables. The maximum population of adult E. rosea is found in the area of high moisture and low sand. In the Ancaster area, a similar type of gradient is found, but here the adults are distributed more or less evenly over the whole area. The only difference between the Dundas and Ancaster areas is that the high sand content in the Ancaster area is associated with an extremely high BD. It seems, therefore, that high bulk density can compensate for the low moisture, so that the two main factors influencing worm distribution are moisture and BD. This conclusion is supported by the results of the principal components analysis. The results for the Dundas area (fig. 5), show that here the distribution of adult worms is determined by moisture (% by volume). In the Ancaster area, the addition of a BD gradient to the first axis, in the opposite direction to the moisture gradient, spreads the worm distribution along the whole axis.

In conclusion, the distribution of E. rosea is mainly determined by moisture, but high BD can compensate for low moisture values.

Behaviour of first instar P. rudis larvae

In the present study, the maximum oviposition by the cluster fly was found to occur in strong light, high relative humidity, and at temperatures of 21 - 27°C. The observation of DeCoursey (1951) indicates that soil structure or some related characteristic may play a secondary

role. Hatching and larval survival are both highly dependent on high humidity. Indeed, in the case of larval survival, water in the liquid phase is essential, indicating that water loss by metabolic processes cannot be replenished by absorption of moisture from the air, through the cuticle. Hatching of the eggs, however, merely requires high humidity.

On hatching, the larva must locate and penetrate a host earthworm within three days in order to survive. In the absence of any reactions to the environmental factors studied, it would appear that a larva locates its host by random locomotion through the soil. This conclusion is supported by the fact that high success of location and penetration can occur in loose, homogeneous soil, at constant temperature and in the absence of significant gradients. The only factor which has been shown to affect the direction of locomotion is the angularity of surface irregularities, and here the effect depends on the angle of approach. It seems that the larvae do not actively burrow, but move through the soil macropores, root channels, worm burrows, and spaces produced as a result of soil disruption by growing vegetation (figs. 18-19). Larvae have never been seen to construct their own burrows.

The reactions of the larvae to crushed earthworms recorded by Pimentel and Epstein (1960) were confirmed in the present study, the larvae feeding on all species tested, namely E. rosea, A. caliginosa, A. chlorotica, L. terrestris,

L. rubellus and Eiseniella tetraedra. It was found that contact with coelomic fluid or slime induced penetration behaviour, slime retaining it activity longer than coelomic fluid. Coelomic fluid is extruded by the worm from the dorsal pores, and it may be that it becomes mixed with the slime on the cuticle, imparting the penetration-inducing properties to the slime, or it is possible that the slime also inherently contains the factor. The factor loses its activity on dilution or on drying, preventing an accumulation of the factor in the soil. Apparently, when the larva is moving randomly through the soil, it contacts earthworms, whereupon penetration is induced by a substance present in the slime on the surface of the worm. Highest host location rates would be expected at 20°C, the temperature at which the rate of locomotion is maximal (table 44).

The host-parasite relationship

The host species of earthworms recorded in the literature are indicated in Table (B). In this area, the naturally-occurring hosts were A. chlorotica and E. rosea, both in the juvenile and adult stages. A. caliginosa, L. rubellus, L. terrestris, and Octolasium lacteum (Orley), all of which occurred commonly, were never found infected. DeCoursey (1932) reported finding A. caliginosa infected in the field, but no other workers have confirmed this. It should be noted however, that recent, as yet unpublished work by Gates and Reynolds (J. W. Reynolds, University of Tennessee,

pers. comm. 1971) has shown that A. caliginosa is actually a complex of at least five distinct species, each with different behaviour and ecology, existing at different levels in the soil. This new information would explain the conflict in the results. That is, DeCoursey was studying a different species of the A. caliginosa complex.

In the laboratory, it was possible to infect E. rosea, A. chlorotica, A. caliginosa, L. rubellus, and L. terrestris, i.e. all the species tested. E. rosea and A. chlorotica were easily infected, but the other species were more difficult to infect. Of five specimens each of L. rubellus and L. terrestris, only one individual of each species was parasitised. Of twenty A. caliginosa, using three hundred larvae, only one individual was parasitised. Infection occurred most often on the upper surface, and rarely towards the ends of the worm, although cases of infection in the pre-clitellar region or in the last two or three segments were noted.

Two or more larvae frequently shared a penetration site under laboratory conditions, up to five larvae being found in a single clump. Up to seven larvae were recorded penetrating a single worm at different points along the body.

In the introduction, the controversy as to whether or not the cluster-fly larvae are parasitic during the whole of their development was mentioned. In the present study,

the observation of DeCoursey (1927), that the larvae frequently withdraw from the worm, was confirmed. In almost all cases, withdrawal occurred when the condition of the worm deteriorated due to the onset of decay. However, larvae were observed to repenetrate undecayed portions of the same worm. Larvae which failed to repenetrate a worm died rapidly.

All larval instars were found feeding within the body of the host, with only the posterior spiracular plate exposed to the surface, but third instar larvae were also found feeding while exposed on the surface of the worm. Third instar larvae were found feeding on worms which showed very little damage, both in the field and in the laboratory. Such instances might occur shortly after a larva had started feeding on a new host suggesting that larvae may parasitize several hosts in the course of their development. First instar larvae which had left infected E. rosea were found free in the soil three weeks after the time of infection, although three weeks is reported as being the time required for the complete larval development (Pimentel and Epstein, 1960). This would be explained by the interruption of development while moving between hosts. Although at first, the larvae must repenetrate a host rapidly or die, towards the end of the first instar they seem to be able to withstand longer separations from a host. This ability is enhanced in the second and third instars.

The time spent in each instar cannot be determined exactly, therefore, as it depends on the number of hosts parasitised and thus on the behaviour of the host.

Autotomy of the infected segments by the host was recorded on many occasions, especially under conditions which were not conducive to encystment by the host.

The effect of multiplicity of infection (MOI) and temperature on the parasitism of E. rosea under standard conditions of loose soil was studied. In general, a constant proportion (14%) of larvae were successful in penetrating a host. This was subject to some qualifications, however, as follows:

At very high MOI levels, and also at higher temperatures, after the three-day infection period, large numbers of uninfected decaying worms, and fewer infected worms, were found. Further observations at 20°C, the higher temperature, showed that penetration rates similar to 13°C could be found after 36 - 38 hr, but at that temperature, by three days decay set in and the larvae left the worms. At 15°C, with high MOI levels, the high mortality of worms was probably due to the greater number of attacks by the cluster-fly larvae.

It had been shown earlier in this study that the main factors affecting the host, E. rosea, were soil moisture and bulk density. A series of experiments was set up to study the effect of these factors on the number of

successful penetrations using constant MOI levels. The number of successful penetrations using these experimental soil mixtures was almost zero, regardless of the levels of the soil factors used, whereas in loosely packed soil mixtures, a high rate of parasitism was observed. A possible reason for the very low penetration rate in the experimental soil mixtures can be found by considering a hypothetical soil in which it is assumed that soil pores are contiguous, worm tunnels being included in the soil macropores. Larvae moving randomly through the pores should eventually encounter a host. The time taken to encounter a host will depend on the rate of locomotion, the number of host worms per unit volume of soil, and the total volume of pore space to be searched. If the number of penetrations is directly proportional to the number of contacts, as can be concluded from Table 53, then within limits, the number of penetrations should increase as the pore space to be searched decreases.

In experimental conditions in which the pore space was maximal, i.e. when the soil was loosely packed, a more or less constant penetration rate of 14% was observed. However, with lower porosity, due to higher BD, the penetration rate fell to almost zero, and did not show the expected increase.

This result is almost certainly due to the fact that in the experimental soil mixtures, the pore spaces were

not contiguous, as was postulated in the hypothetical soil, and as would occur to a large extent in natural soils; thus the larvae would be unable to get down into the soil to contact the worms. At present, no method of simulating the pore morphology in the laboratory is known, so that nothing more can be said about this aspect than to observe that the pore morphology is important, rather than simply the absolute value of the porosity determined by the BD level.

The interpretation of the few results available regarding penetration rates in experimental soil mixtures is made even more complicated by the fact that the earthworm behaviour varies with the soil conditions; casting, burrowing and encystment behaviour all changing with soil moisture and bulk density.

The same problem was encountered in evaluating the effects of the soil factors on the maintenance of the infection in the laboratory. It was found that the success of the infection was largely dependent on the encystment behaviour of the host, as unencysted, infected worms either decayed or lost the infection by autotomy. As was indicated above, the encystment behaviour of the host depends on the levels of the soil factors, and probably also on the size of the host and its physiological condition. That is, the success of the infection depends on the host behaviour, which in turn depends on the soil conditions.

When infected E. rosea were kept under conditions which induced high encystment rates, it was possible to rear cluster-fly larvae through to the last larval instar, feeding on the outside of a host. This is the last stage of the host-parasite relationship. In the course of this study, the host-parasite relationship has thus been examined at all stages, and an insight obtained into the main factors which influence its success.

The possibility that the success of infection was determined by soil atmospheric conditions, i.e. either the worm or larva being adversley affected by high CO_2 levels or low O_2 levels, or both, was ruled out by a series of experiments in which the O_2, CO_2 and N_2 levels in the atmosphere were varied.

The main study of the effects of known atmospheres on earthworms is by Nagano (1934), on E. foetida, in which the effect of 100% N_2 was studied. In pure N_2, he found that E. foetida could live for 15 days. This result is highly suspect, as he only flushed the N_2 throught the apparatus for 5 min and failed to check whether this was adequate. In the present study, it was found for E. rosea that 100% N_2 was lethal over a period of 24 hr. However, E. rosea survived at extremely low levels of O_2 (down to 0.5%) and high levels of CO (up to 15% at the 0.5% O_2 level). It would thus appear that Nagano's experiments allowed some air to remain in the system, permitting the worms to survive for a long period.

In the present study, it could be seen that E. rosea has an extremely high tolerance of low O_2 and high CO_2 levels (which would be expected in streambank areas), although it was impossible to define limits to the tolerance, as this changed with site of collection, and also with time (Table 57). The cluster-fly larvae also can tolerate low O_2 and high CO_2 levels, levels much more extreme than reported in the soil (Russel and Appleyard, 1915). It thus appears very unlikely that the soil atmosphere affects the host-parasite relationship. This conclusion is supported by the fact that the infection can be maintained in distilled water for 6 - 10 days. This tolerance by the P. rudis larvae is a feature unusual in Cyclorrhaphous Diptera. The soil atmosphere, however, may have an indirect effect on the host-parasite relationship by influencing wound healing. Stephenson (1930) states that regeneration of earthworm tissue is greatly impaired when the O_2 level falls much below the normal atmospheric level. However, he gives no reference to the method and results on which this conclusion is based.

The host-parasite relationship has thus been traced from the initial penetration of the host by the first instar larva, to the final stage when the third instar larva was feeding on the outside of the host. At all stages, it appears that the relationship is affected by the behaviour of the host, which in turn is affected

by soil conditions. Before any further conclusions can be reached, it is evident that a detailed study of the effects of soil conditions on the behaviour of the host, is required.

One fact remains to be discussed; that P. rudis larvae infect only a few host species in the field, although other species of worms may be present in large numbers. No experimental investigation has been conducted on this aspect, but a few reasons may be suggested. (It should be remembered that many non-host species have slime which induces penetration behaviour in the laboratory).

Oviposition is influenced by high humidity, which will be maintained both by high soil moisture and by dense surface vegetation. Soil in which the surface vegetation is insufficiently dense will tend to have lower surface humidity. This situation would be found in many woodland sites; thus woodland species of worms are unlikely to become hosts.

With highly active worm species, the larva may be unable to penetrate the worm before it is rubbed off. Worm activity can be related to the availability of food, to temperature, and to moisture (Evans, 1947). One other factor of worm behaviour is of importance, the depth of burrowing in the soil. Of the commonly occurring worm species in the Hamilton area, only E. rosea and A. chlorotica are surface-dwelling species, occurring mainly within the top 10 cm of soil.

Where activity is not limiting, the integument of some worm species may provide a mechanical barrier to penetration. In some worm species, there may be a reaction against the larva as indicated by Keilin (1915), although no such reaction was observed in the present study. Thus at all stages of the host-location and penetration behaviour, factors may be operating to restrict the host range.

The results of this study could be used as a basis for predicting cluster-fly population explosions from climatological data once the effects of climatic and pedological factors on E. rosea behaviour is known. The evaluation of these effect on E. rosea would be a logical next step in the study of cluster-fly ecology.

SUMMARY

The distribution of E. rosea, the main host species of earthworm of the cluster-fly, Pollenia rudis, was determined mainly by soil moisture and bulk density. The cluster-fly larva located a host by random locomotion through the soil pores, whereupon penetration was induced by a substance present in the worm slime.

In the laboratory, slime of several species of worms induced penetration behaviour, namely E. rosea, A. chlorotica, A. caliginosa, L. rubellus, L. terrestris and Eiseniella tetraedra, i.e. all the species tested. All these species were infected in the laboratory with the exception of E. tetraedra, which was not tested. In the field, only E. rosea and A. chlorotica were found infected. Both adult and juvenile worms acted as hosts.

Larvae pentrated mainly on the upper surface, with fewer penetrations occurring towards the ends of the worms. Up to seven larvae were found penetrating a single host. It was often found that larvae would share a penetration wound, up to five being found in a single clump.

Under standard conditions of loose soil, a more or less constant proportion of larvae succeeded in locating and penetrating a host. Under a high number of attacks,

however, or at temperatures much greater than 15°C, the infected worms rapidly decayed.

The penetration rate was influenced by the pore morphology of the soil, and by the burrowing, casting, and encystment behaviour of the host worms, which in turn was affected by the soil conditions, especially soil moisture and bulk density. The success of maintenance of the infection in the laboratory was likewise dependent on these aspects of the host behaviour.

It is unlikely that the success of the infection was affected by soil atmospheric conditions, as both larvae and hosts could tolerate much lower O_2 and much higher CO_2 levels than occur naturally in soil.

The time spent in each instar was found to vary considerably, depending on the number of hosts used. All instars were found feeding within the worm, with only the posterior spiracular plate exposed. The third instar was also found feeding on the outside of the host's body.

It was often found that infected E. rosea autotomised the segments containing the larvae. This behaviour was restricted almost completely to unencysted worms.

At all stages, the host-parasite relationship was affected mainly by the host behaviour, which in turn was influenced by soil factors, mainly moisture and bulk density.

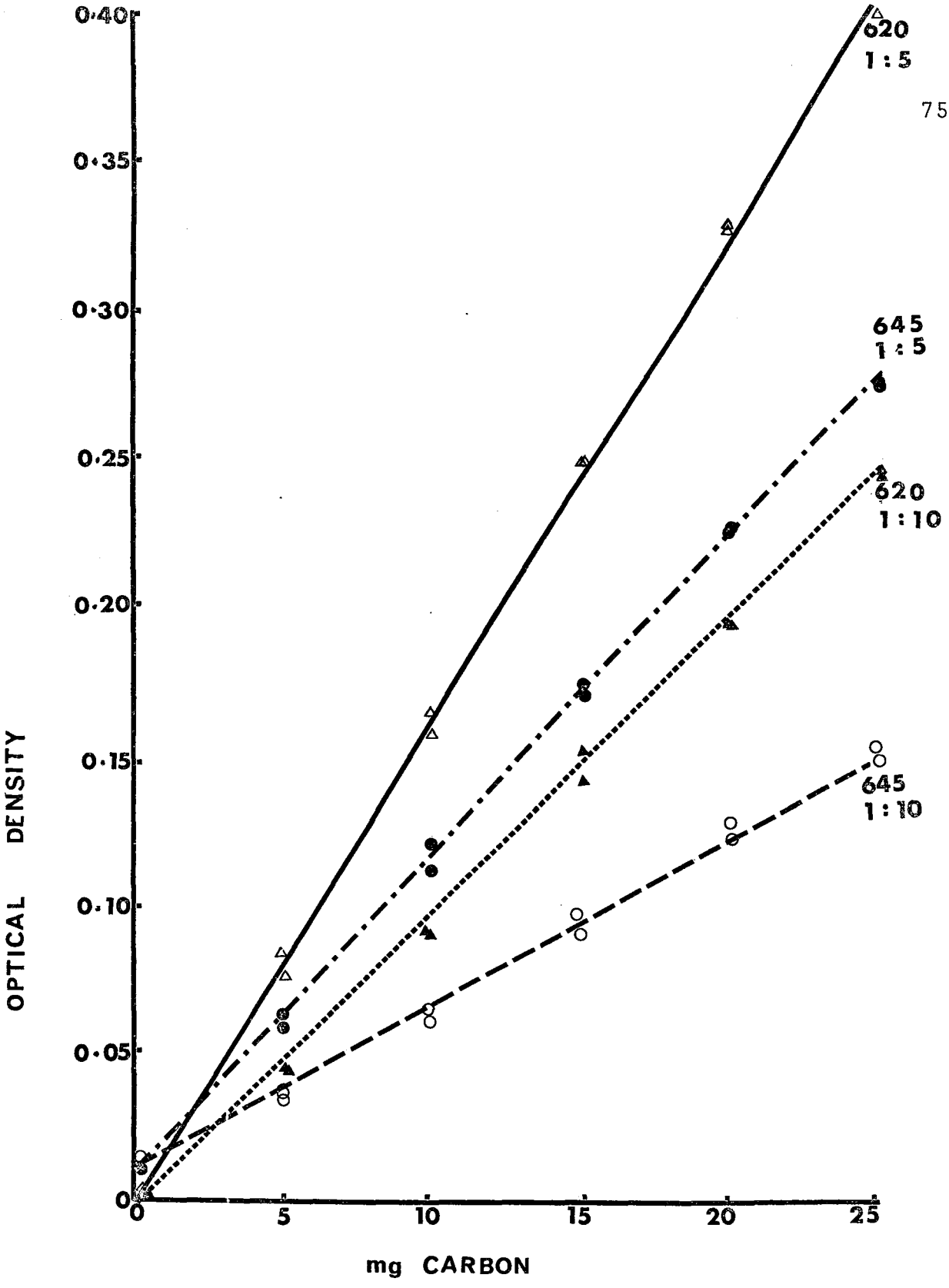


Figure 1

Sucrose calibration curves

Wavelength and dilution factor indicated

Figure 2

Dundas area.

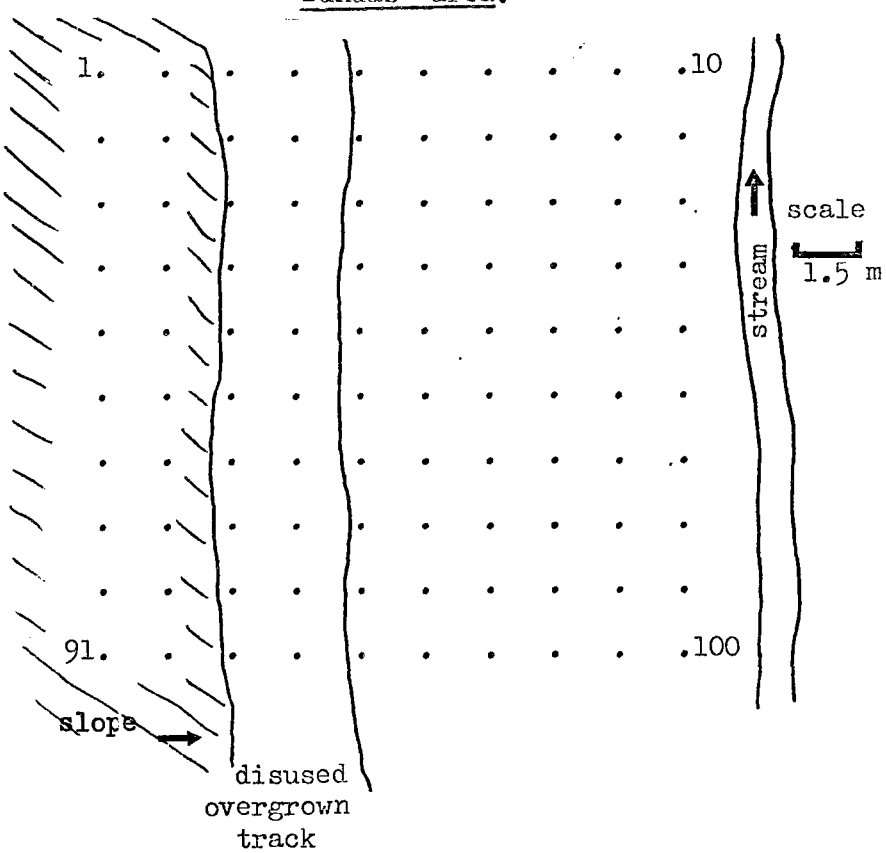
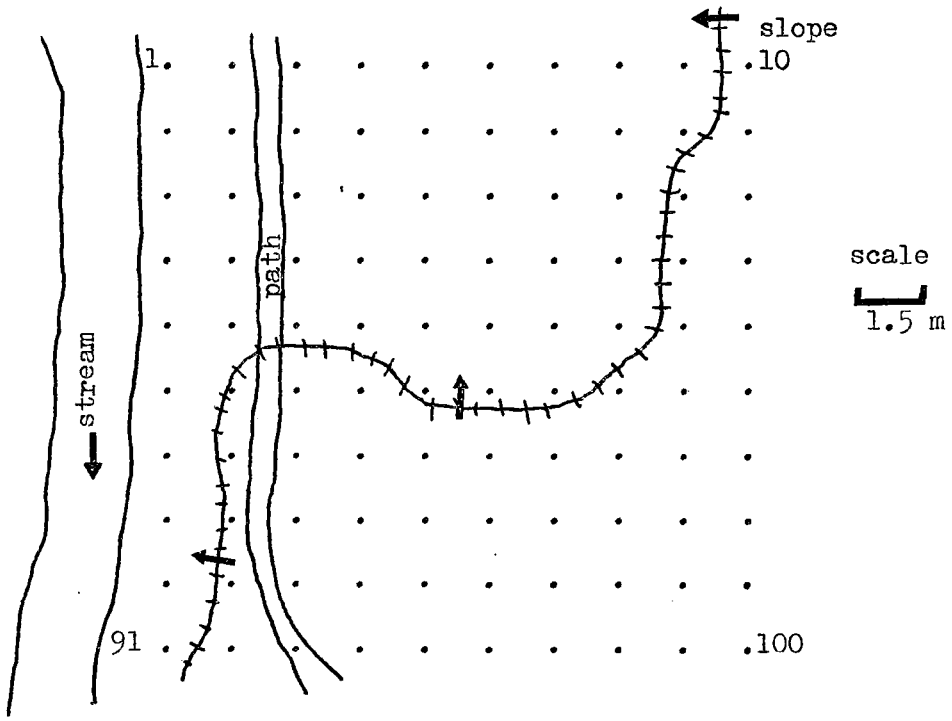


Figure 3

Ancaster area.



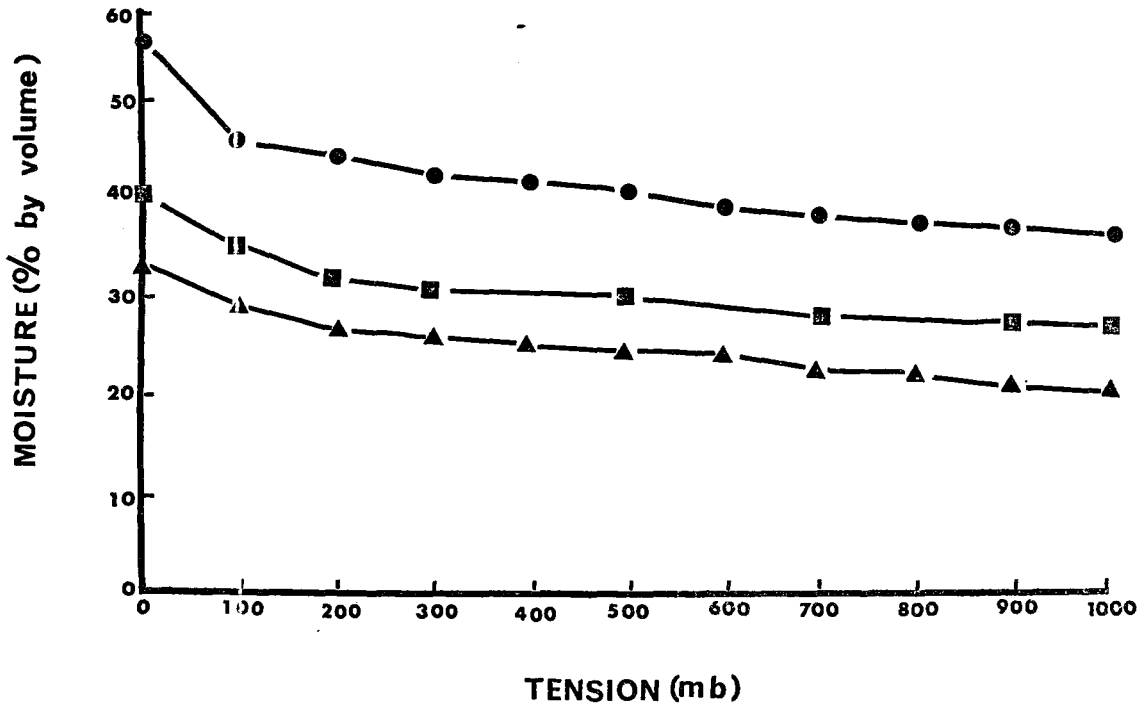


Figure 4

Moisture tension curves.

(● — ●) Dundas site #6, (■ — ■) Ancaster site #33, (▲ — ▲) Ancaster site #85.

Figure 5

Environmental gradients corresponding to the first three axes of the Principal Components Analysis, Dundas area.

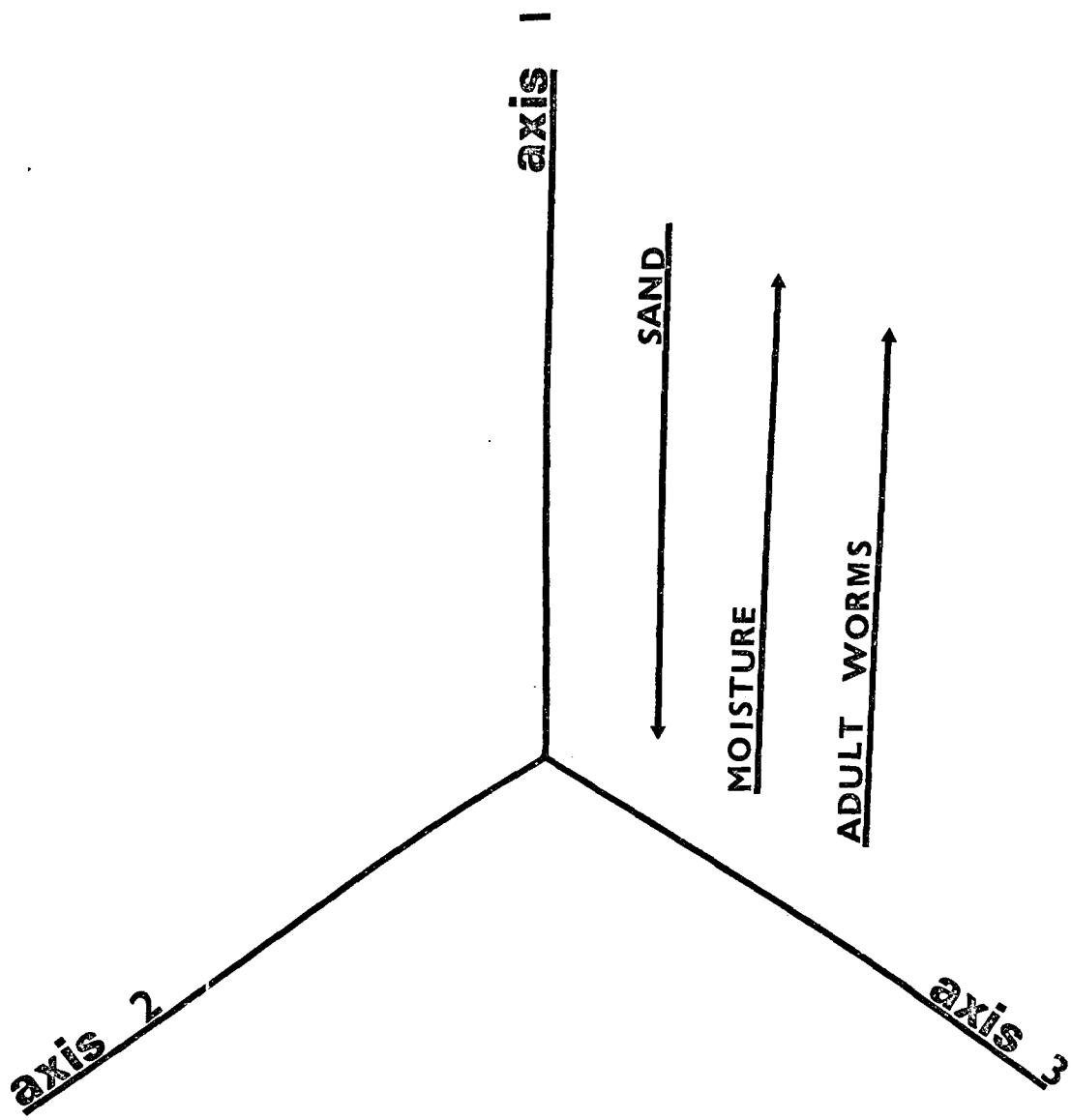
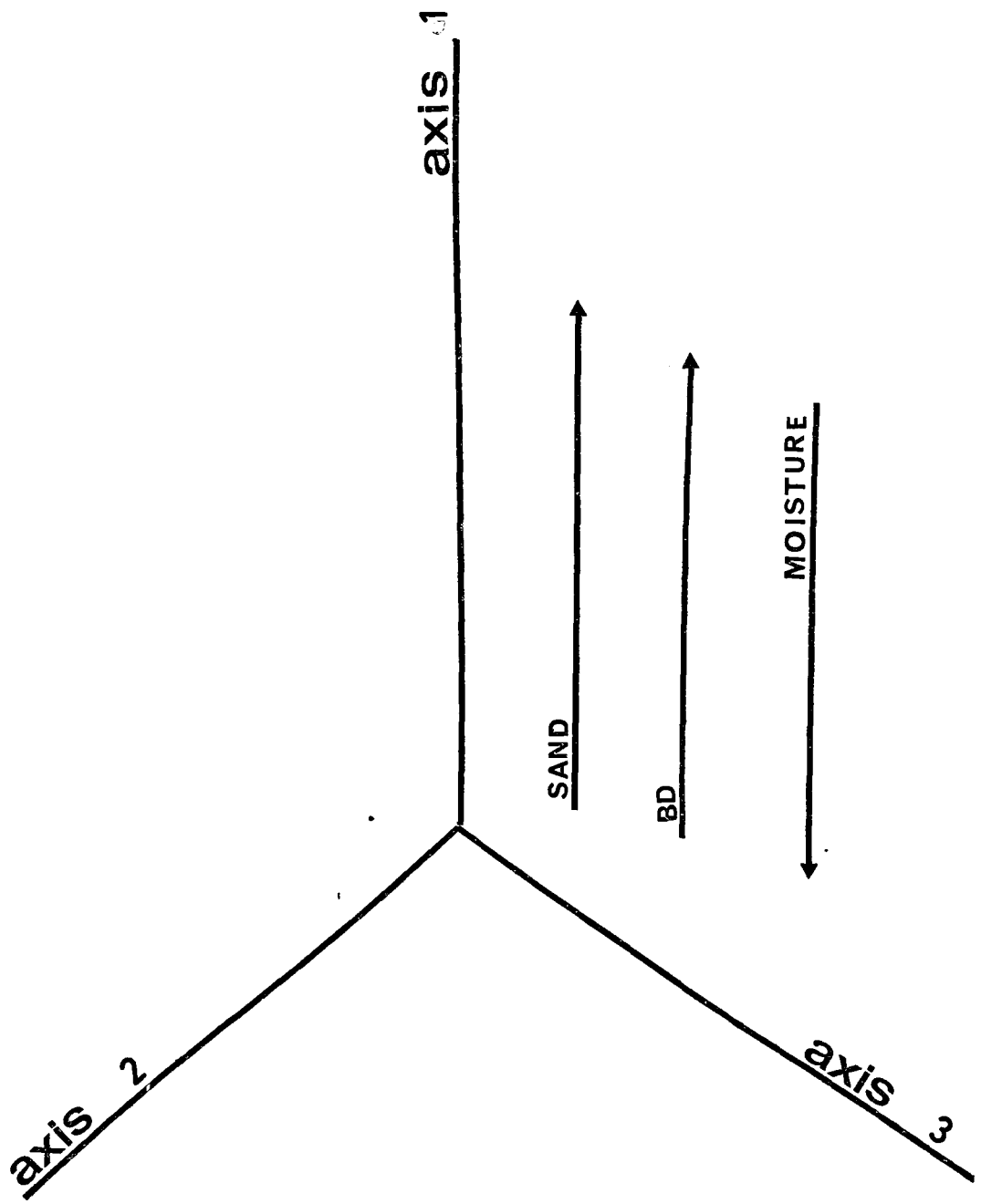


Figure 6

Environmental gradients corresponding to the first three axes of the Principal Components Analysis, Ancaster area.



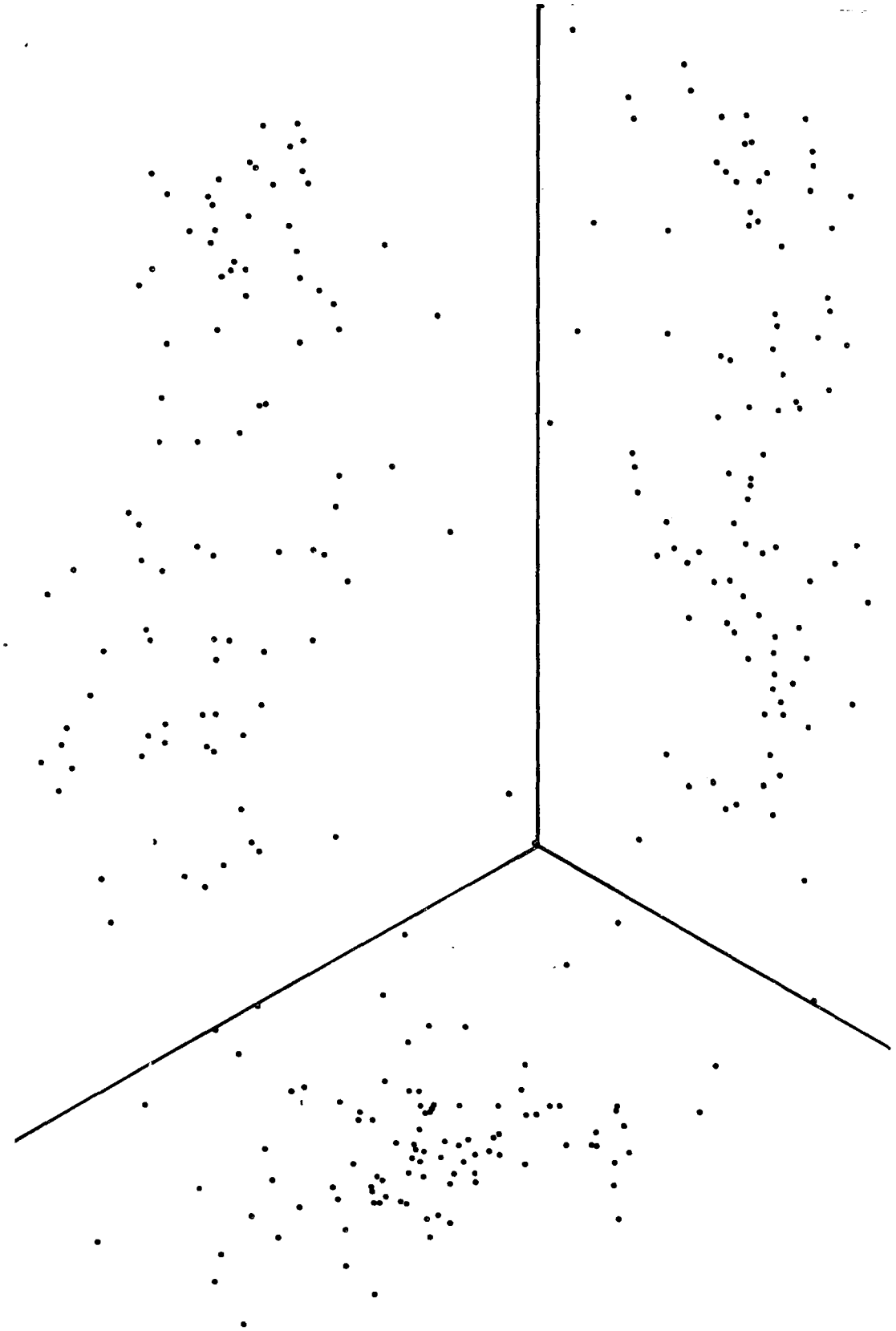


Figure 7. Ordination study - Dundas plot distribution based on Principal axes.

Figure 8. Ordination study - Ancaster plot distribution based on Principal axes.

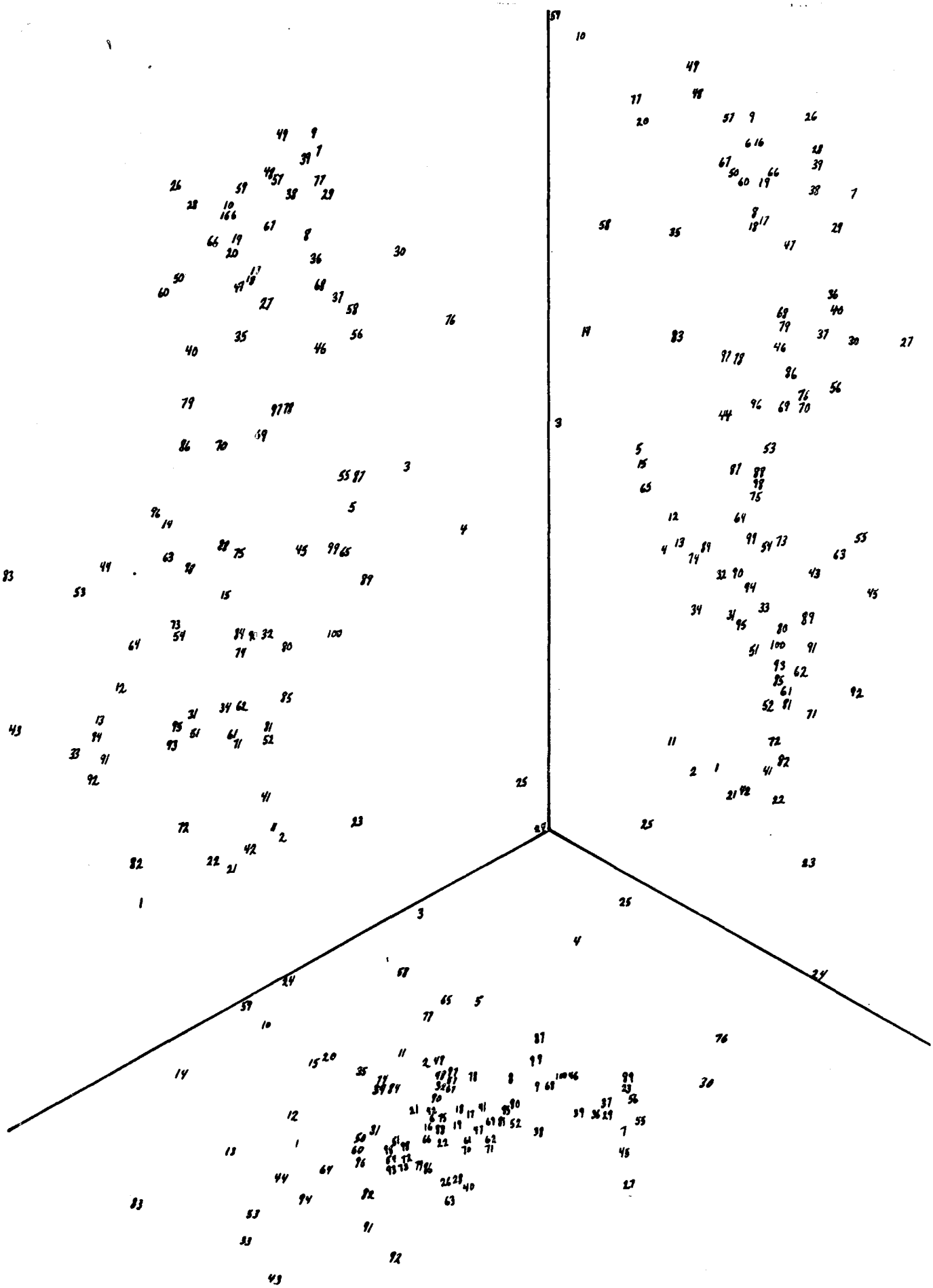


Figure 9. Ordination study - Dundas sample site numbers.

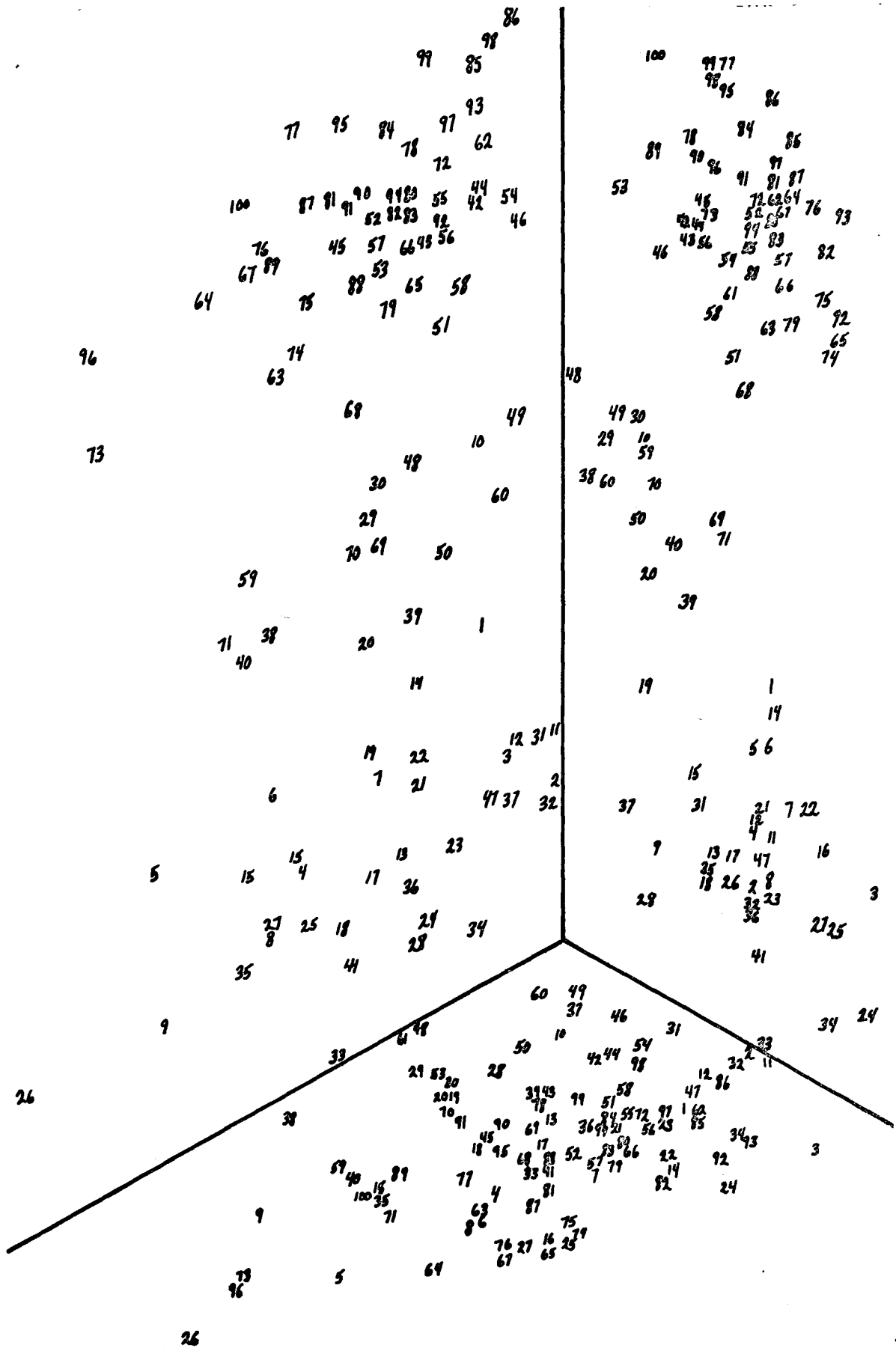


Figure 10. Ordination study - Ancaster sample site numbers.

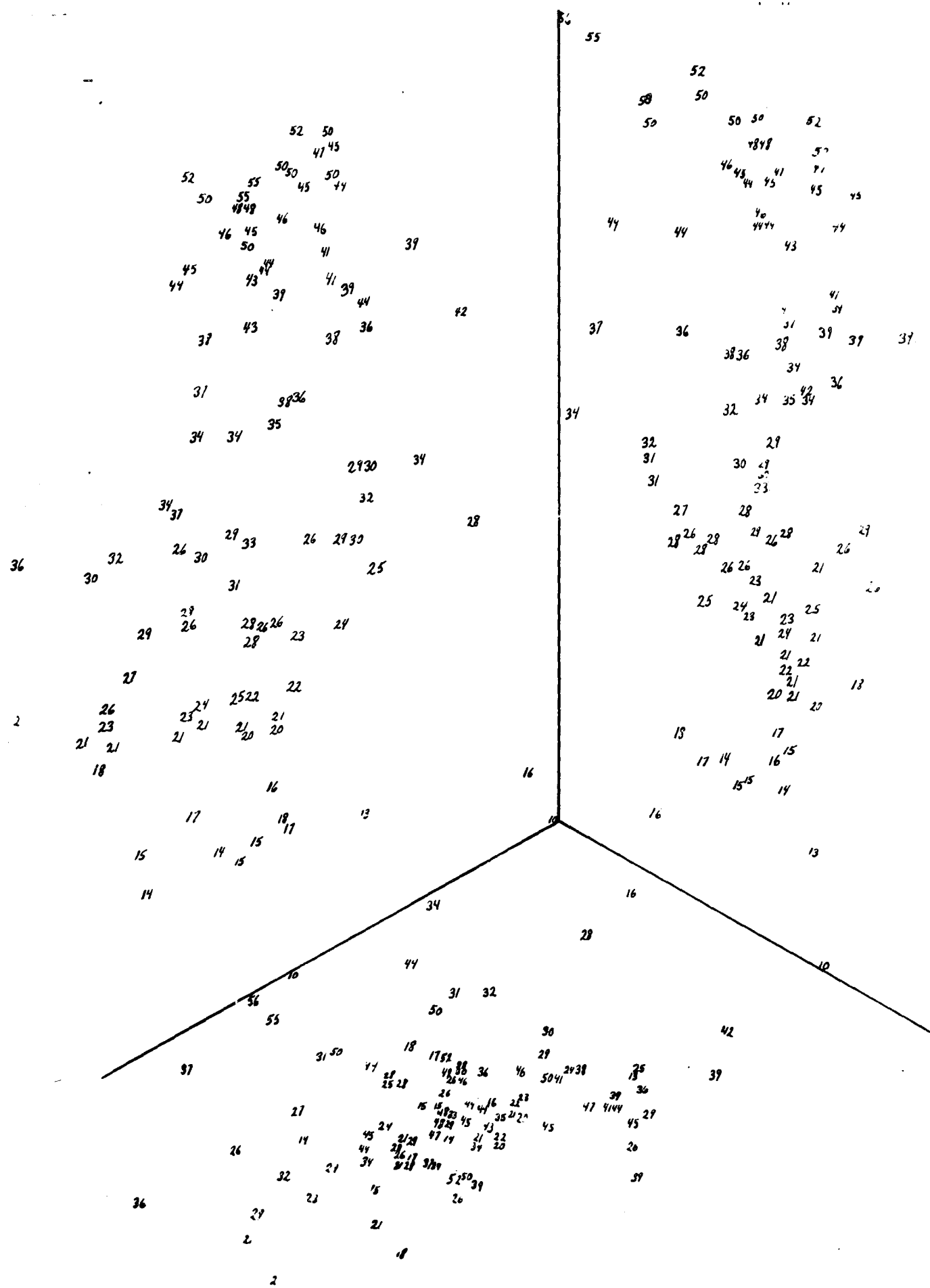


Figure 11. Ordination study - Dundas soil moisture values.

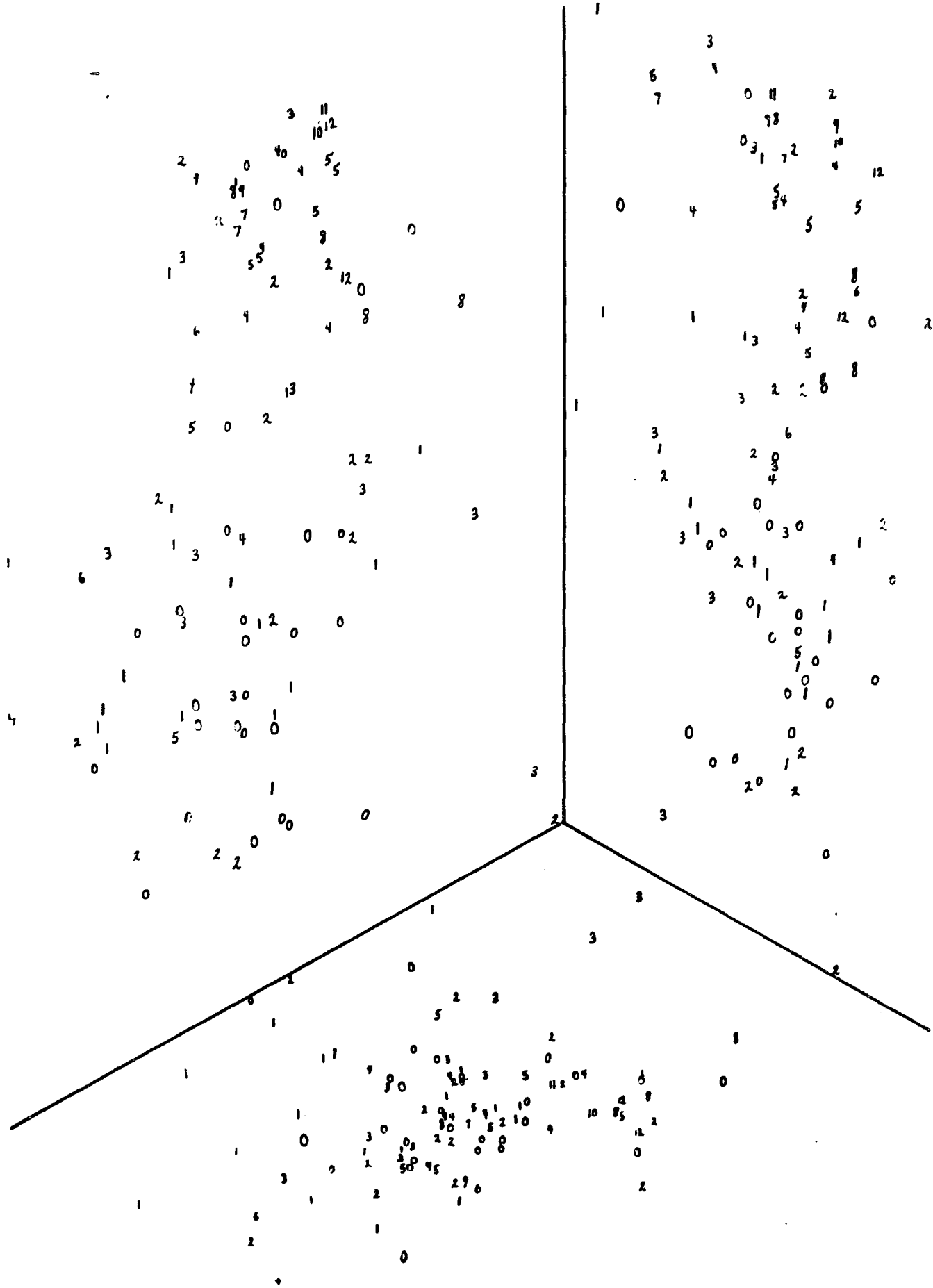


Figure 12. Ordination study - Dundas, number of adult E. rosea per site.

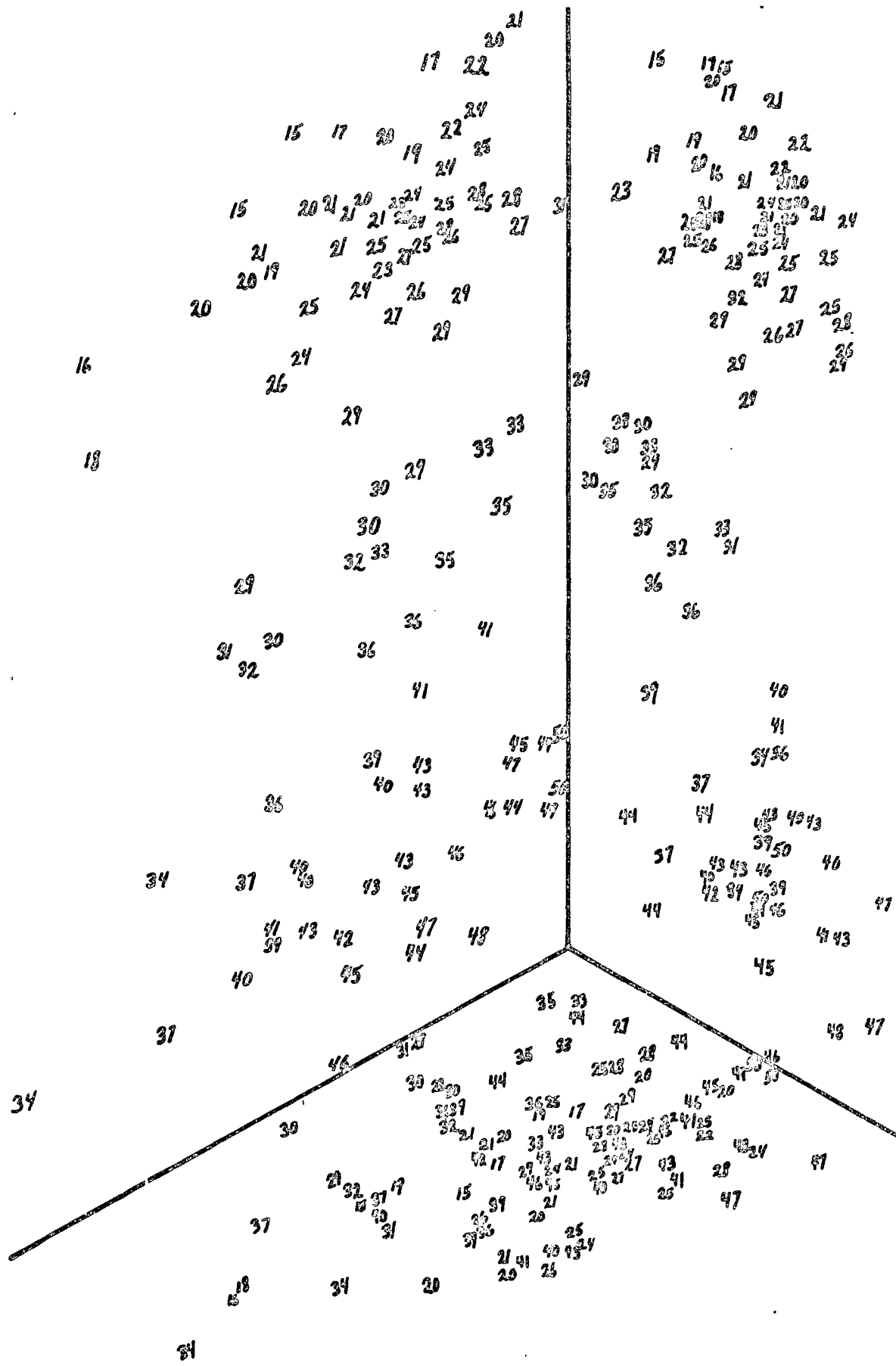


Figure 13. Ordination study - Ancaster soil moisture values.

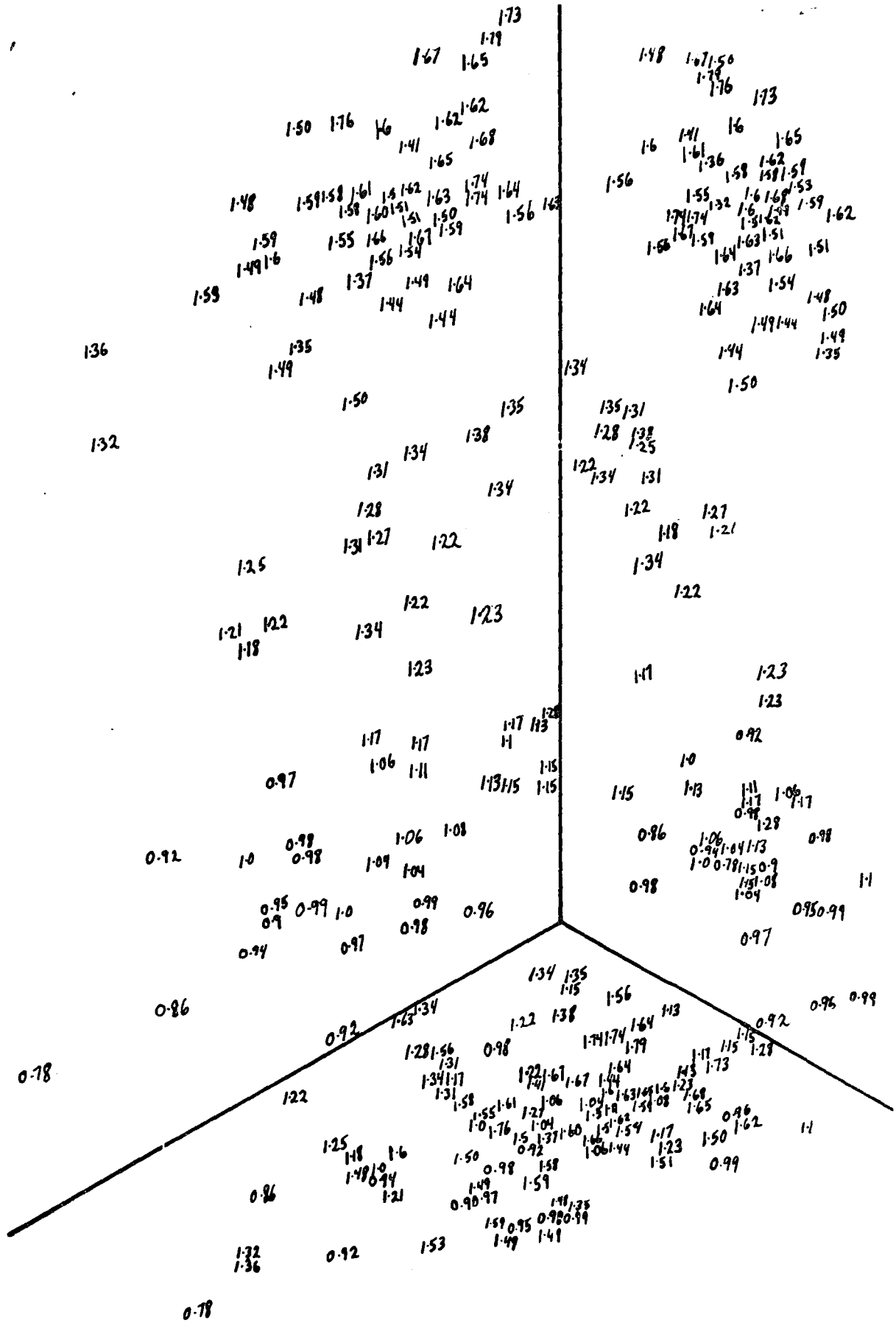


Figure 14. Ordination study - Ancaster bulk density values.

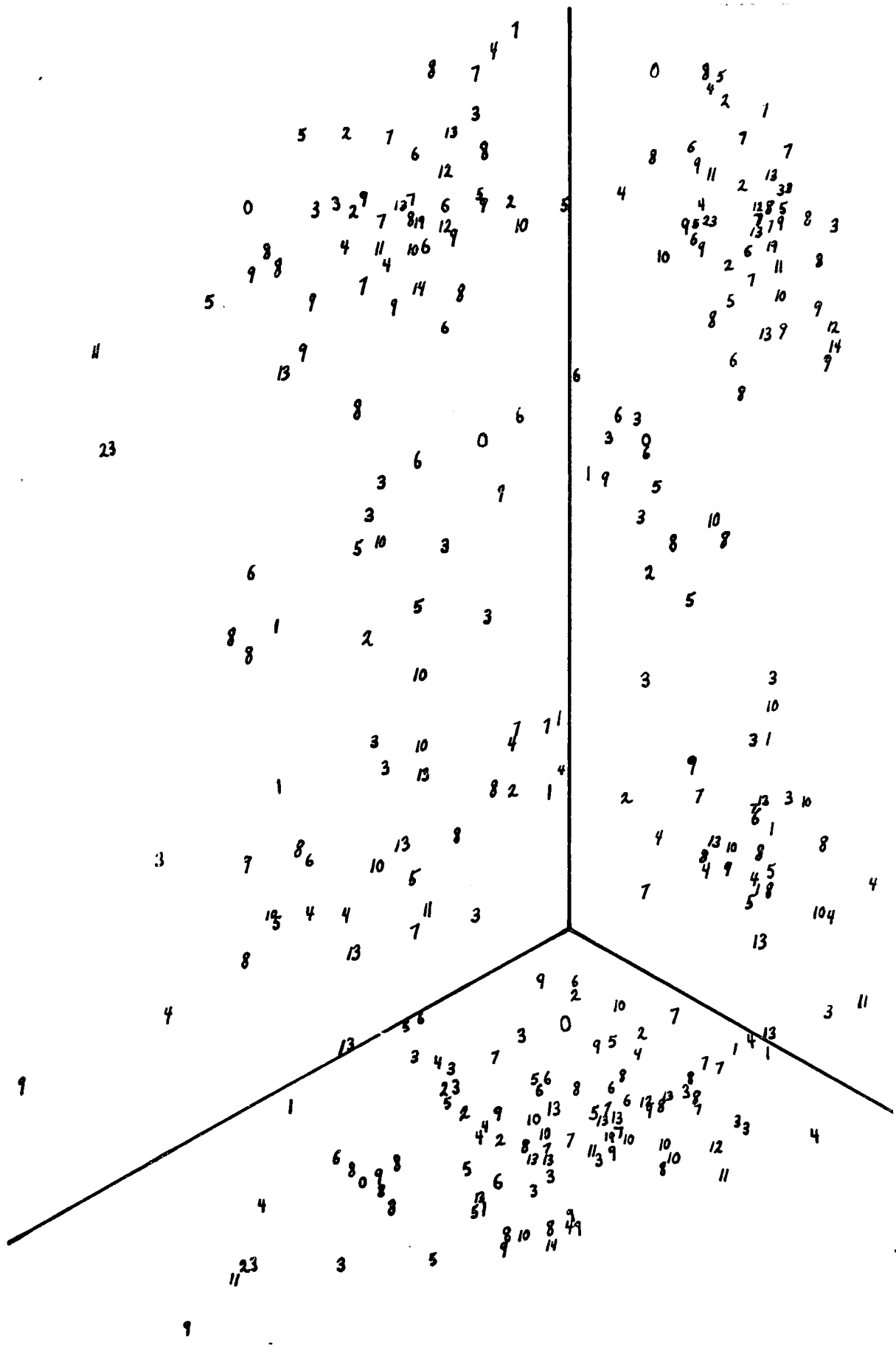
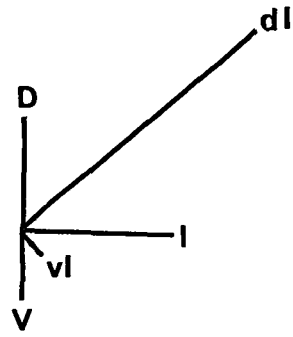


Figure 15. Ordination study - Ancaster, number of adult E. rosea per site.

Figure 16

Preferred penetration site of first-instar cluster-fly larvae around the circumference of E. rosea.



larvae penetrating in this sector

1 5 10

D dorsal
dl dorso-lateral
l lateral
vl ventro-lateral
V ventral

Figure 17

Preferred penetration site of first-instar cluster-fly larvae along the length of E. rosea.

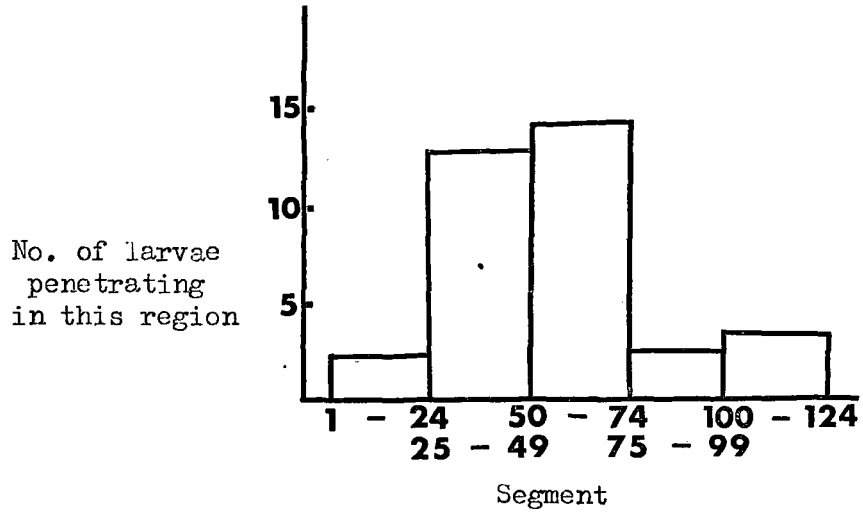


Figure 18

Soil section illustrating the movement of first-instar cluster-fly larvae through the soil. Larva (1) can be seen emerging from a soil macropore. Larva (2) is following the space produced by the growth of root (3).

Figure 19

Dorsal view of larva moving through a soil macropore, across the surface of a soil ped. The opaqueness is due to the impregnating medium, which filled the soil pores.

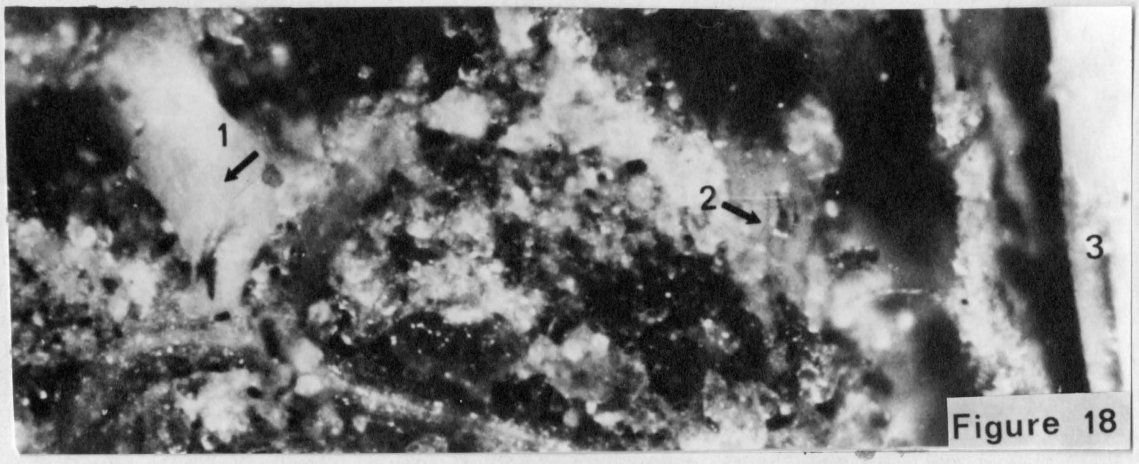


Figure 18

Figure 19

Figure 20

Daily progress of the infected E. rosea of table 47, in loose soil at room temperature (21 - 24°C).

——— worm healthy. - - - - worm decaying. X worm dead.

↑ larva left worm. ⊓ larva repenetrated in different position.

A worm lost infection by autotomising the infected segments.

Table 1

Code for worm stage and soil factors

Stage or Factor	Alphabetic code
Juvenile *E. rosea*	WORMJ
Adult *E. rosea*	WORMA
Temperature (°C)	TEMP
pH	PH
Compaction	COMP
Bulk density	BD
Moisture (% by weight)	H2OW
(% by volume)	H2OV
Porosity	POROS
Gas-filled porosity	GFPOR
Loss on Ignition	LOI
Organic carbon	OC
Sand	SAND
Silt (total)	SILTT
Clay	CLAY

Table 2

Distribution of juvenile E. rosea, Dundas area.

4.0	0.0	8.0	3.0	7.0	16.0	8.0	11.0	8.0	0.0
0.0	5.0	15.0	2.0	5.0	8.0	6.0	24.0	4.0	8.0
2.0	8.0	9.0	6.0	8.0	2.0	5.0	6.0	7.0	5.0
2.0	6.0	11.0	11.0	13.0	4.0	5.0	6.0	6.0	9.0
2.0	6.0	3.0	6.0	7.0	6.0	16.0	4.0	6.0	3.0
3.0	2.0	4.0	7.0	6.0	7.0	6.0	6.0	3.0	6.0
0.0	2.0	5.0	3.0	6.0	8.0	6.0	7.0	2.0	0.0
3.0	0.0	2.0	2.0	1.0	11.0	10.0	0.0	5.0	0.0
3.0	0.0	5.0	1.0	6.0	5.0	2.0	3.0	1.0	7.0
7.0	0.0	8.0	5.0	4.0	1.0	1.0	10.0	6.0	2.0

Key to map intervals

blank		
0.00 - 4.04	4.05 - 8.99	9.00 - 24.00

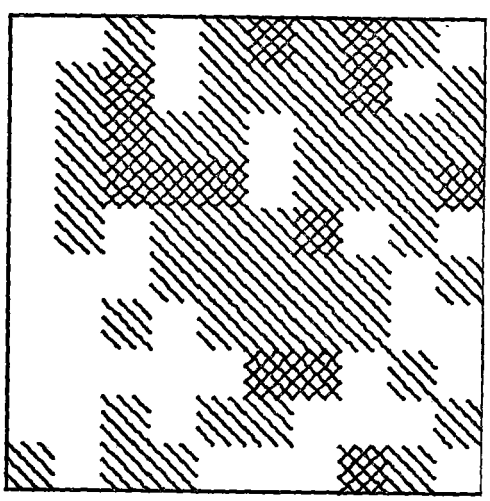


Table 3

Distribution of adult E. rosea, Dundas area.

0.0	0.0	1.0	3.0	3.0	9.0	12.0	5.0	11.0	1.0
0.0	1.0	1.0	1.0	1.0	8.0	4.0	5.0	7.0	7.0
2.0	2.0	0.0	2.0	3.0	2.0	2.0	9.0	5.0	0.0
0.0	2.0	2.0	3.0	4.0	8.0	12.0	4.0	10.0	6.0
1.0	0.0	4.0	3.0	0.0	4.0	5.0	4.0	3.0	3.0
0.0	0.0	6.0	3.0	2.0	8.0	0.0	0.0	0.0	1.0
0.0	0.0	1.0	0.0	2.0	2.0	0.0	2.0	2.0	0.0
0.0	0.0	0.0	0.0	4.0	8.0	5.0	3.0	4.0	0.0
1.0	2.0	1.0	0.0	1.0	5.0	2.0	0.0	1.0	1.0
1.0	0.0	5.0	1.0	1.0	2.0	1.0	3.0	0.0	0.0

Key to map intervals

blank — 0.00 - 2.04

2.05 - 5.99

6.00 - 12.00

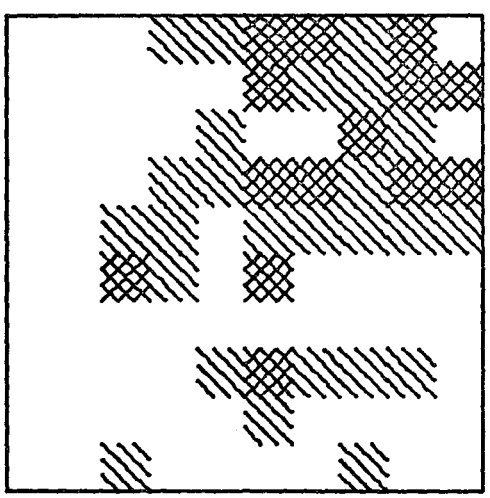


Table 4

Distribution of soil temperature values(°C), Dundas area.

18.0	16.5	17.2	16.0	14.5	13.0	13.2	15.0	15.0	15.5
18.0	17.5	17.0	17.0	17.0	14.5	16.0	14.5	15.5	15.0
15.5	16.0	16.0	16.0	14.8	14.5	14.5	14.5	15.0	15.0
17.0	16.0	17.0	17.5	15.0	16.8	15.5	16.0	17.0	17.0
20.0	20.0	19.5	19.5	17.0	16.5	15.0	15.2	16.0	17.0
20.5	19.5	18.5	19.0	18.5	17.0	17.3	16.9	16.5	17.0
20.0	20.0	19.0	19.0	17.8	14.0	13.0	13.0	12.7	12.5
14.0	14.5	14.0	14.5	14.0	14.5	14.0	13.8	14.0	15.0
17.0	17.0	15.8	17.5	15.5	15.5	15.0	15.5	15.0	16.0
18.5	18.0	15.0	15.0	15.0	14.8	14.0	13.8	13.5	13.5

Key to map intervals

blank		
12.50 - 15.00	15.01 - 17.00	17.01 - 20.50

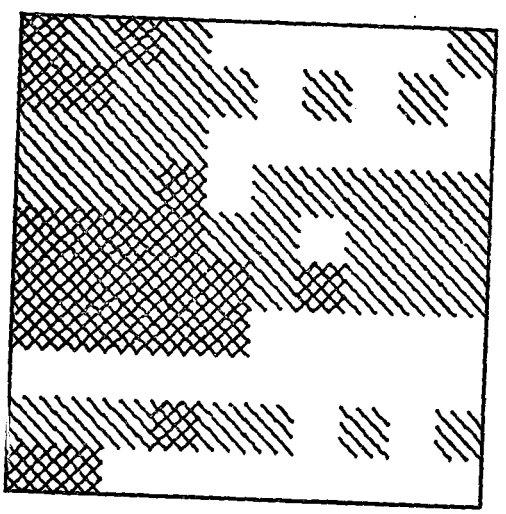


Table 5

Distribution of pH values, Dundas area.

7.5	7.6	7.8	7.8	7.9	8.0	8.0	8.0	7.9	8.2
7.4	7.5	8.0	7.8	7.9	7.8	7.9	8.0	8.1	7.8
7.5	7.6	7.8	7.5	7.9	8.0	8.0	8.0	7.9	8.0
7.5	7.4	8.0	7.8	8.1	7.8	7.8	7.8	8.0	8.1
8.0	8.0	7.9	8.2	8.1	7.8	8.1	7.8	8.0	8.0
7.8	7.8	8.5	7.8	7.8	8.3	7.9	8.4	8.1	7.9
7.9	8.3	8.2	7.8	7.6	8.1	8.1	8.2	8.1	7.8
8.0	8.1	8.0	7.8	8.1	8.3	8.0	8.0	8.2	8.3
8.0	8.2	7.9	7.9	7.9	8.0	8.1	8.1	8.0	8.0
7.8	7.5	8.1	8.3	8.2	8.3	7.9	7.9	8.1	8.0

Key to map intervals

blank — 7.40 - 7.75

 — 7.76 - 8.04

 — 8.05 - 8.50

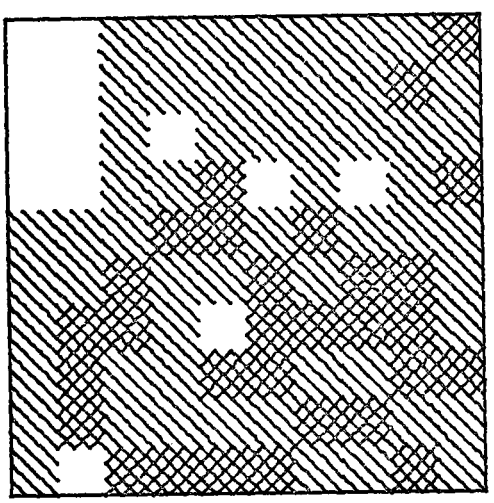


Table 6

Distribution of compactness values (penetrometer readings), Dundas area.

2.0	1.7	1.5	1.8	1.6	1.0	1.0	1.3	0.8	0.8
2.5	2.0	1.7	1.3	1.3	1.0	1.0	0.9	0.9	0.8
2.0	1.8	2.0	1.3	0.8	1.0	1.0	1.0	0.9	0.8
1.3	1.6	2.8	2.1	1.5	1.0	0.9	0.9	0.8	1.3
2.5	2.2	2.8	2.2	1.2	1.1	0.9	0.8	1.0	1.2
2.5	2.8	2.8	2.2	1.8	1.2	1.0	1.3	1.1	1.2
2.5	2.5	2.5	2.0	1.0	0.5	0.6	0.8	1.0	0.9
3.0	1.0	2.0	1.3	0.8	0.5	0.7	0.9	0.8	1.0
2.0	3.2	1.2	1.5	1.8	0.5	0.8	1.0	1.2	1.5
2.2	2.5	2.5	3.0	2.0	1.2	1.0	1.4	1.5	1.5

Key to map intervals

blank

0.50 - 1.05

1.06 - 1.89

1.90 - 3.20

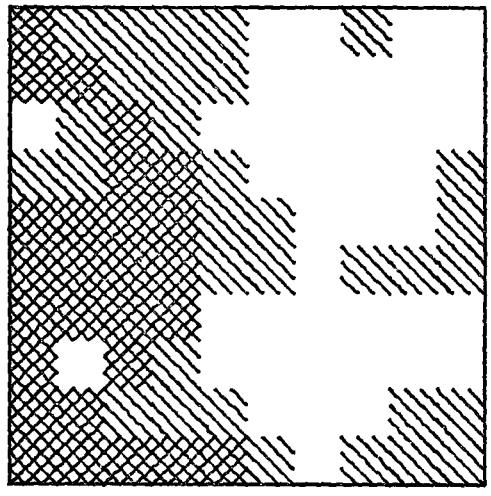


Table 7

Distribution of soil bulk density values, Dundas area.

1.2	1.0	0.8	0.7	0.8	1.1	1.0	1.0	1.0	1.1
0.9	1.2	1.3	1.1	1.1	1.1	1.2	1.0	1.0	1.1
1.1	1.1	0.8	0.5	0.5	1.3	1.1	1.2	1.0	0.9
1.2	1.0	1.4	1.2	1.1	1.0	0.9	1.0	1.0	1.1
0.9	1.0	1.3	1.2	1.0	0.9	1.1	1.0	1.0	1.1
1.1	1.0	1.4	1.1	0.9	0.9	1.1	0.9	1.0	1.1
1.2	1.1	1.3	1.4	0.9	1.1	1.0	1.0	1.0	1.0
1.2	1.2	1.2	1.1	1.2	0.9	0.9	0.9	1.0	0.9
1.1	1.3	1.5	1.0	1.1	1.1	0.8	0.9	0.8	1.0
1.4	1.4	1.2	1.3	1.1	1.2	1.0	1.1	0.9	0.9

Key to map intervals

blank — 0.51 - 0.96

 — 0.97 - 1.15

 — 1.16 - 1.46

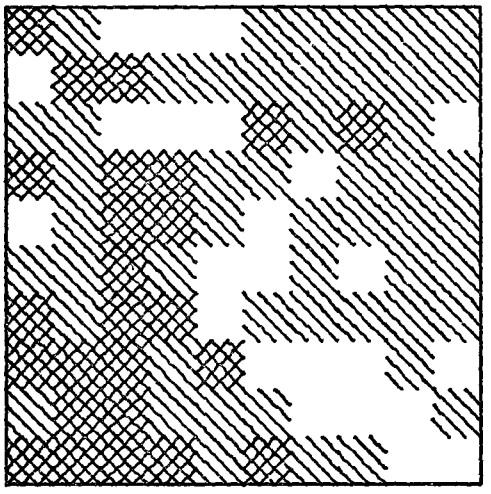


Table 8

Distribution of soil moisture values (percent by weight), Dundas area.

11.9	17.0	45.0	40.0	38.3	43.8	45.2	44.3	50.7	49.6
19.2	21.9	19.5	32.9	29.3	43.5	42.2	41.4	43.7	44.6
13.6	12.6	15.6	19.8	29.0	41.2	35.1	41.0	45.1	43.9
20.1	26.2	15.6	21.3	41.0	41.1	40.6	45.2	47.6	34.9
16.4	14.5	15.6	25.9	26.4	41.4	40.2	50.4	51.5	40.9
19.0	19.6	21.3	23.4	31.6	39.4	47.0	48.9	54.3	38.7
17.6	18.9	21.1	20.8	34.7	41.8	46.5	40.5	33.3	32.6
16.6	13.6	22.5	26.4	28.2	44.7	54.2	39.2	35.2	25.8
19.3	11.4	24.4	26.5	20.9	32.3	38.5	30.6	29.5	26.5
15.1	12.6	16.6	17.7	19.5	27.9	37.8	27.4	30.7	26.6

Key to map intervals

blank		
11.41 - 25.00	25.01 - 35.99	36.00 - 54.32

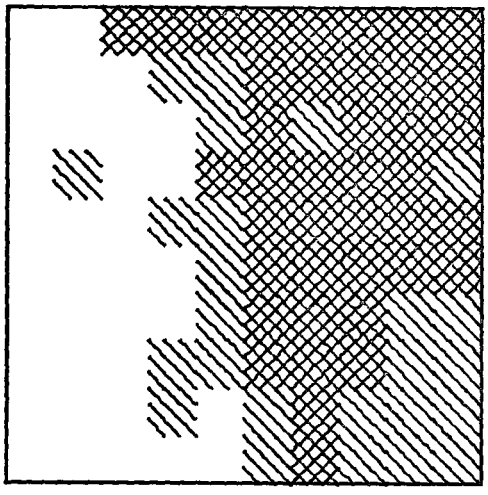


Table 9

Distribution of soil moisture values (percent by volume), Dundas area.

14.3	16.9	33.8	28.0	32.0	47.8	44.7	45.6	49.6	54.9
18.2	27.2	26.1	37.4	31.5	47.9	44.4	43.9	45.3	50.0
15.0	13.8	13.2	10.1	15.6	52.0	39.0	49.5	43.9	39.0
23.8	26.5	21.2	25.1	43.5	41.4	38.9	45.1	47.0	38.8
16.4	15.0	20.8	31.8	25.8	38.1	43.0	49.7	51.9	44.6
20.5	19.9	29.3	26.1	29.0	36.0	50.0	43.9	55.5	44.2
20.8	21.6	26.4	28.5	30.5	46.8	46.4	40.6	34.7	34.2
19.8	16.6	27.6	28.3	33.3	41.5	49.6	35.8	37.2	22.9
20.5	14.8	35.7	27.6	22.4	34.4	30.1	29.2	25.1	25.8
20.5	17.7	20.5	23.0	22.6	34.0	37.8	29.7	29.0	23.8

Key to map intervals

blank		
10.12 - 22.90	22.91 - 43.87	43.88 - 55.47

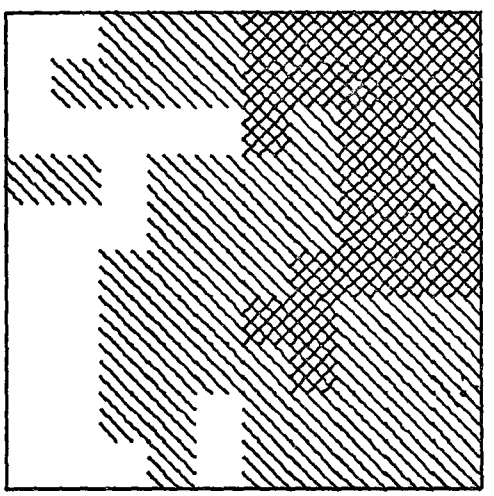


Table 10

Distribution of porosity values (% of total soil volume), Dundas area.

45.8	57.5	64.4	68.5	60.3	50.9	55.6	56.3	56.1	50.3
57.3	44.2	43.2	46.0	51.8	50.5	52.6	52.4	50.7	52.4
53.1	50.7	64.1	77.1	74.4	43.2	52.8	45.7	56.2	62.2
49.8	54.6	39.3	53.1	52.3	57.2	58.0	52.5	55.6	47.2
54.8	53.5	33.3	41.7	56.2	58.6	51.8	53.2	54.8	45.6
48.7	54.6	38.2	47.0	58.7	58.9	54.4	59.7	49.2	46.0
52.8	53.5	46.9	45.1	60.5	49.6	53.0	57.3	53.0	50.2
52.7	48.5	48.1	51.6	49.8	58.2	56.8	54.5	47.3	55.6
55.5	44.9	34.3	53.2	57.2	46.7	58.9	49.9	61.9	53.8
42.5	40.7	47.8	41.7	48.1	46.4	55.1	48.4	59.8	60.0

Key to map intervals

blank		
33.30 - 48.09	48.10 - 56.29	56.30 - 77.10

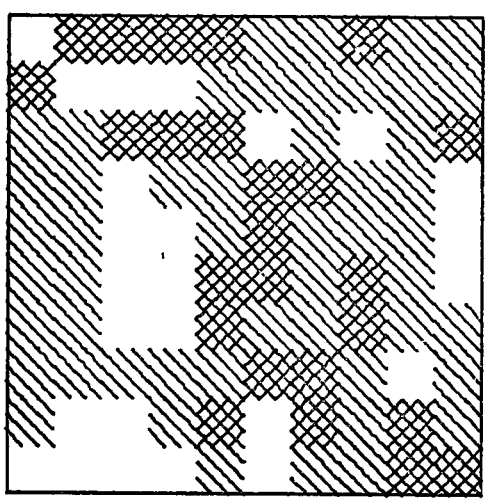


Table 11

Distribution of gas-filled porosity values (% of total soil volume), Dundas area.

31.5	40.6	30.7	40.5	28.3	3.2	11.0	10.7	6.6	1.0
39.2	17.0	17.1	8.7	20.4	2.6	8.2	8.6	5.5	2.4
38.1	37.0	47.0	67.0	58.8	1.0	13.9	1.0	12.4	23.2
26.1	28.2	18.1	28.1	8.4	15.9	19.2	7.4	8.6	8.4
38.5	38.5	12.5	9.9	30.4	20.5	8.8	3.6	3.0	1.1
28.2	34.8	8.9	20.9	29.8	22.9	4.4	15.9	1.0	0.9
32.0	32.0	20.6	16.6	30.1	2.8	6.7	16.7	18.3	16.1
33.0	31.9	20.6	28.1	25.3	37.5	7.3	18.8	10.2	32.7
35.1	30.1	1.0	25.7	34.8	12.3	28.8	20.8	36.9	28.1
22.1	23.1	27.4	18.8	25.5	12.5	17.4	18.7	30.8	36.3

Key to map intervals

blank		
0.90 - 22.98	22.99 - 44.98	44.99 - 67.00

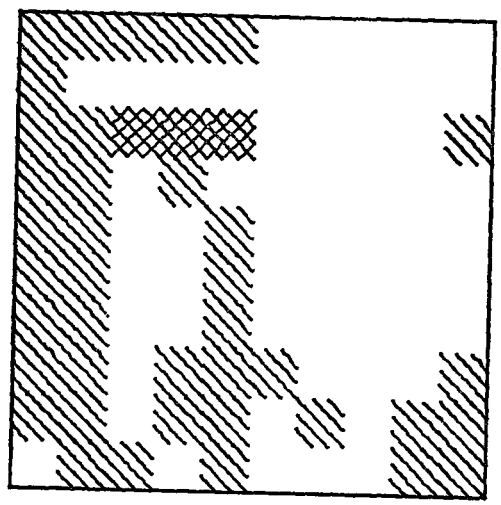


Table 12

Distribution of loss on ignition values (% by weight), Dundas area.

7.2	6.0	8.6	10.2	13.0	12.0	11.2	11.0	10.8	9.4
6.0	7.6	7.0	8.4	11.4	11.2	10.6	9.6	10.4	8.4
7.2	7.2	7.2	9.0	11.2	10.8	10.8	10.0	10.6	11.0
5.6	8.8	10.6	9.4	10.0	9.2	9.4	9.6	9.0	9.2
9.6	9.6	10.8	12.2	10.8	10.4	10.8	12.0	11.4	12.0
10.8	9.8	10.8	8.4	11.6	11.4	11.4	12.6	11.4	10.2
7.6	7.0	6.2	6.2	11.0	9.2	11.4	11.0	10.4	9.8
6.4	4.6	4.6	6.8	8.4	10.4	11.8	11.0	10.2	9.8
6.4	5.0	6.0	8.0	9.4	10.4	11.2	10.0	10.4	9.6
6.2	6.8	6.6	7.4	7.6	10.8	11.6	11.4	10.6	10.2

Key to map intervals

blank — 4.60 - 7.88

 — 7.89 - 10.18

 — 10.19 - 13.00

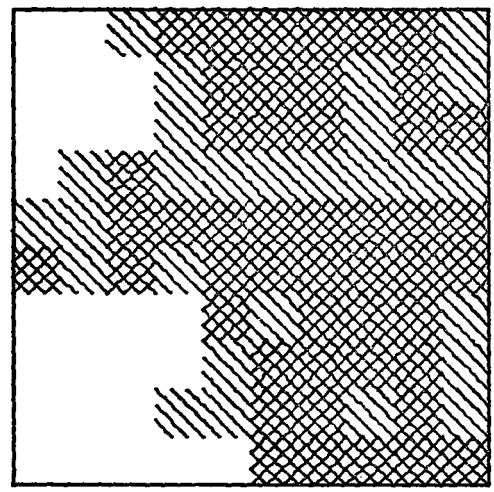


Table 13

Distribution of organic carbon values (% by weight), Dundas area.

1.9	1.5	2.0	2.8	3.8	3.2	2.9	3.8	2.3	2.7
2.2	1.9	1.6	2.1	3.5	2.9	2.7	2.9	3.4	2.6
2.1	2.1	1.4	2.3	2.4	2.3	2.1	2.6	1.7	1.4
1.8	2.7	2.3	3.1	3.3	3.1	3.0	2.7	2.9	2.5
2.3	2.3	1.7	3.2	2.2	2.4	2.2	2.4	2.5	2.4
2.3	2.3	1.7	1.9	2.8	2.7	2.2	2.8	2.5	2.4
2.0	1.8	1.5	1.7	3.0	2.4	2.9	2.9	2.5	2.5
1.8	1.4	1.4	2.0	2.1	2.7	2.8	2.9	2.9	2.2
1.4	0.8	1.2	1.5	2.0	1.5	1.6	2.5	2.7	1.6
1.5	1.5	1.3	1.2	1.8	2.3	2.5	2.3	2.5	2.4

Key to map intervals

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0.80 - 1.81 1.82 - 2.72 2.73 - 3.83

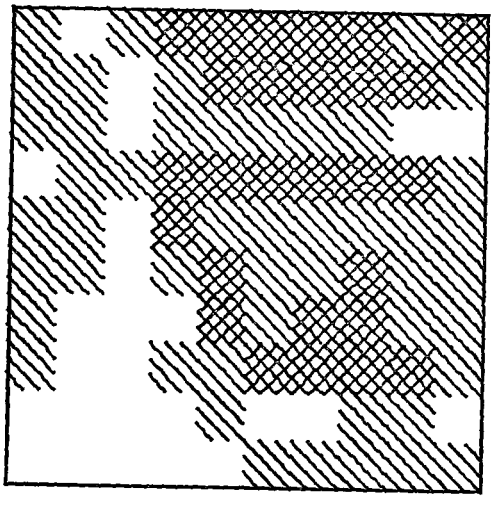

Table 14

Distribution of sand values (% by weight), Dundas area.

67.9	68.1	67.7	61.8	60.4	43.2	33.2	44.7	40.5	54.0
68.0	63.1	63.5	65.1	64.7	42.4	43.0	45.7	42.2	53.3
65.6	62.9	59.8	61.1	72.1	36.0	34.2	37.2	37.0	38.0
60.8	61.2	58.0	63.9	53.8	38.0	40.8	36.7	35.3	37.6
62.0	64.8	52.7	56.3	42.9	45.5	40.7	43.9	43.9	45.5
60.1	59.2	52.7	55.1	45.0	41.1	43.5	58.6	57.2	45.6
57.2	55.8	48.0	56.2	62.9	40.3	44.2	44.3	47.3	44.8
56.5	61.1	50.5	60.5	51.1	39.7	49.7	48.3	41.3	52.8
56.5	59.0	57.4	57.5	54.6	44.7	52.2	49.7	48.2	55.1
52.5	48.7	56.0	56.9	57.8	49.7	50.1	50.3	54.0	52.7

Key to map intervals

blank		
33.21 - 46.00	46.01 - 57.99	58.00 - 72.12

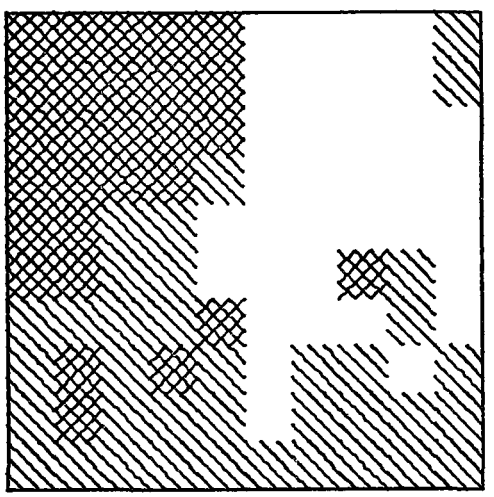

Table 15

Distribution of total silt values (% by weight), Dundas area.

27.3	28.0	28.8	34.6	34.5	51.7	57.2	51.0	52.4	40.3
27.7	33.1	33.6	30.7	31.8	51.8	50.8	52.8	52.5	43.0
29.7	34.3	36.8	35.1	25.0	57.9	61.0	57.7	58.3	57.2
36.4	36.6	38.4	33.3	42.7	57.0	55.1	58.9	59.0	52.7
35.7	32.7	42.9	41.3	50.6	50.5	53.3	50.8	50.9	49.9
37.0	37.9	43.8	39.6	51.9	55.3	52.1	38.6	38.9	51.3
40.3	40.7	48.1	40.3	34.8	54.4	50.9	52.1	49.0	50.1
40.2	35.9	39.8	34.3	40.9	52.1	44.1	43.3	48.3	39.2
38.5	34.1	35.6	33.8	38.2	47.7	40.8	41.4	41.8	36.5
39.0	41.8	38.5	38.1	35.3	43.6	43.4	40.3	40.2	39.4

Key to map intervals

blank		
24.98 - 34.10	34.11 - 46.99	47.00 - 60.98

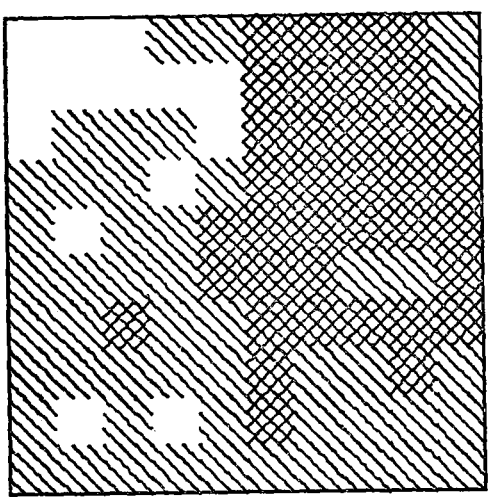

Table 16

Distribution of clay values (% by weight), Dundas area.

4.8	3.9	3.5	3.5	5.1	5.1	9.6	4.3	7.1	5.7
4.3	3.8	3.0	4.2	3.5	5.8	6.2	1.5	5.3	3.8
4.7	2.8	3.3	3.8	2.9	6.1	4.8	5.2	4.7	4.8
2.7	2.2	3.5	2.8	3.5	5.0	4.2	4.4	5.7	9.6
2.3	2.5	4.4	2.4	6.4	4.0	5.9	5.3	5.2	4.6
2.8	2.9	3.5	5.3	3.0	3.5	4.4	2.8	3.8	3.1
2.5	3.5	3.9	3.5	2.3	5.3	4.9	3.6	3.7	5.1
3.3	3.0	9.7	5.2	8.0	8.2	6.2	8.4	10.4	7.9
4.9	6.9	7.0	8.7	7.2	7.6	7.1	8.9	10.0	8.4
8.6	9.5	5.5	5.0	6.8	6.7	6.5	9.4	5.8	7.9

Key to map intervals

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1.50 - 4.98 4.99 - 7.68 7.69 - 10.40

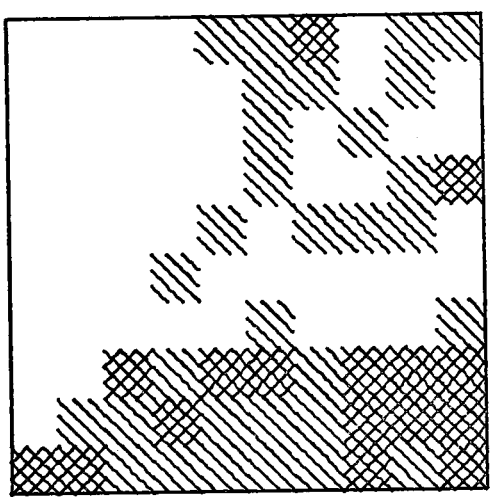

Table 17

Distribution of juvenile E. rosea, Ancaster area.

4.0	6.0	26.0	22.0	27.0	22.0	35.0	28.0	27.0	9.0
6.0	18.0	34.0	30.0	19.0	40.0	31.0	22.0	44.0	22.0
7.0	21.0	44.0	36.0	31.0	26.0	26.0	16.0	18.0	27.0
13.0	5.0	35.0	5.0	31.0	27.0	19.0	27.0	41.0	27.0
13.0	14.0	34.0	33.0	27.0	29.0	48.0	14.0	33.0	18.0
24.0	39.0	48.0	26.0	22.0	27.0	31.0	13.0	20.0	28.0
12.0	34.0	52.0	27.0	27.0	16.0	25.0	19.0	11.0	13.0
14.0	30.0	25.0	22.0	17.0	12.0	19.0	23.0	25.0	21.0
3.0	10.0	23.0	13.0	8.0	4.0	12.0	15.0	20.0	11.0
10.0	7.0	4.0	15.0	3.0	20.0	18.0	13.0	18.0	13.0

Key to map intervals

blank		
3.00 - 19.29	19.30 - 35.69	35.70 - 52.00

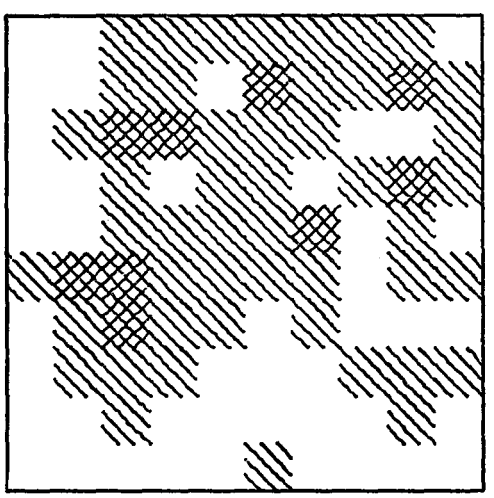

Table 18

Distribution of adult E. rosea, Ancaster area.

3.0	4.0	4.0	6.0	3.0	1.0	3.0	5.0	4.0	0.0
1.0	7.0	13.0	10.0	9.0	8.0	10.0	4.0	3.0	2.0
13.0	10.0	8.0	11.0	4.0	9.0	10.0	7.0	3.0	3.0
7.0	1.0	13.0	3.0	8.0	5.0	2.0	1.0	5.0	8.0
13.0	9.0	6.0	5.0	4.0	10.0	8.0	6.0	6.0	3.0
6.0	7.0	4.0	2.0	6.0	9.0	11.0	8.0	6.0	9.0
5.0	8.0	13.0	5.0	14.0	10.0	9.0	8.0	10.0	5.0
8.0	12.0	23.0	9.0	9.0	8.0	5.0	6.0	9.0	7.0
3.0	8.0	19.0	7.0	7.0	7.0	3.0	7.0	8.0	9.0
2.0	12.0	3.0	13.0	2.0	11.0	13.0	4.0	8.0	0.0

Key to map intervals

blank		
0.00 - 3.49	3.50 - 9.99	10.00 - 23.00

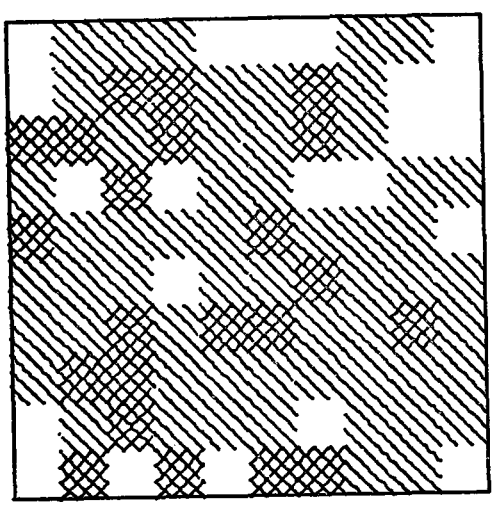

Table 19

Distribution of soil temperature values (°C), Ancaster area.

18.0	15.0	14.5	13.5	13.0	13.0	13.0	13.0	13.0	13.0
19.0	19.0	18.0	17.0	18.0	17.0	17.0	16.0	16.0	16.0
18.5	17.5	16.0	16.0	15.5	15.5	16.0	15.0	16.0	15.0
17.5	17.5	16.5	16.2	15.8	15.5	15.0	16.0	16.0	16.0
18.5	19.5	18.5	18.0	18.0	18.0	17.0	17.0	17.3	17.0
18.0	18.0	18.5	18.3	18.0	18.0	18.0	17.5	17.2	17.2
17.0	18.5	18.0	18.5	16.0	16.5	16.0	15.5	15.5	15.5
17.8	18.2	20.0	19.5	18.5	19.0	18.2	18.0	17.0	17.0
18.5	19.0	20.5	20.5	18.5	18.2	18.2	19.0	18.5	18.0
18.5	18.5	19.5	18.5	18.0	18.0	18.0	18.0	18.0	17.5

Key to map intervals

blank		
13.00 - 15.48	15.49 - 17.98	17.99 - 20.50

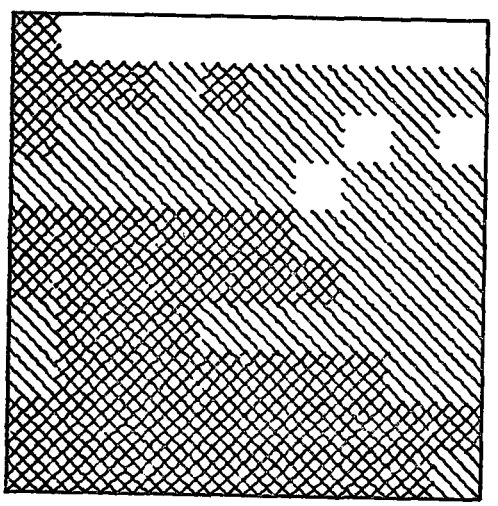

Table 20

Distribution of pH values, Ancaster area.

7.8	7.7	7.9	7.9	7.9	7.5	7.5	7.7	7.4	6.9
7.7	7.7	7.6	7.7	7.6	7.6	7.3	7.4	7.3	6.9
7.9	7.8	7.7	7.5	7.6	7.4	6.8	7.2	6.8	6.8
7.5	7.5	7.5	7.4	7.3	7.4	7.5	7.3	6.9	7.1
7.5	7.6	7.7	7.8	8.0	7.7	7.5	7.4	7.5	7.4
7.7	8.1	7.8	7.8	7.5	7.8	7.9	7.6	7.6	7.1
7.6	7.8	7.9	7.8	8.1	7.9	7.9	7.7	7.3	7.3
7.8	7.9	8.0	8.0	8.1	7.9	7.8	8.0	7.9	7.8
7.8	7.9	7.8	7.8	7.8	7.8	7.8	7.8	7.9	7.9
8.0	8.2	8.1	8.0	8.0	8.0	7.9	7.9	8.0	8.1

Key to map intervals

blank — 6.80 - 7.49

 — 7.50 - 7.89

 — 7.90 - 8.19

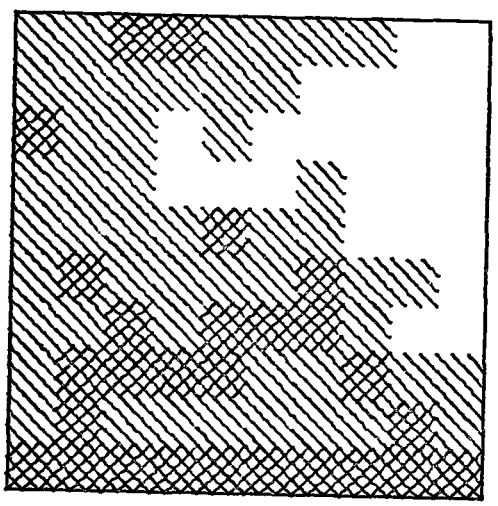

Table 21

Distribution of compactness values (penetrometer readings). Ancaster area.

1.7	3.0	0.8	1.0	1.2	0.7	0.8	0.8	0.8	1.2
2.8	2.5	1.3	0.8	0.6	0.8	0.7	0.9	0.6	0.6
1.8	2.0	0.6	0.5	0.6	0.8	0.8	0.4	0.8	0.7
2.0	2.2	0.5	0.3	0.4	0.3	0.3	0.8	0.6	0.5
1.0	2.4	0.5	0.7	0.8	0.6	0.7	0.5	0.3	0.3
0.8	2.5	1.0	0.8	0.7	0.7	0.9	0.9	0.8	0.7
1.2	1.8	1.0	1.0	0.8	1.5	1.5	1.0	0.6	0.8
0.9	0.8	1.8	1.3	1.5	1.2	1.2	0.9	0.8	1.1
0.8	0.8	1.6	1.8	0.8	1.3	1.5	1.5	0.8	0.8
1.5	0.5	4.5	1.2	2.2	1.8	0.8	1.0	1.6	1.5

Key to map intervals

blank		
0.25 - 0.83	0.84 - 1.94	1.95 - 4.50

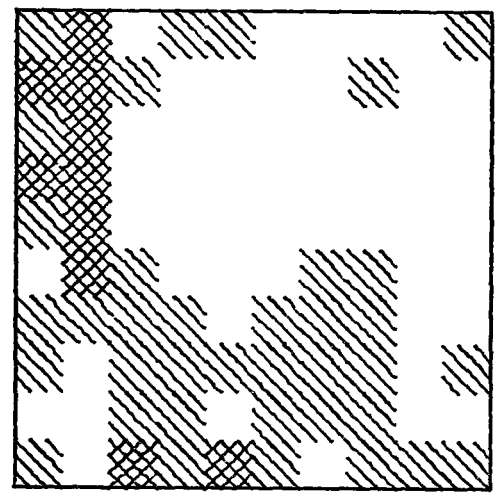

Table 22

Distribution of soil bulk density values, Ancaster area.

1.2	1.1	1.1	1.0	0.9	1.0	1.1	0.9	0.9	1.4
1.3	1.2	1.1	1.2	1.0	1.0	1.0	1.0	1.2	1.3
1.1	1.2	1.1	1.0	1.0	0.8	0.9	1.0	1.3	1.3
1.1	1.1	0.9	1.0	0.9	1.0	1.1	1.2	1.2	1.2
1.0	1.7	1.7	1.7	1.5	1.6	1.1	1.3	1.4	1.2
1.4	1.6	1.6	1.6	1.6	1.6	1.7	1.6	1.3	1.3
1.6	1.7	1.5	1.5	1.5	1.5	1.5	1.5	1.3	1.3
1.2	1.6	1.3	1.4	1.5	1.6	1.5	1.4	1.4	1.6
1.6	1.5	1.5	1.6	1.6	1.7	1.6	1.4	1.6	1.6
1.6	1.5	1.6	1.5	1.8	1.4	1.6	1.8	1.7	1.5

Key to map intervals

blank — 0.78 - 0.99

 — 1.00 - 1.47

 — 1.48 - 1.79

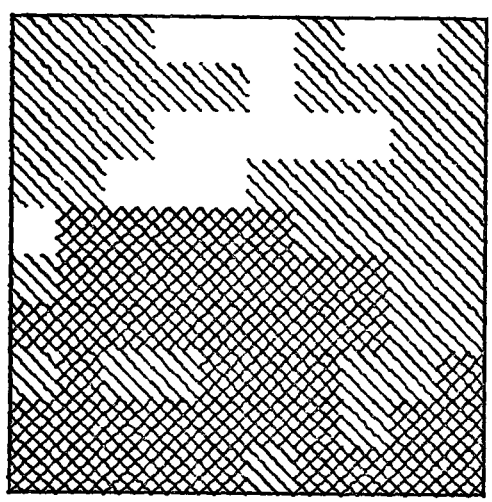

Table 23

Distribution of soil moisture values (percent by weight), Ancaster area.

33.0	43.2	42.2	39.8	36.3	36.7	37.6	43.1	42.1	23.8
38.8	38.4	40.3	33.3	36.8	40.4	40.8	42.2	33.0	27.0
38.5	36.8	42.2	47.5	42.9	42.9	42.4	44.0	23.7	22.8
39.1	42.2	49.8	49.8	41.8	43.1	38.6	24.3	29.3	26.8
45.7	14.4	15.0	16.0	13.7	17.1	40.6	21.5	24.0	28.5
20.2	13.2	14.3	17.1	15.2	16.3	14.8	17.8	23.2	25.6
19.0	14.8	17.0	13.0	17.5	17.1	13.6	19.4	26.1	24.5
25.9	14.6	13.4	17.4	16.5	13.0	9.9	13.5	18.6	14.8
13.2	16.3	15.8	12.6	13.2	11.9	12.5	17.5	12.1	12.4
13.4	18.7	14.7	15.1	9.8	11.6	13.7	11.2	10.3	10.2

Key to map intervals

blank		
9.81 - 19.00	19.01 - 31.99	32.00 - 49.81

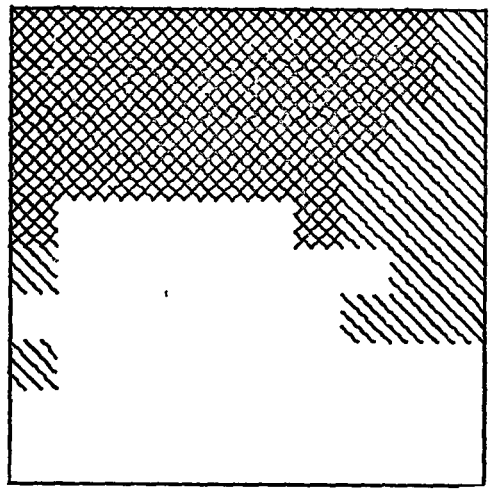

Table 24

Distribution of soil moisture values (percent by volume), Ancaster area.

40.8	49.9	46.7	39.4	33.7	35.8	40.2	38.8	36.5	32.9
49.9	45.0	42.9	41.0	37.0	39.7	42.6	42.4	38.9	36.4
42.8	43.4	45.9	47.2	42.6	33.5	40.6	43.5	30.4	30.0
44.4	48.5	46.3	48.0	39.6	45.0	44.4	29.7	36.0	31.8
44.5	25.2	25.1	27.9	21.3	26.9	45.9	29.0	32.5	35.0
29.1	21.2	22.5	28.1	24.8	26.1	24.6	29.3	29.0	34.6
31.1	24.9	25.5	19.9	26.2	26.5	20.3	29.3	33.2	32.2
31.4	24.2	17.7	23.5	24.5	20.8	15.0	19.1	26.9	24.0
20.9	24.6	24.0	20.2	21.8	20.6	19.9	24.0	19.4	20.1
21.3	28.1	23.9	23.2	17.3	15.8	22.3	20.1	17.3	15.2

Key to map intervals

blank		
15.00 - 25.00	25.01 - 40.99	41.00 - 49.86

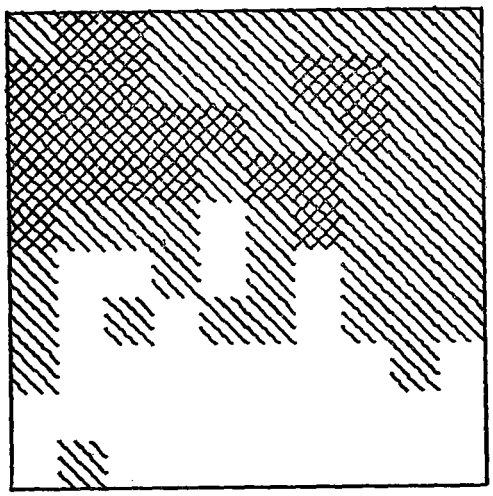

Table 25

Distribution of porosity values (% of total soil volume), Ancaster area.

44.3	45.1	47.3	53.0	56.0	51.2	49.2	55.0	58.9	37.9
45.4	47.3	52.1	47.7	54.8	53.5	52.1	55.2	50.2	47.8
50.0	49.9	51.0	52.7	55.3	63.9	55.9	53.0	42.5	40.7
46.6	48.2	58.2	51.8	57.5	53.0	48.3	48.1	44.9	49.9
56.2	30.1	33.2	25.9	33.8	29.5	49.2	39.4	35.7	41.7
35.0	36.6	33.4	30.0	30.5	32.1	33.6	34.3	47.0	39.3
26.2	28.2	40.2	38.7	40.1	34.1	36.5	40.0	42.8	44.2
49.9	29.5	43.7	42.4	36.7	36.2	29.5	28.0	35.0	31.1
32.8	31.9	32.0	27.7	25.7	22.1	32.4	34.7	36.1	31.5
32.5	32.2	27.1	31.0	29.6	41.9	26.9	23.7	24.8	33.3

Key to map intervals

blank		
22.10 - 36.02	36.03 - 49.96	49.97 - 63.90

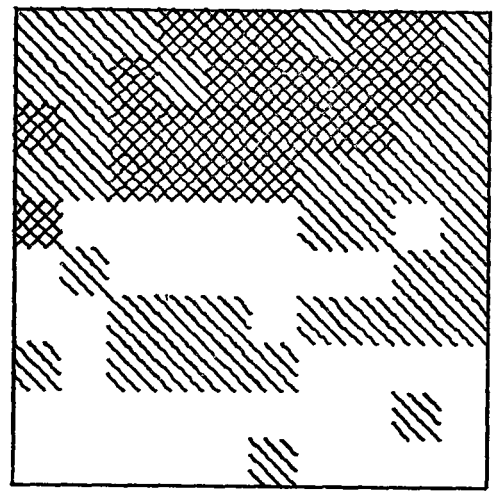

Table 26

Distribution of gas-filled porosity values (% of total soil volume), Ancaster area.

3.5	1.0	0.6	13.7	22.4	15.4	9.0	16.2	22.5	5.1
1.0	2.3	9.2	6.8	17.8	13.8	10.6	12.8	11.4	11.5
7.3	6.5	5.1	5.5	12.7	30.4	15.3	9.6	12.2	10.7
1.2	1.0	11.9	3.8	18.0	8.0	3.9	18.5	8.9	18.1
11.7	4.9	8.2	8.0	12.5	2.7	3.3	10.5	3.2	6.8
6.0	5.5	10.9	1.9	5.8	6.0	9.0	5.0	18.1	4.7
1.0	3.3	14.8	18.8	14.0	7.6	16.2	10.7	9.6	12.1
18.6	5.4	26.0	8.9	12.2	15.5	14.5	9.0	8.2	7.1
11.9	7.3	8.1	7.5	3.9	1.6	12.5	10.7	16.7	11.4
11.3	4.1	3.3	7.9	12.4	26.1	4.7	3.6	7.5	18.2

Key to map intervals

blank — 0.60 - 7.50

 — 7.51 - 12.99

 — 13.00 - 30.40

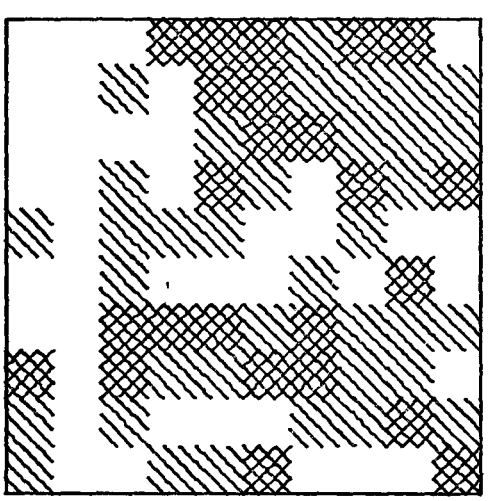

Table 27

Distribution of loss on ignition values (% by weight), Ancaster area.

7.6	9.0	9.6	12.2	12.4	13.2	13.6	12.4	16.4	6.0
7.2	9.4	9.8	7.8	13.2	10.4	12.6	13.2	9.4	7.6
9.2	7.6	10.6	14.6	12.0	14.4	12.2	14.4	5.6	6.6
8.4	9.4	15.8	11.2	12.4	12.0	12.6	5.4	6.8	6.4
8.6	7.4	4.4	4.4	3.6	4.8	7.4	6.4	7.6	7.8
6.2	4.8	5.6	5.6	5.6	4.4	4.2	5.0	6.0	8.4
6.4	5.2	5.2	4.6	5.2	5.0	4.8	6.4	8.0	6.6
6.4	5.0	5.0	5.8	5.0	4.2	4.4	5.8	4.6	6.0
4.0	5.0	4.8	5.4	5.2	4.0	5.2	5.8	4.6	5.4
5.0	4.4	5.6	5.2	5.6	4.8	5.0	4.2	4.8	4.8

Key to map intervals

blank — 3.60 - 6.30

 — 6.31 - 11.39

 — 11.40 - 16.40

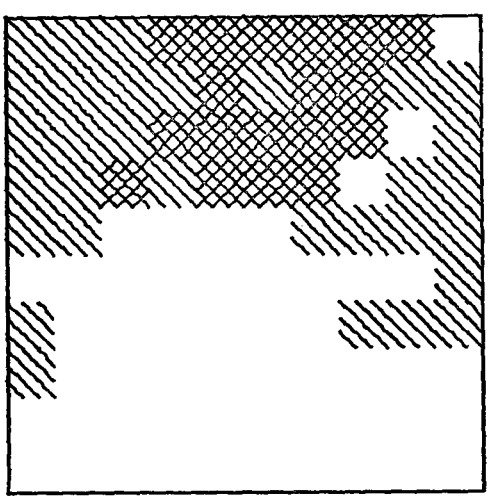

Table 28

Distribution of organic carbon values (%by weight), Ancaster area.

2.0	2.5	1.8	2.0	1.7	3.6	4.1	3.1	4.4	1.1
2.2	2.6	2.6	2.2	3.2	3.5	4.2	4.0	2.3	1.8
2.0	2.2	2.8	3.5	3.7	4.7	3.8	4.2	1.7	2.0
2.3	2.3	4.6	3.1	3.7	3.9	3.8	1.6	2.2	1.8
2.6	1.4	0.9	1.0	0.7	1.0	1.8	2.2	1.7	2.2
1.6	0.7	1.0	0.9	1.0	1.0	0.6	1.2	1.5	1.8
2.4	1.0	1.0	1.4	1.0	1.0	0.8	1.9	2.0	2.0
1.8	1.5	1.4	1.2	1.0	0.9	1.0	1.4	1.2	1.4
0.8	0.8	0.8	1.0	0.6	0.6	0.7	1.0	0.8	1.2
1.0	0.8	1.3	1.0	1.1	1.0	1.0	0.8	1.0	0.9

Key to map intervals

blank		
0.60 - 1.94	1.95 - 3.29	3.30 - 4.66

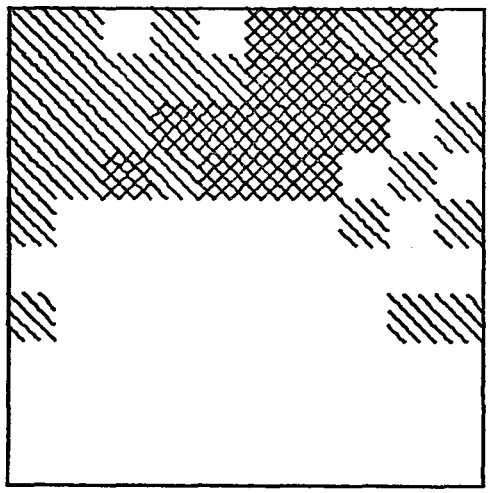

Table 29

Distribution of sand values (% by weight), Ancaster area.

55.9	51.8	59.5	56.1	59.3	58.7	56.7	55.6	51.2	52.7
53.1	52.0	51.8	58.7	53.2	59.3	53.0	50.3	50.5	52.9
54.3	57.2	53.5	58.0	58.5	57.9	58.7	45.7	51.8	54.1
48.4	51.0	51.7	54.2	52.7	51.6	44.5	50.4	52.6	55.5
55.3	58.2	59.2	58.4	60.6	55.1	52.3	49.6	51.4	52.3
60.5	65.1	55.8	60.8	62.7	58.6	65.2	59.9	57.0	48.4
59.8	64.5	65.0	69.2	69.0	64.5	67.8	61.2	57.0	55.0
60.3	64.3	64.8	68.6	67.9	70.0	66.3	59.8	64.6	65.2
67.1	68.2	65.1	64.5	66.5	64.7	67.6	63.7	61.8	61.9
65.0	67.4	68.4	63.6	64.5	67.0	65.1	62.0	62.8	63.7

Key to map intervals

blank		
44.45 - 52.00	52.01 - 62.29	62.30 - 69.98

Table 30

Distribution of total silt values (% by weight), Ancaster area.

37.2	42.0	34.1	38.5	35.5	36.1	36.8	36.6	42.0	40.8
41.4	41.0	42.6	36.6	40.7	34.8	41.0	42.0	42.3	41.9
39.7	36.3	41.0	36.0	35.5	37.2	33.1	47.8	43.0	40.8
44.3	42.1	41.8	38.7	41.3	41.3	48.0	41.2	41.1	38.6
40.4	36.3	35.5	35.1	31.9	38.6	41.5	44.9	44.5	43.2
35.2	31.7	40.0	34.7	32.1	34.5	29.9	35.9	39.0	43.7
35.7	30.4	29.5	26.0	23.3	30.5	27.1	33.6	35.8	40.0
34.6	30.3	29.6	27.2	27.8	26.1	29.7	33.8	30.3	30.1
28.3	28.1	30.5	32.3	29.3	29.4	27.8	31.3	31.8	33.2
31.0	28.6	27.4	31.4	29.5	28.7	30.9	32.1	31.6	30.8

Key to map intervals

blank		
23.26 - 31.50	31.51 - 40.59	40.60 - 48.00

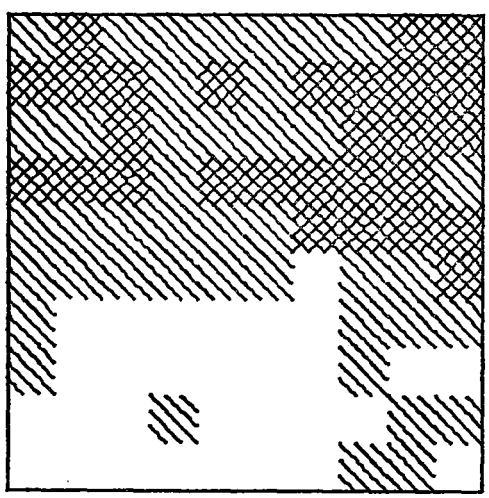

Table 31

Distribution of clay values (% by weight), Ancaster area.

6.8	6.2	6.4	5.4	5.2	5.2	6.4	7.8	6.8	6.5
5.6	7.1	5.7	4.7	6.1	5.8	6.1	7.7	7.2	5.2
5.9	6.6	5.4	6.0	5.9	4.9	8.3	6.5	5.2	5.1
7.3	6.9	6.4	7.2	5.9	7.1	7.6	8.4	6.3	5.8
6.3	5.5	5.3	6.4	7.6	6.3	6.2	5.4	4.1	4.4
4.3	3.2	4.3	4.6	5.2	6.9	4.9	4.2	4.1	7.8
4.5	5.1	5.5	4.8	7.7	5.1	5.1	5.2	7.2	4.9
5.1	5.4	5.6	4.2	4.3	3.9	4.1	6.4	5.2	4.8
4.6	3.7	4.3	3.2	4.2	5.9	4.6	5.1	6.3	4.9
4.0	4.0	4.3	5.0	5.9	4.3	4.0	5.9	5.6	5.5

Key to map intervals

blank		
3.20 - 4.71	4.72 - 6.39	6.40 - 8.40

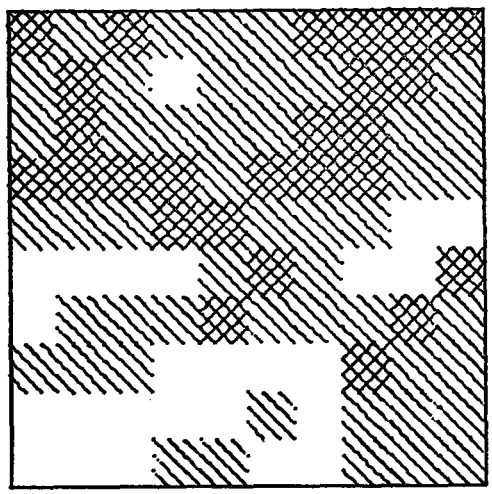

Table 32

Correlation coefficients, Dundas area

	WORMJ	WORMA	TEMP	PH	COMP	BD	H2OW	H2OV	POROS	GFPOR	LOI	OC	SAND	CLAY	SILTT
WORMJ	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
WORMA	*	*	*	*	*	*	*	.50	*	*	*	*	-.56	*	.57
TEMP	*	*	*	*	.61	*	*	*	*	*	*	*	*	*	*
PH	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
COMP	*	*	.61	*	*	*	-.74	-.67	*	*	*	*	.51	*	*
BD	*	*	*	*	*	*	*	*	-.92	*	*	*	*	*	*
H2OW	*	*	*	*	-.74	*	*	.93	*	-.61	.66	.58	-.62	*	.64
H2OV	*	.50	*	*	-.67	*	.93	*	*	-.83	.55	.51	-.69	*	.71
POROS	*	*	*	*	*	-.92	*	*	*	.55	*	*	*	*	*
GFPOR	*	*	*	*	*	*	-.61	-.83	.55	*	*	*	.60	*	-.61
LOI	*	*	*	*	*	*	.66	.55	*	*	*	.67	*	*	*
OC	*	*	*	*	*	*	.58	.51	*	*	.67	*	*	*	*
SAND	*	-.56	*	*	.51	*	-.62	-.69	*	.60	*	*	*	*	-.97
CLAY	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SILTT	*	.57	*	*	*	*	.64	.71	*	-.61	*	*	-.97	*	*

Table 33

Correlation coefficients, Ancaster area

	WORMJ	WORMA	TEMP	PH	COMP	BD	H2OW	H2OV	POROS	GFPOR	LOI	OC	SAND	CLAY	SILTT
ORMJ	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
ORMA	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
EMP	*	*	*	*	*	.61	-.57	*	-.58	*	-.63	-.57	*	*	*
H	*	*	*	*	*	*	*	-.50	*	*	*	-.54	.70	*	-.70
OMP	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D	*	*	.61	*	*	*	-.92	-.80	-.96	*	-.87	-.85	.64	*	-.62
2OW	*	*	-.57	*	*	-.92	*	.95	.89	*	.89	.87	-.72	.52	.69
2OV	*	*	*	-.50	*	-.80	.95	*	.78	*	.76	.76	-.76	.52	.73
OROS	*	*	-.58	*	*	-.96	.89	.78	*	*	.83	.82	-.61	*	.58
FPOR	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
OI	*	*	-.63	*	*	-.87	.89	.76	.83	*	*	.93	-.62	*	.60
C	*	*	-.57	-.54	*	-.85	.87	.76	.82	*	.93	*	-.66	*	.63
AND	*	*	*	.70	*	.64	-.72	-.76	-.61	*	-.62	-.66	*	-.58	-.99
LAY	*	*	*	*	*	*	.52	.52	*	*	*	*	-.58	*	*
ILTT	*	*	*	-.70	*	-.62	.69	.73	.58	*	.60	.63	-.99	*	*

Table 34

The regression of number of worms against raw data of soil factors

Area	Stage	Variable entered	Multiple r	r^2	Increase in r^2
Dundas	Juvenile	OC	.3046	.0928	.0928
		SILTT	.3468	.1203	.0275
		SAND	.3884	.1508	.0305
		TEMP	.4089	.1672	.0164
	Adults	SILTT	.5685	.3232	.3232
		OC	.6200	.3844	.0612
Ancaster	Juvenile	COMP	.3794	.1440	.1440
		POROS	.4260	.1815	.0375
		BD	.4577	.2094	.0280
	Adults	TEMP	.3709	.1376	.1376
		POROS	.4503	.2028	.0652
		SILTT	.5202	.2707	.0679
		COMP	.5662	.3094	.0387

Table 35

The regression of the square root transformation of numbers of worms against raw data of soil factors

Area	Stage	Variable entered	Multiple r	r^2	Increase in r^2
Dundas	Juveniles	LOI	.3315	.1099	.1099
		CLAY	.3770	.1421	.0322
		SAND	.4192	.1757	.0336
	Adults	SILTT	.5129	.2630	.2630
		OC	.5791	.3354	00723
Ancaster	Juveniles	COMP	.4187	.1753	.1753
		POROS	.4599	.2115	.0362
		BD	.4868	.2370	.0255
	Adults	TEMP	.3639	.1324	.1324
		POROS	.4408	.1943	.0619
		SILTT	.5027	.2527	.0584
		COMP	.5572	.3105	.0577

Table 36

The regression of the logarithmic transformation of numbers of worms against raw data of soil factors

Area	Stage	Variable entered	Multiple r	r^2	Increase in r^2
Dundas	Juveniles	LOI	.3147	.0990	.0990
		CLAY	.3748	.1405	.0414
		SAND	.4259	.1814	.0409
	Adults	SILTT	.5704	.3253	.3253
		OC	.6373	.4061	.0808
Ancaster	Juveniles	COMP	.4452	.1982	.1982
		POROS	.4790	.2294	.0313
		H2OW	.5156	.2658	.0364
	Adults	TEMP	.3356	.1126	.1126
		COMP	.4164	.1734	.0608
		POROS	.4562	.2081	.0347
		SILTT	.5244	.2750	.0669
		LOI	.5347	.2859	.0109

Table 37

The regression of number of worms against the square root transformation of the raw data of soil factors

Area	Stage	Variable entered	Multiple r	r^2	Increase in r^2
Dundas	Juveniles	LOI	.2695	.0879	.0879
		SILTT	.3383	.1145	.0266
		CLAY	.3920	.1536	.0392
		TEMP	.4166	.1735	.0199
	Adult	SAND	.5740	.3294	.3294
		OC	.6283	.3948	.0653
Ancaster	Juveniles	COMP	.3716	.1381	.1381
		POROS	.4168	.1737	.0356
		BD	.4474	.2002	.0265
	Adult	TEMP	.3700	.1369	.1369
		POROS	.4459	.1988	.0619
		SILTT	.5178	.2681	.0693
		COMP	.5443	.2963	.0282

Table 38

The regression of number of worms against logarithmic transformation of the raw data of soil factors

Area	Stage	Variable entered	Multiple r	r^2	Increase in r^2
Dundas	Juveniles	OC	.2866	.0822	.0822
		CLAY	.3331	.1109	.0288
		SAND	.4085	.1669	.0559
		TEMP	.4357	.1898	.0230
	Adults	SAND	.5833	.3402	.3402
		OC	.6282	.3946	.0544
		SILTT	.6364	.4050	.0103
		CLAY	.6450	.4160	.0110
Ancaster	Juveniles	COMP	.3714	.1167	.1167
		POROS	.3921	.1537	.0370
		BD	.4159	.1729	.0192
		GFPOR	.4288	.1839	.0109
	Adults	TEMP	.3689	.1361	.1361
		POROS	.4405	.1940	.0580
		SILTT	.5128	.2630	.0690
		COMP	.5287	.2795	.0165

Table 39

Correlation coefficients for isonome-interval selection methods, Dundas area

Factor	Method	r	r^2
WORMJ	THRDS	.8933	.6577
	QRTLS	.8312	.6910
	CLCVE	.8790	.7726
WORMA	THRDS	.9351	.8534
	QRTLS	.8704	.7576
	CLCVE	.9349	.8740
TEMP	THRDS	.8979	.8062
	QRTLS	.9012	.8122
	CLCVE	.9133	.8341
PH	THRDS	.7479	.5594
	QRTLS	.8834	.7805
	CLCVE	.8849	.7830
COMP	THRDS	.9093	.8269
	QRTLS	.9093	.8269
	CLCVE	.9325	.8696
BD	THRDS	.8396	.7050
	QRTLS	.8705	.7577
	CLCVE	.8615	.7421
H2OW	THRDS	.9227	.8513
	QRTLS	.8911	.7940
	CLCVE	.9491	.9007
H2OV	THRDS	.9061	.8210
	QRTLS	.9125	.8327
	CLCVE	.8922	.7960
POROS	THRDS	.8623	.7071
	QRTLS	.8573	.7349
	CLCVE	.8311	.6907
GFPOR	THRDS	.8877	.7826
	QRTLS	.8678	.7530
	CLCVE	.8372	.7010
LOI	THRDS	.9207	.8757
	QRTLS	.9162	.8395
	CLCVE	.8984	.8071

.....Continued

Table 39 Continued

Factor	Method	r	r^2
OC	THRDS	.8969	.8044
	QRTLS	.9132	.8340
	CLCVE	.8664	.7507
SAND	THRDS	.9220	.8501
	QRTLS	.9056	.8202
	CLCVE	.9291	.8632
CLAY	THRDS	.9288	.8486
	QRTLS	.9135	.8346
	CLCVE	.8964	.8035
SILTT	THRDS	.9173	.8415
	QRTLS	.8932	.7979
	CLCVE	.9325	.8696

THRDS = Thirds method.
QRTLS = Quartiles method.
CLCVE = Clinographic curve method.

Table 40

Correlation coefficients for isonome-interval selection methods, Ancaster area

Factor	Method	r	r^2
WORMJ	THRDS	.8995	.8171
	QRTLS	.8916	.7949
	CLCVE	.8774	.7699
WORMA	THRDS	.8540	.7293
	QRTLS	.8562	.7330
	CLCVE	.8553	.7315
TEMP	THRDS	.8982	.8179
	QRTLS	.8749	.7655
	CLCVE	.8635	.7457
PH	THRDS	.7863	.6183
	QRTLS	.8773	.7697
	CLCVE	.8617	.7426
COMP	THRDS	.8961	.7529
	QRTLS	.8382	.7025
	CLCVE	.8882	.7889
BD	THRDS	.9088	.8258
	QRTLS	.9086	.8255
	CLCVE	.9270	.8593
H2OW	THRDS	.9400	.8837
	QRTLS	.8798	.7740
	CLCVE	.9673	.9356
H2OV	THRDS	.8601	.7399
	QRTLS	.9098	.8278
	CLCVE	.9170	.8409
POROS	THRDS	.9289	.8567
	QRTLS	.8990	.8082
	CLCVE	.9194	.8453
GFPOR	THRDS	.8928	.7971
	QRTLS	.8855	.7841
	CLCVE	.8958	.8024
LOI	THRDS	.9508	.8719
	QRTLS	.9157	.8385
	CLCVE	.9454	.8938

.....Continued

Table 40 Continued

Factor	Method	r	r^2
OC	THRDS	.9395	.8831
	QRTLS	.9037	.8166
	CLCVE	.9296	.8641
SAND	THRDS	.8896	.7913
	QRTLS	.9004	.8107
	CLCVE	.9081	.8247
CLAY	THRDS	.8965	.8037
	QRTLS	.9115	.8308
	CLCVE	.9112	.8302
SILTT	THRDS	.9048	.8186
	QRTLS	.8982	.8068
	CLCVE	.9243	.8544

THRDS = Thirds method.
QRTLS = Quartiles method.
CLCVE = Clinographic curve method.

Table 41

Percentage variation between soil sample sites accounted for by the axes of the Principal Component Analysis

Area	Axis	% by each axis	Cumulative %
Dundas	1	69.58	69.58
	2	17.06	86.64
	3	8.47	95.11
	4	3.24	98.35
	5	.48	98.83
Ancaster	1	78.59	78.59
	2	13.33	91.92
	3	4.75	96.67
	4	1.25	97.92
	5	.92	98.84

Table 42

Effect of atmospheric temperature and relative humidity on hatching of cluster-fly eggs

Day	T°C	Moist filter paper	Relative humidity(%) 100	90	75	50	30
2	15	-	-	-	-	-	-
	27	-	-	-	-	-	-
	35	9,9,8	8,7,5	3,6,4	3,2,2	-	-
3	15	-	-	-	-	-	-
	27	7,6,6	6,4,5	1,0,1	-	-	-
	35	9,9,8	8,7,5	3,6,4	3,2,2	-	-
4	15	7,5,6	6,6,6	-	-	-	-
	27	8,8,8	8,7,7	1,0,1	-	-	-
	35	9,9,8	8,7,5	3,6,4	3,2,2	-	-
5	15	7,5,6	6,6,6	-	-	-	-
	27	9,9,8	8,7,8	1,0,1	-	-	-
	35	9,9,8	8,7,5	3,6,4	3,2,2	-	-

- = None hatched.

Table 43

Effect of atmospheric humidity on

cluster-fly larval survival at 21°C

The mean time of three tests, using ten larvae each is recorded

% R. H.	Time to:	
	50% Mortality	100% Mortality
75	18 min	22 min
90	52 min	65 min
100	102 min	112 min
Moist filter paper	3 days	3+ days

Table 44

Effect of temperature on rate of locomotion (mm/min) of first instar cluster-fly larvae

Trial	Temperature (°C) 15	20	25
1	5.25	14.57	9.43
2	5.40	13.20	8.33
3	5.05	11.21	8.14
4	4.65	17.75	7.80
5	5.38	15.60	7.30
6	5.35	14.60	9.50
7	4.93	17.50	8.87
8	5.53	-	-
9	5.53	-	-

Analysis as one-way classification experimental design:

$T_1 = 47.07$ $T_2 = 104.73$ $T_3 = 59.37$

$n_1 = 9$ $n_2 = 7$ $n_3 = 7$

$\bar{T}_1 = 5.23$ $\bar{T}_2 = 14.96$ $\bar{T}_3 = 8.48$

$T = T_1 + T_2 + T_3 = 211.17$

$N = n_1 + n_2 + n_3 = 23$

$\Sigma\Sigma\, x_{ij}^2 = 2353.365$

Correction Factor (C.F.) $= 1/N(T)^2 = 1938.816$

Temperature Sum of Squares (S.S.) $= T_1^2 / n_1 + T_2^2 / n_2 + T_3^2 / n_3$ - C.F.

$= 377.812$

$\Sigma\Sigma(x_{ij} - \bar{x}..)^2 = \Sigma\Sigma x_{ij}^2$ - C.F.

$= 414.549$

.....Continued

Analysis of Variance of temperature effects : $H_o : t_1 = t_2 = t_3$

Source	S.S.	d.f.	M.S.S.	F	d.f.
Temperature	377.812	2	188.906	102.834	(2,20)
Error (by subtraction)	36.737	20	1.837		
Total = $\Sigma\Sigma (x_{ij} - \bar{x}..)^2$	414.549	22			

Tabulated 5% F value for (2,20) d.f. = 3.49 reject H_o

Student's t-test of temperature effects

Tabulated 5% t value for 20 d.f. = 1.725

(i) To test $H_o : t_2 = t_3$, where S_E + error M.S.S.

$$t = (\bar{T}_2 - \bar{T}_3) / S_E \quad 1/n_2 + 1/n_3$$

$$= 8.60$$

reject H_o i.e. $t_2 > t_3$

(ii) To test $H_o : t_3 = t_1$

$$t = (\bar{T}_3 - \bar{T}_1) / S_E \quad 1/n_3 + 1/n_1$$

$$= 4.80$$

reject H_o i.e. $t_3 > t_1$

i.e. $t_2 > t_3 > t_1$

Table 45

Behaviour of first-instar cluster fly larvae in temperature gradients

Trial	Temperatures (°C) of the 6 isotherms						Temperature of experimental isotherm	Number of larvae used	% moving up gradient	% moving down gradient	% not moving off isotherm
	1	2	3	4	5	6					
1	9.0	10.1	12.2	14.2	18.5	25.0	12.2	50	56	32	12
2	10.0	12.0	14.0	16.2	20.0	26.1	16.2	50	40	52	8
3	8.7	10.1	11.2	13.0	16.2	18.6	11.2	20	10	20	70
4	12.0	14.0	16.2	18.7	22.8	32.0	18.7	20	25	70	5
5	12.5	14.5	16.8	19.2	21.6	26.8	19.2	20	65	30	5
6	10.0	10.6	12.0	13.0	14.3	18.5	12.0	20	50	50	0
7	9.0	10.5	12.0	14.0	16.5	20.7	12.0	20	30	30	40

Table 46

Site of infection by first-instar cluster-fly larvae at room temperature, in glass vials containing a few small pieces of filter-paper, using 12-15 larvae per vial

Species	Worm #	Number of larvae	Site
L. terrestris	1	1	103D
L. rubellus	1	1	86Ldl
A. chlorotica	1	2 together	60Rl
	2	1	43Ldl
	3	1	81Rvl
E. rosea	1	1	30Ll
		1	32Ll
		1	37Ll
		1	49Ll
		1	63V
	2	2 together	59Rdl
	3	1	105V
	4	1	26Ldl
	5	1	43Rdl
	6	1	16Ldl
	7	1	40D

Site code:segment number; right(R) or left(L) side; dorsal(D), ventral(V), Lateral(l); dorsolateral(dl) or ventrolateral(vl).

Table 47

Site of infection by first-instar cluster-fly larvae, on *E. rosea*, using 8-10 larvae per vial

Worm #	Number of larvae	Site
1	2 together	63Rd1
2	1	29Ld1
3	2 together	118R1
4	1	34Ld1
5	2 together, feeding on open wound	42-45D
6	1	15L1
7	1	41R1
	1	60Rd1
	2 together	63Rd1
	1	75Rd1
8	2 together	72Ld1
9	1	38Rd1
10	1	51Rv1
11	1	86D
12	1	53V
13	2 together	72D

Site code and experimental conditions as in table 46.

Table 48

Effect of multiplicity of infection and temperature on rate of infection of E. rosea by first-instar cluster-fly larvae under standard soil conditions

M.O.I.	Trial	IH	INH	NIH	NINH	% of larvae penetrating
13°C						
0.5:1	1	3	0	16	1	30
1 : 1	1	3	0	17	0	15
	2	2	0	17	1	10
	3	2	0	18	0	10
2 : 1	1	5	0	14	1	12
	2	4	0	16	0	10
	3	9	0	10	1	22
3 : 1	1	4	0	15	1	6
	2	11	0	9	0	18
	3	9	0	9	2	15
	4	14	0	6	0	23
	5	9	0	11	0	15
	6	9	0	11	0	15
	7	5	0	15	0	8
	8	9	0	11	0	15
4 : 1	1	9	0	11	0	10
	2	13	0	7	0	16
5 : 1	1	16	0	4	0	16
	2	18	0	2	0	18
	3	7	0	13	0	7
	4	13	0	6	1	13
	5	10	0	8	2	10
7.5:1	1	17	0	1	2	11
10:1	1	9	0	3	8	4

.....Continued

Table 48 Continued

M.O.I.	Trial	IH	INH	NIH	NINH	% of larvae penetrating
20°C						
1 : 1	1	5	1	11	3	30
	2	2	0	18	0	10
	3	2	0	16	2	10
2 : 1	1	2	2	12	4	10
	2	9	0	10	1	22
5 : 1	1	5	5	10	0	10
	2	6	8	6	0	14
10:1	1	0	14	6	0	7

Table 49

Effect of multiplicity of infection (MOI) on rate of infection of *E. rosea* by first-instar cluster-fly larvae at 13°C, under standard soil conditions

MOI	1	2	3	4	5	6	7	8	Totals	# of Trials
0.5:1	30								30	1
1:1	15	10	10						35	3
2:1	12	10	22						44	3
3:1	6	13	15	23	15	15	8	15	115	8
4:1	10	16							26	2
5:1	16	18	7	13	10				64	5
7.5:1	11								11	1
									325	23

$\Sigma x^2 = 5281$ $\quad (\Sigma x)^2 = 105625$

Correction Factor (C.F.) = 1/23(105625) = 4592

MOI Sum of Squares (S.S.) = $\frac{900}{1} + \frac{1225}{3} + \frac{1936}{3} + \frac{13225}{8} + \frac{676}{2}$

$$\frac{4095}{5} + \frac{121}{1} - \text{C.F.}$$

$$= 292$$

Total S.S. = 5281 - 4592 = 689

Analysis of Variance $H_o : t_1 = t_2 \ldots = t_n = 0$

.....Continued

Table 49 Continued

Source	S.S.	d.f.	M.S.S.	F	d.f.
MOI	292	6	48.66	1.93	(6,16)
Error (by subtraction)	397	16	24.81		
Total	689	22			

Tabulated 5% F for (6,16) degrees of freedom = 2.74 $\therefore$ acept H_o

$\therefore$ MOI has no effect.

$\therefore$ Mean infection rate = 325/23 = 14% of larvae successfully penetrat

Table 50

Effects of soil conditions on the host location and penetration success of first-instar cluster-fly larvae at 15°C

No.[1]	BD	Moisture %	Larvae penetrating				
			Pilot experiment	Soil type-I (i)	(ii)	(iii)	Soil type-II (iv)
1	1.75	15	0	0	3	0	-
2	1.75	30	4	1	0	0	1
3	1.75	45	0	2	0	0	0
4	1.50	15	0	0	0	0	-
5	1.50	30	1	1	0	3	1
6	1.50	45	0	1	0	0	0
7	1.25	15	0	0	0	0	-
8	1.25	30	0	2	0	0	1
9	1.25	45	0	0	0	0	0

- = no result due to death of worms

1 : Twenty E. rosea and 60 first-instar cluster-fly larvae were used in each test.

Table 51

The effects of soil conditions on the maintenance of the cluster-fly infection in E. rosea, for soil type-II after one week. Five worms were used in each test.

			Experiment Number			
No.	BD.	moisture %	1 (15°C)	2 (15°C)	3 (15°C)	4 (20°C)
1	1.75	25	X X X X □	X X X X X	X X X X X	X X X X X
2	1.75	45	X X X X |	X X ○ □ ●	X X X X X	X X X X X
3	1.75	65	X X □ ■ ▼	X X X X ●$_A$	X X X X ■	X X X X □
4	1.50	25	X X ○ ▼ ▼	X X X ○ ●	X X X X X	X X X X X
5	1.50	45	X X ○ □ ■$_A$	X X X X X	X X X X X	X X X X X
6	1.50	65	X X □ ■ ■$_A$	X X □ □ ■	X X X ■$_A$ ■$_A$	X X X ○ □
7	1.25	25	X X X X ●$_A$	X X X X X	X X X X X	X X X X X
8	1.25	45	X X X X ■$_A$	X X X X X	X X X X ○	X X X X X
9	1.25	65	X X | ▼ ■$_A$	X X □ □ ■	X □ □ ■$_A$ ■$_A$	X X □ ■ ■$_A$

X worm dead

| worm left soil

○ I,H,E

□ I,H,NE

△ I,NH,E

▽ I,NH,NE

● NI,H,E

■ NI,H,NE

▲ NI,NH,E

▼ NI,NH,NE

A worm had lost infection by autotomy

(N)I = (Not) Infected

(N)H = (Not) Apparently healthy. (In conjunction with I, H means healthy apart from infection by the larva)

(N)E = (Not) Encysted

Table 52

Effect of soil conditions on uninfected E. rosea

for soil type-II, after one week at 15°C

No.	BD	Moisture %	Fate of the five worms, in three trials											
			Dead			Left Soil			Healthy encysted			Healthy non-encysted		
			1	2	3	1	2	3	1	2	3	1	2	3
1	1.75	25	0	1	1	0	4	4	3	0	0	2	0	0
2	1.75	45	0	0	0	0	0	0	1	0	0	4	5	5
3	1.75	65	0	0	0	0	0	0	0	1	0	5	4	5
4	1.50	25	0	0	0	2	0	3	3	3	0	0	2	2
5	1.50	45	0	0	0	0	0	0	1	0	0	4	5	5
6	1.50	65	0	0	0	0	0	0	0	0	0	5	5	5
7	1.25	25	1	0	1	0	0	2	4	5	2	0	0	0
8	1.25	45	0	0	0	0	0	0	2	0	0	3	5	5
9	1.25	65	0	5	0	0	0	0	0	0	0	5	0	5

Table 53

Number of E. rosea encysting under drying conditions, ten worms per test

		Moisture content 25% Trial 1	25% Trial 2	25% Total	45% Trial 1	45% Trial 2	45% Total	Overall Totals
Bulk density	1.75	5	7	12	0	3	3	15
	1.50	10	5	15	2	0	2	17
	1.25	3	5	8	4	4	8	16
	1.00	5	9	14	3	1	4	18
Totals				49			17	

Analysis as two-way classification experimental design

$\Sigma\Sigma\, x_{ij} = 66$

$\Sigma\Sigma\, x_{ij}^2 = 394$

$(\Sigma\Sigma\, x_{ij})^2 = 4356$

$N = 16$

Correction Factor (C.F.) $= 4356 / 16$

$= 272.25$

"Between moisture" S.S. $= 1/8(49^2 + 17^2) - C.F.$

$= 65.25$

"Between BD" S.S. $= 1/4(15^2 + 17^2 + 16^2 + 18^2) - C.F.$

Error S.S. $= 394 - 1/2(12^2 + 15^2 + 8^2 + 14^2 + 3^2 + 2^2 + 8^2 + 4^2)$

$= 33.00$

Total S.S. $= 394 - 272.50$

$= 121.50$

Interaction S.S. (by subtraction) $= 121.50 - 33.00 - 1.25 - 65.25$

$= 22.00$

.....Continued

Table 53 Continued

H_o : $b_i = 0$ for all i

$t_j = 0$ for all j

$\tau_{ij} = 0$ for all i and j

Analysis of Variance

Source	S.S.	d.f.	M.S.S.	F	d.f.
Moisture	65.25	1	65.25	15.80	1,8
BD	1.25	3	0.41	0.10	3,8
Interaction	22.00	3	17.33	1.78	3,8
Error	33.00	8	4.125		
Total	121.50	15			

Tabulated 5% value for (1,8) d.f. = 5.32

Tabulated 5% F value for (3,8) d.f. = 4.07

$\therefore$ Accept H_o ; $b_i = 0$ for all i

and $\tau_{ij} = 0$ for all i and j

Reject H_o : $t_j = 0$ for all j

i.e. moisture levels alone affect encystment under drying conditions.

Table 54

Survival of infected worms under optimal encystment conditions for soil type-II

Tray #	Temperature (°C)	No. of worms used	Results
1	20	77	Only 5 worms still alive after 3 weeks. All NI,H,E.
2	20	88	44 still alive. 3 NI,H,NE. 2NI,H,NE. 39 NI,H,E.
3	15	120	55 still alive. 11 NI,H,NE. 2 NI,NH,NE. 41 NI,H,E. 1 I,H,E (a 3rd instar larva on the outside of an encysted worm. The worm appeared to be only slightly eaten, and was still alive and quite healthy).
4	15	140	53 still alive. 32 NI,H,E. 20 NI,H,NE. 1 I,H,E. (a 3rd instar larva still within the worm). A large P. rudis 1st instar larva was found free in the soil

(N) I = (Not) Infected

(N) H = (Not) Healthy (apart from P. rudis infection)

(N) E = (Not) Encysted

Table 55

Date and location of infected worms and cluster-fly pupae in the field

Where relevant, site numbers as in figs. 2-3 are given

Date	Site	Host species	Cluster fly
1969			
May 23	Ancaster	E. rosea	3rd instar
26	"	E. rosea	"
June 16	"	E. rosea	"
	"	E. rosea	"
July 27	"	E. rosea	"
	"	A. chlorotica	"
	"	E. rosea	"
August 11	"	E. rosea	"
	"	A. chlorotica	"
1970			
June 11	Dundas #53	-	pupa
22	" #74	-	pupa
July 1	Ancaster #21	E. rosea(adult)	3rd instar
2	" #31	A. chlorotica (juvenile)	" (still inside host)
3	" #42(i)	E. rosea(juvenile)	3rd instar(feeding on outside)
	(ii)	A. chlorotica(juv.)	"

.....Continued

Table 55 Continued

Date	Site	Host species	Cluster fly
July 4	Ancaster #59	-	pupa
7	" #71	-	pupa
	" #76	-	pupa
	" #80	-	3rd instar (free in soil)
9	" #94	-	pupa
	" #97	-	pupa

Note: no infected individuals of the following species were found: A. caliginosa, L. rubellus, L. terrestris, Octolasium lacteum or Eiseniella tetraedra.

Table 56

Behaviour of earthworms in the field

Site[1]	BD	Moisture %	Relative abundance	% encystment	Maximum depth of occurrence(cm)
1	1.01	12.15	+	100	15
2(i)	1.04	36.50	+	25	15
(ii)	1.61	32.22	+++	20	20
3	1.10	9.16	+	100	10
4	0.75	13.68	+	100	10
5	0.65	17.22	+++	100	10
6	1.36	9.37	++	100	10
7	0.99	23.30	+++	100	10

1 All results are for E. rosea except site #5 which contained A. chlorotica.

2 Each site was examined to a depth of 60 cm.

3 Each site was 25 x 25 cm. The scale for relative abundance indicates the number of worms found in the sample site:

\+ 1-10
++ 11-20
+++ > 20

Table 57

Effect of atmospheric conditions on survival of earthworms and first-instar cluster-fly larvae over a 24-hr period

Source	Date	$\%N_2$	$\%O_2$	$\%CO_2$	No.used	%mortality
E. rosea						
Culture	2/5/70	100	0	0	15	100
Culture	3/5/70	99.5	0.5	0	15	0
Culture	4/5/70	84.5	0.5	15	15	0
Ancaster	10/5/70	84.5	0.5	15	12	88.3
Ancaster	11/5/70	84.5	0.5	15	6	100
Culture					6	0
Ancaster	30/6/70	62.5	2.5	35	12	91.6
Ancaster	2/7/70	57	3.0	40	12	91.6
Ancaster	24/6/70	0	8.0	92	12	91.6
Ancaster	6/7/70	73.5	1.5	25	12	66.0
Dundas					12	41.6
Ancaster #100	27/7/70	73.5	1.5	25	12	100
# 1					12	0
Ancaster	3/7/70	79	1.0	20	12	41.6
Dundas	1/6/70	79	1.0	20	12	75.0
Dundas	8/6/70	73.5	1.5	25	12	41.6
Dundas	22/6/70	57	3.0	40	12	50.0

.....Continued

Table 57 Continued

Source	Date	$\%N_2$	$\%O_2$	$\%CO_2$	No.used	%mortality
P. rudis First instar larvae:						
Culture	22/8/70	79	21	0	15	0
Culture	24/8/70	95	5	0	15	13.3
Culture	25/8/70	90	5	5	15	0
Culture	26/8/70	80	5	15	15	13.3

Parentheses indicate that these worms were studied together in one experiment.

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Appendix A

Behaviour of worms in experiments gauging penetration success of Pollenia larvae

Table 1

Pilot experiment

No.	BD	H_2O	Remarks
1	1.75	15	Worms were still pressed against bottom of tray. No burrowing had occurred.
2	1.75	30	The worms burrowed between the soil and the walls of the container. One corner of the soil had cracked. Two worms at the bottom of the crack were each infected with two larvae.
3	1.75	45	The worms only burrowed between the soil and the walls of the container.
4	1.50	15	The worms burrowed normally through the soil.
5	1.50	30	Surface casts were produced which blocked the openings of the burrows.
6	1.50	45	
7	1.25	15	The worms were aggregated into two clumps at the bottom of the tray.
8	1.25	30	
9	1.25	45	Many worms were lying partly exposed on the surface of the soil. The burrows were often closed with flowing soil. Five worms had completely left the soil.

Table 2

(i) Soil Type - I

No.	BD	H_2O	Remarks
1	1.75	15	sc+(when initially wet); E-; all worms dried up, dead.
2	1.75	30	sc+; E-.
3	1.75	45	sc+; E-. The soil surface was slightly drier than the rest of the soil due to drainage. This set-up had been standing longer than the equivalent in (ii) and (iii), before the larvae were added.
4	1.50	15	sc-; E-. All worms dead except for 2.
5	1.50	30	sc+; E-.
6	1.50	45	sc+; E-.
7	1.25	15	sc-; E+. Two worms left soil, one died in soil.
8	1.25	30	sc-; E-.
9	1.25	45	sc-; E-. Two worms left soil.

sc-	surface casting behaviour
++	many surface casts formed
+	few surface casts formed
-	no surface casts formed
E-	Encystment behaviour
++	present in almost all worms
+	present in some worms
-	absent

Table 3

(ii) Soil Type - I

No.	BD	H_2O	Remarks
1	1.75	15	sc+; E+. Two of the 3 were on dead worms, the larvae dead at the edge of the penetration wound. Non-encysted worms dead, dried up.
2	1.75	30	sc+; E-.
3	1.75	45	sc+; E-.
4	1.50	15	sc-; E++.
5	1.50	30	sc+; E-.
6	1.50	45	sc-; E-.
7	1.25	15	sc-; E-. All dried up, dead.
8	1.25	30	sc-; E-.
9	1.25	45	sc-; E-. Some worms exposed on surface.

Table 4

(iii) Soil Type - I

No.	BD	H_2O	Remarks
1	1.75	15	sc+; E-. Worms dried up, dead.
2	1.75	30	sc+; E-.
3	1.75	45	sc+; E-.
4	1.50	15	sc-; E-. All dried up, dead.
5	1.50	30	sc-; E-. Two of the larvae were in the same penetration wound of one worm.
6	1.50	45	sc-; E-.
7	1.25	15	sc-; E+. None dead (compared with (ii) above - dried more slowly than in (ii)).
8	1.25	30	sc-; E-.
9	1.25	45	sc-; E-.

Table 5

(iv) Soil Type - II

No.	BD	H_2O	Remarks
1	1.75	15	sc++ (when initially wet). E++. Although all the worms were encysted, when removed from the cysts they were inactive - they did not burrow into loose, moist soil.
2	1.75	30	sc+; E+. Eight worms left the soil. The infected worm was half out of the soil.
3	1.75	45	sc++; E++. Eight worms encysted in 1 clump.
4	1.50	15	sc+; E++. The encysted worms were inactive when removed from their cysts. They appeared dehydrated.
5	1.50	30	sc+; E+. Seven worms left the soil.
6	1.50	45	sc+; E-.
7	1.25	15	sc-; E++. All worms were encysted in 3 clumps. The worms were active when removed from the cysts.
8	1.25	30	sc+; E+.
9	1.25	45	sc-; E-.

Table 6

(v) Soil Type - II

No.	BD	H_2O	Remarks
1	1.75	15	sc+; E-. All dried up dead.
2	1.75	30	sc+; E+. 13 dead.
3	1.75	45	sc+; E+.
4	1.50	15	sc+. Worms too dried up to see whether or not encysted.
5	1.50	30	sc+; E+. Five worms dead.
6	1.50	45	sc+; E-.
7	1.25	15	sc-. Too dried up to see whether or not encysted. Four worms left soil.
8	1.25	30	sc-; E++. Seven worms left soil.
9	1.25	45	sc-; E-.

Table 7

(vi) Soil Type - II

No.	BD	H_2O	Remarks
1	1.75	15	sc+ (when initially wet). Encysted in clumps, but all dead.
4	1.50	15	sc+ (when initially wet). E-. All dead.
7	1.25	15	sc-; E-. All dead.