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ECOLOGY OF THE MYCOPHAGOUS NEMATODE, *APHELENCHUS AVENAE*

by

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Thesis submitted to The University of Adelaide  
in fulfilment of the requirements for the  
degree of Doctor of Philosophy

July, 1984.

*Awarded Feb. 1985*

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SUMMARY

The mycophagous nematode *Aphelenchus avenae* Bastian, 1865 was found to be abundant in soil from South Australian wheat-fields. Mean population density from 57 fields under wheat was 349, 242 m<sup>-2</sup> in the top 20cm of soil and comprised 9% of all nematodes. Population density was positively correlated with soil pH and the nematode tended to be relatively more abundant in sandier soils and in soils with higher levels of soluble salts. Studies on the distribution and abundance of *A. avenae* in saline soils surrounding brackish lagoons suggested that type of plant cover - and associated soil mycoflora - was a more important influence than salinity *per se* on population density of the nematode. The effects of salinity and pH of culture medium on population increase of *A. avenae* in monoxenic culture suggested that these factors were more likely to influence population density in soil by their actions upon host fungi rather than on the nematode. Subsequent studies concentrated on the relationship between the soil mycoflora and population density of *A. avenae*.

Abundance of *A. avenae* was studied in two soils, a field under wheat and a pine-forest, which, because of the differences in their plant covers, were expected to differ in composition of the mycoflora. *A. avenae* was more abundant in the wheat-field soil than in the pine-forest soil and litter where it was comparatively rare. *Aphelenchoides* spp. appeared to be the most common mycophagous nematodes in the pine-forest, particularly in litter. Various methods were used to compare mycofloras of soil, roots and organic debris from the two sites. It was concluded that the sites possessed distinct mycofloras and that the mycoflora of the wheat-field soil was more diverse than that of

the pine-forest soil which was dominated by *Penicillium* spp. It is suggested that composition of the soil mycoflora is a major factor influencing abundance of *A. avenae*.

To test the hypothesis that composition of the soil mycoflora influenced abundance of *A. avenae*, feeding trials were conducted using isolates of *A. avenae* and various fungi derived from wheat-field and pine-forest soils. Other mycophagous nematodes - *Aphelenchoides* spp. - from these soils were also tested to determine the degree of resource overlap between them and *A. avenae*. Genera and species groups of fungi, and individual isolates, varied markedly in their suitability as food-hosts for *A. avenae* and other mycophagous nematodes. The more diverse mycoflora of wheat-field soil afforded a greater range of suitable fungal hosts than that of pine-forest soil. *Penicillium* spp., the fungi which dominated pine-forest soil, were comparatively poor hosts for the nematode. Individual isolates of *A. avenae* from the wheat-field and pine-forest soils varied greatly in their rates of population increase in feeding trials, suggesting an underlying genetic diversity, apparently in the form of genetically distinct clones. Little host specialization appeared to exist between *A. avenae* and *Aphelenchoides* spp. that would preclude the possibility of competition for food resources. Feeding trials indicated that actinomycetes and bacteria from the two soils were little used, if at all, by *A. avenae* as food sources.

All mainland isolates of *A. avenae* examined were apparently parthenogenetic, however, a population from Dangerous Reef, an island in Spencer Gulf, proved to be amphimictic. Parthenogenetic isolates of *A. avenae*, even when derived from the same field, varied markedly in their propensity to produce males at temperatures above 25°C.

Populations of the nematode sampled north of 34°S latitude were found to have  $n = 9$  chromosomes while those to the south had  $n = 8$  chromosomes. There was no relationship between karyotype and propensity to produce males at high temperatures, nor were the two karyotypes correlated with any known environmental variables. Intraspecific variation between parthenogenetic populations was also observed in rates of egg-laying and population growth in monoxenic cultures. It was concluded that evolutionary development had not halted with the adoption of parthenogenesis in *A. avenae* and that mutation rates and the automictic mode of reproduction of this nematode allowed for considerable genetic flexibility. A mechanism by which heterozygosity could be maintained in this nematode is discussed.

At 25°C rates of egg-laying by females of a parthenogenetic isolate of *A. avenae* were equal to those of amphimictic females, prompting an expectation of a twofold advantage in reproductive rate for the parthenogenetic isolate. However, when inoculated together on to fungal cultures the amphimictic isolate was not selectively displaced by the parthenogenetic isolate, possibly because of an inhibitory effect on egg-laying of parthenogenetic females induced by amphimictic males. Thus, amphimictic populations may be able to maintain themselves in nature in the presence of parthenogenetic competitors. The parthenogenetic population appeared to be better adapted to higher temperatures than the amphimictic population, the rates of egg-laying and egg-hatching at 30°C being higher for the former population. The ecological correlates of parthenogenesis in this nematode are discussed.

DECLARATION

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, no material described herein has been previously published or written by another person except when due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

.....19<sup>th</sup> July 2 1984.....  
Date

ACKNOWLEDGEMENTS

I wish to thank Professor H.R. Wallace, who supervised me during this work with a great deal of intelligence and enthusiasm. His encouragement and direction are gratefully acknowledged. I thank Dr. J.H. Warcup for providing constructive criticism. The congeniality of the staff and students of the Department of Plant Pathology, Waite Agricultural Research Institute, was a constant blessing throughout this work.

I thank Ms. A. Smith and other staff of the Biometry Department for their assistance with statistical analyses, Mr. B. Palk for preparing photographs and Mrs. M. Brock for typing the thesis.

This work was carried out under a Commonwealth Postgraduate Research Award and with additional support from the University of Adelaide.

## I. INTRODUCTION

Although *A. avenae* has been found in lesions on plant roots (Christie and Arndt, 1936), within root parenchyma tissue (Steiner, 1936) and to feed upon root hairs (Chin and Estey, 1966) and to multiply to some extent upon plant callus tissue (Barker and Darling, 1965), it is regarded as being primarily mycophagous (Hooper, 1974). Evans and Womersley (1980) considered that the reproduction of *A. avenae* in tomato root tissues observed by Chin and Estey (1966) conclusively demonstrated that at least some populations of the nematode may act as facultative plant pathogens. However, Chin and Estey allowed that the nematodes may have entered the roots to feed upon *Verticillium albo-atrum* with which the test plants had also been inoculated. Nevertheless, it is clear that the classification of *A. avenae* as a strict, obligate fungivore made by Mankau and Mankau (1963) was premature. The high rate of reproduction of this nematode upon a wide range of fungi (Townshend, 1964), certainly suggests that fungi are its preferred food. That some browsing on roots may also occur is not surprising and is in accord with the hypothesis put forward by taxonomists (Maggenti, 1971; Triantaphyllou and Hirschmann, 1980) in deriving the evolutionary development of plant parasitic nematodes from mycophagous ancestors.

Plant pathologists have concentrated mainly on the effects of *A. avenae* on root-pathogenic fungi (Rhoades and Linford, 1959; Barker, 1964; Klink and Barker, 1968; Barnes *et al.*, 1981; Caubel *et al.*, 1981) in the hope of enlisting the nematode as an agent for biological control and, more recently, on possible adverse effects on plants through feeding on mycorrhizal fungi (Sutherland and Fortin, 1968; Hussey and Roncadori, 1981). Apart from these aspects its ecological role in the soil has re-

ceived scant attention. Evans and Womersley (1980) have recently reviewed information on its behaviour, physiology and experimental uses. The factors controlling its distribution and abundance are largely unknown and are liable to remain so until there is a better understanding of the ecology of its food resources - soil fungi - and of its relationships with other soil fauna. Studies of soil fungi pose many problems owing to their enormous diversity and variety of form and all isolation methods are more or less selective for particular groups and/or types of propagules. New techniques, which aim to quantify the amount of living and active mycelium in soil (Frankland, 1975; Söderström, 1977), may eventually be of use in studies on soil fauna which feed on fungal protoplasm, but present great analytical problems when comparing different soils (Bååth and Söderström, 1982).

In surveying the literature on this nematode it was found that at least 92 species of fungi from 50 genera and including all sub-divisions of the Eumycota have been recorded as food sources for various *A. avenae* isolates in culture. Studies in which a single *A. avenae* isolate has been tested on a variety of fungi in culture (Mankau and Mankau, 1963; Townshend, 1964) also suggest that this nematode acts as an ecological generalist (that is, utilizing a wide range of resources), but that it prefers plant pathogenic fungi. However, a study has not previously been made using *A. avenae* and fungi isolated from the same soil. The present study aims to test the hypothesis that abundance of *A. avenae* is influenced by composition of the soil mycoflora. Population densities of *A. avenae* and soil mycofloras have been examined in two habitats - a field under wheat and a pine-forest - which differ markedly in the composition of their surface vegetation and hence are expected to differ in the composition

of their soil mycofloras (Waid, 1960). The suitability of these mycofloras to support population growth of *A. avenae* was compared by measuring rates of population growth of *A. avenae* reared on fungi from both soils. Other mycophagous nematodes from the same soils were tested on these fungi to determine the extent of resource overlap and, hence, the likelihood of competitive interactions between these nematodes and *A. avenae*.

Parthenogenetic plant parasitic nematodes (Triantaphyllou and Hirschmann, 1980) and, indeed, all parthenogenetic organisms, (White, 1973), are commonly regarded as having strictly limited evolutionary potential. In this study, the genetic diversity amongst parthenogenetic populations of *A. avenae* from soils given over to the same regime of land use - to minimize differences in the composition of the soil mycoflora - was examined as an indication of the genetic flexibility allowed by this mode of reproduction. In these soils, planted to wheat, population density of *A. avenae* was compared with physico-chemical properties of the soil to determine their influence on abundance of the nematode. Different populations of the nematode were reared in culture under different environmental conditions to investigate the possible adaptive significance of existing genetic variation in relation to soil factors. Genetic variation between parthenogenetic isolates of *A. avenae* from wheat-field and pine-forest soils was also studied by comparing reproductive rates of the nematode reared on various fungi.

Amphimictic populations of *A. avenae* have been found from Western Australia (Evans, 1968; Evans and Fisher, 1970) and Malawi (Evans and Womersley, 1980; Hooper and Clark, 1980). All other populations examined from various parts of the world including Australia (Evans and Fisher, 1970), North America (Triantaphyllou and Fisher, 1976), South America



(Dao, 1970) and Europe (Goodey and Hooper, 1965; Dao, 1970) have apparently been parthenogenetic. Three parthenogenetic *A. avenae* populations examined by Triantaphyllou and Fisher (1976) were found to undergo meiosis and their mode of reproduction can thus be classified as automictic or meiotic parthenogenesis (White, 1973). The transition from amphimixis to meiotic parthenogenesis is viewed as a relatively simple phenomenon by cytogeneticists (White, 1973; Suomalainen *et al.*, 1979), spontaneous or tycho-parthenogenesis - thought to involve automictic mechanisms - being reported from a variety of otherwise bisexual species (White, 1973; Cuellar, 1977). The nature of the selective forces which maintain sexual reproduction in nature and the advantages accruing to an organism which adopts parthenogenesis have become central issues in evolutionary biology (Williams, 1975; Cuellar, 1977; Maynard Smith, 1978; Charlesworth, 1980; Tooby, 1982). Although parthenogenesis has arisen repeatedly amongst all families of plant parasitic nematodes (Triantaphyllou and Hirschmann, 1980), nematologists have contributed little to this discussion (but see Triantaphyllou and Hirschmann, 1964, and Poinar and Hansen, 1983, for reviews of reproduction in nematodes). Species, like *A. avenae*, which exist in both amphimictic and parthenogenetic forms may present opportunities to test some of the predictions made by evolutionary biologists. In this study, the supposed two-fold advantage of parthenogenesis (Maynard Smith, 1978) is examined with respect to *A. avenae*.

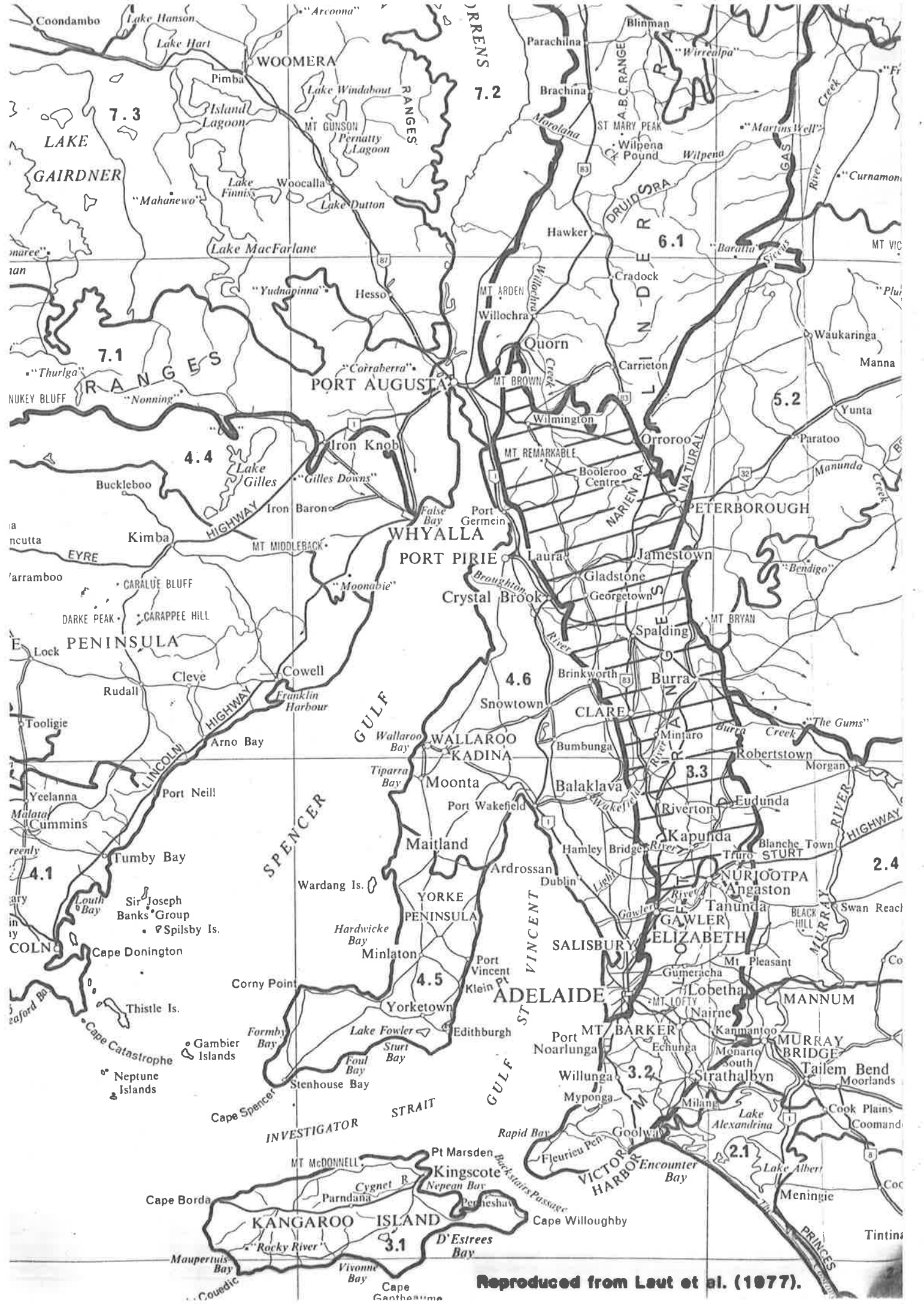
## II. MATERIALS AND METHODS

### 1. SAMPLING SITES

#### (a) Mid-North Wheatlands Environmental Region

In 1977 the CSIRO Division of Land Use Research published the results of its ecological survey of South Australia, a study intended to be used as a test case for assessing the suitability of LANDSAT imagery for this purpose (Laut *et al.*, 1977). The approach used to map and describe the environments of South Australia was a modified form of integrated reconnaissance survey in which regions were characterized by combinations of attributes (rather than one single attribute such as soils, vegetation or land use) but with emphasis being given to geomorphology. A simple four-level hierarchy of areal units was used to provide the necessary scales to describe environmental conditions, ranging from the smallest, "environmental units", through "environmental associations" and "environmental regions" to "environmental provinces". This system is suitable for use in organizing field surveys of soil fauna, more so than mapping systems which use an arbitrary grid because the areal units relate to actual environmental conditions. The northern-most region of the Mt. Lofty Block Province, named the Mid-North Wheatlands Environmental Region (Fig. 1) after its predominant land use, provided an ideal basis for a survey of *Aphelenchus avenae* in wheat-fields. It extends from Adelaide to Quorn, the northern boundary marked by the approximate northern limit of wheat cultivation, and encompasses considerable climatic heterogeneity. Mean annual rainfall ranges from 250-700 mm while mean annual evaporation ranges from 1800-2400 mm. It consists of 19 environmental associations covering an area of 13,180 km<sup>2</sup>; the associations ranging in size from 20 km<sup>2</sup> to 2,090 km<sup>2</sup>. The geomorphology is characterized by narrow ridges separated by wide plains. The dominant

FIG. 1: The Mid-North Wheatlands Environmental  
Region (hatched area) of South Australia



Reproduced from Laut et al. (1977).

soil type is a hardsetting pedal red duplex soil, Dr 2.23 in the classification of Northcote (1974). This red-brown earth is the mainstay of wheat growing in South Australia (Stace *et al.*, 1968).

Fifty-seven soil samples were taken from fields under wheat in the Mid-North Wheatlands Environmental Region during the 1980 wheat season between 28 July and 17 October. All 19 environmental associations were sampled at least once, some as many as 8 times (Table 1, Fig. 2).

(b) Williamstown Wheat-Field and Pine-Forest

The wheat-field and pine-forest chosen for a more detailed study of the abundance of *A. avenae* were selected on the basis of their proximity, both being located near Williamstown (34°40'S, 138°53'E), South Australia in the Rosedale Environmental Association (3.3.1, see Fig. 2). Mean annual rainfall in this area is about 560 mm.

The pine-forest, planted with *Pinus radiata* D. Don in 1966, is located on the northern shore of the South Para Reservoir. The field, under wheat at the time of sampling, is situated 5 km north-west of the forest on the northern face of a hill sloping at 10-15°. It had had a rotation of cereals and pasture grasses since at least before 1960. Woodland dominated by *Eucalyptus leucoxylon* and *E. odorata* originally covered much of the surrounding region (Laut *et al.*, 1977) while the dominant soil type is a hard setting pedal red duplex soil (Dr 2.23 in the classification of Northcote, 1974).

## 2. SOIL SAMPLING

(a) Mid-North Wheatlands Environmental Region

In the survey of mid-north wheat-fields, soil samples 18.5 cm wide

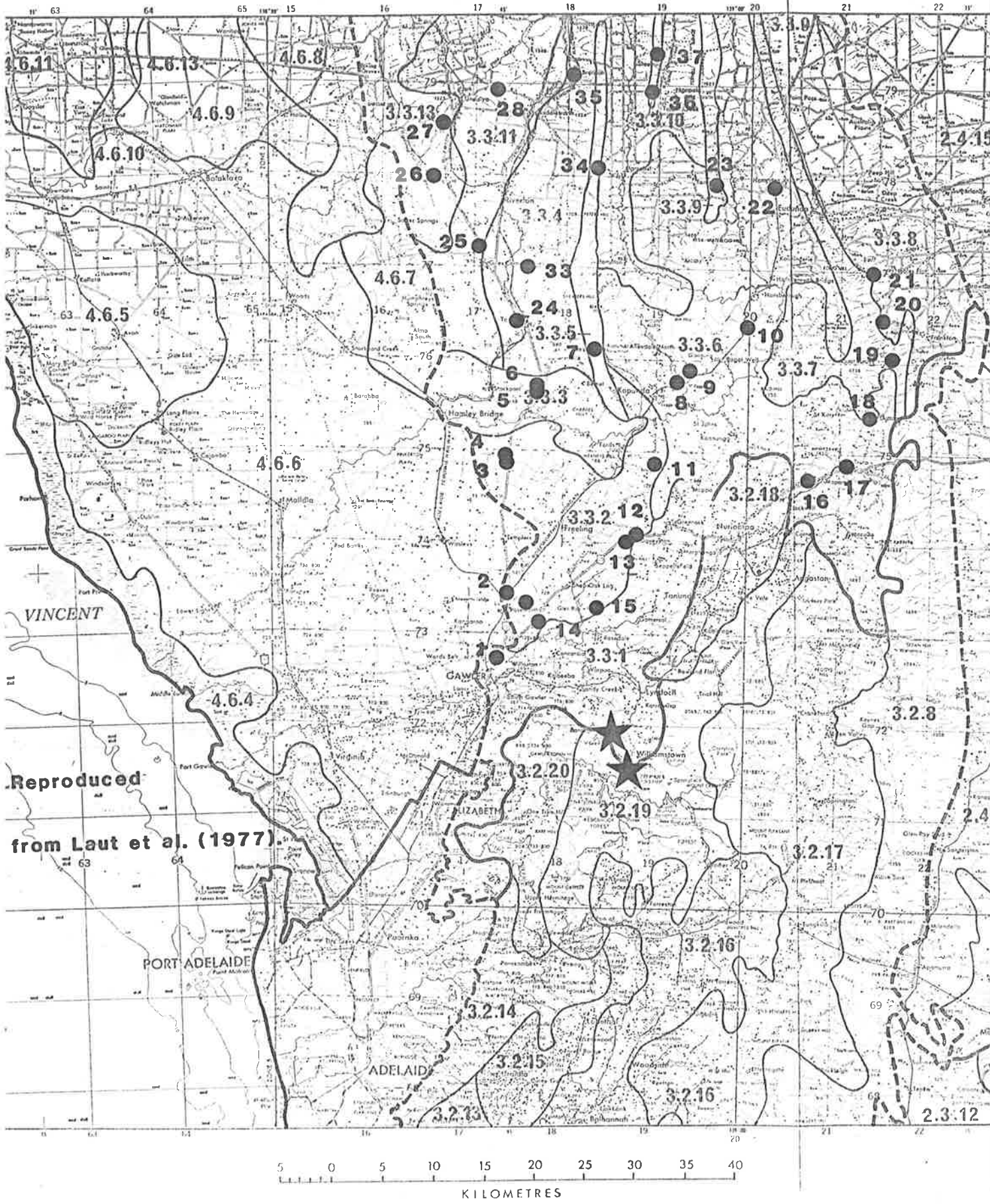
TABLE 1: Soil samples taken from fields under wheat from Environmental Associations of the Mid-North Wheatlands Environmental Region during the 1980 growing season

Environmental Association	No. Samples	Dates Sampled
Rosedale (3.3.1)	1	28/7
Freeling (3.3.2)	8	28/7(7), 3/9(1)
Stockport (3.3.3)	2	28/7
Tarlee (3.3.4)	3	22/9(1), 16/10(2)
Shearers Hill (3.3.5)	2	28/7 16/10
Mopami (3.3.6)	6	28/7(3), 3/9(3)
Rufus (3.3.7)	2	3/9
Neales Flat (3.3.8)	2	3/9
Burra Hill (3.3.9)	2	3/9 17/10
Apoinga (3.3.10)	2	16/10
Hansen (3.3.11)	7	22/9(2), 16/10(5)
Bald Hill (3.3.12)	2	16/10
Clare (3.3.13)	3	22/9(2), 17/10(1)
Yongala (3.3.14)	2	22/9 17/10
Yacka (3.3.15)	2	22/9
Appila (3.3.16)	4	22/9(1), 17/10(3)
Tarcowie (3.3.17)	1	17/10
Wirrabara (3.3.18)	2	17/10
Mt. Remarkable (3.3.19)	4	17/10

FIG. 2 (A,B & C): Mid-North Wheatlands Environmental Region showing Environmental Associations (3.3.1 - 3.3.19) and the locations (numbered) of the 57 fields under wheat sampled. The wheat-field and pine-forest sampled near Williamstown (in Environmental Association 3.3.1) are marked by stars, the more southerly of which corresponds to the pine-forest.

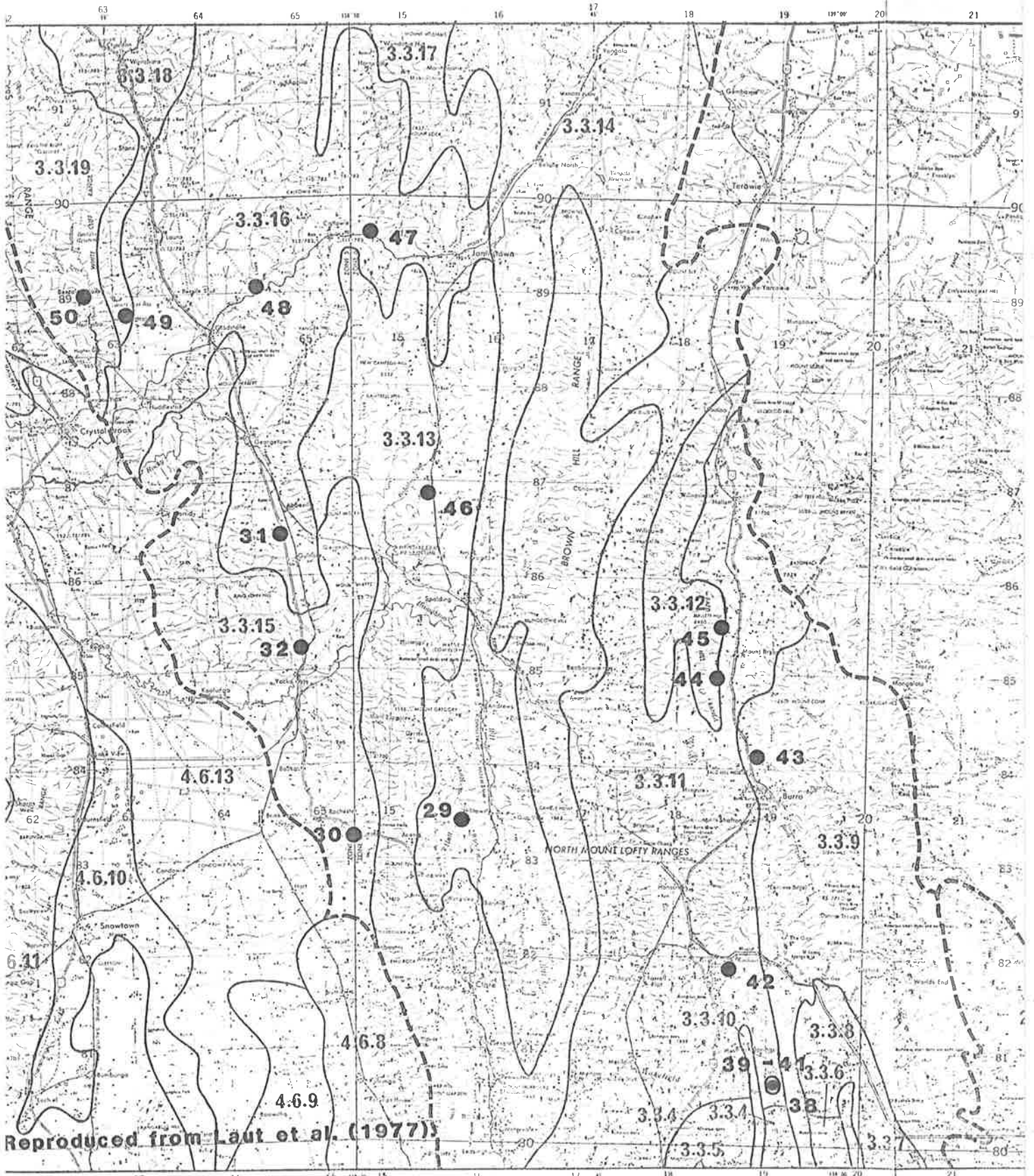
The northern boundary (top of page) of Map A joins the southern boundary of Map B; Map C similarly joins Map B.

ADELAIDE





BURRA



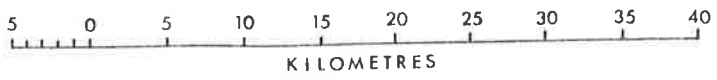
Reproduced from Laut et al. (1977)



# PORT AUGUSTA - ORROROO



Reproduced from Laut et al. (1977)



and 20 cm deep, were taken with a spade. Three sub-samples were arbitrarily taken from each wheat-field sampled and bulked to give a combined sample of 5-7 kg in weight. Large stones were removed and soil clods broken by hand and the sample was thoroughly mixed by shaking in a polythene bag for one minute.

(b) Williamstown Wheat-Field and Pine-Forest

A 3-hectare area, 200 by 150 m, was arbitrarily chosen for study at each site and soil sampled from each of 12 quadrats, 50 m square. The locality within each quadrat sampled was chosen by a random-walk technique in which numbers of paces (from 0-60, 60 paces corresponding to about 50 m) north and east of the south-west corner of each quadrat were assigned by random number tables. At the locality so chosen, six sub-samples were taken with a 5 cm diameter auger to a depth of 10 cm from an area of one-metre square and bulked together. Large stones were removed and soil clods broken by hand and the sample was thoroughly mixed by shaking in a polythene bag for one minute. An additional sample was taken from each quadrat of the wheat-field with a spade from the nearest row of wheat to measure plant height.

The surface litter was brushed away from the floor of the pine-forest before sampling of the mineral horizons but coverings of mosses and liverworts were not removed. Samples of litter from each of the L, F and H-layers defined by Hesselman (1926) and Kubiena (1953) were also taken from each quadrat in the pine-forest. These layers could be distinguished by the stage of decomposition of the pine-needles. The L-layer was characterized by undecomposed, non-compacted litter composed of brown, intact needles. The F-layer was more compacted and was composed of darker brown, partially fragmented needles. Where the litter was sufficiently deep it could be

divided into F<sub>1</sub>- and F<sub>2</sub>-layers. Needles were often intact in the F<sub>1</sub>-layer but were more fragmented and compressed in the F<sub>2</sub>-layer. Fungal hyphae were very dense in the F<sub>2</sub>-layer, with basidiomycetes, especially *Amphinema byssoides*, forming a continuous mat. The H-layer was composed of dark, amorphous humus.

### 3. PHYSICO-CHEMICAL CHARACTERISTICS OF SOILS

#### (a) Mid-North Wheatlands Environmental Region

##### (1) Soil pH

Soil pH was measured with soil suspended in 0.01 M CaCl<sub>2</sub> solution (Peech, 1965).

##### (2) Total soil salinity

Electrical conductivity of 1:5 soil:water extracts in  $\mu\text{ S cm}^{-1}$  at 25°C was determined to give an indication of total soil salinity.

##### (3) Soil texture

Soil texture was estimated using the method of Jaenike *et al.* (1980). Soil was first dried at 90°C for 72 hours and sifted through a 2 mm sieve. Ten g of soil was added to a large test-tube, mixed with 30 ml distilled water and agitated for 2 min. at maximum speed on a laboratory test-tube shaker. The suspension was allowed to settle for 30 sec., the components separated, dried at 90°C for 72 hours and weighed. The component that settled in 30 sec. was designated sand, and that remaining in suspension was designated silt and clay.

The results obtained by this simple technique were found to be reproducible. For example, results from 5 replicate assays on soil from sample no. 40 are given on the next page.

<u>Replicate</u>	<u>% Silt and Clay</u>	<u>% Sand</u>	<u>Total</u>
1	60.1	39.5	99.6
2	59.5	40.0	99.5
3	59.8	39.8	99.6
4	57.8	41.6	99.4
5	54.3	45.0	99.3

Totals do not attain 100% due to small losses of material, ranging from 0.04-0.07 g, during separation of components. Results obtained by this method on wheat-field soils are very similar to those obtained with more elaborate hydrometer methods (Table 2).

(b) Williamstown Wheat-Field and Pine-Forest

Soil pH and total soil salinity were determined for soil samples from these sites by the methods described above. In addition, % soil moisture was determined for 2 replicate 50 g sub-samples per sample by drying in a 90°C oven for 72 hours. Soil texture was determined for a sample from each site by both the methods of Jaenike *et al.* (1980) and the hydrometer method (Piper, 1950), allowing a comparison of these methods to be made. Results obtained by the two methods were similar, especially with the wheat-field soil (Table 2). With the much coarser pine-forest soil the hydrometer methods gave somewhat higher readings for the silt and clay component.

#### 4. EXTRACTION OF NEMATODES

A modified Baermann method (after Whitehead and Hemming, 1965) was used throughout for extraction of nematodes from soil. Large numbers of samples can be processed simultaneously by this method. Fifty ml of soil were evenly spread over a 2-ply facial tissue supported by a stainless-steel wire mesh placed in water in a 140 mm Petri dish. Nematodes were extracted over 48 hours at room temperature and counted in Doncaster dishes. *Aphelenchus avenae* could be readily identified under a stereoscopic dissecting

TABLE 2: Comparison of hydrometer (H) method and method of Jaenike *et al.* (1980) for determining soil texture in wheat-field (W) and pine-forest (P) soils

Soil	Replicate	% silt and clay		% sand	
		H	J	H	J
W	1	50.36	53.5	49.64	46.2
	2	51.96	50.6	48.04	49.2
	3	52.16	54.0	47.84	45.8
	4	-	53.0	-	46.4
	Mean	51.49	52.8	48.51	46.9
P	1	14.56	8.7	85.44	91.0
	2	14.76	8.5	85.24	91.4
	3	15.16	8.5	84.84	91.2
	Mean	14.82	8.6	85.17	91.2



microscope and specimens removed for starting cultures. Two replicate 50 ml soil samples were processed for each sample from the Williamstown wheat-field and pine-forest.

Nematodes were extracted from basidiomycete fructifications using a thermal misting apparatus or the modified Baermann method. Thermal misting and a modified Minderman (1956) technique were used to extract nematodes from pine-forest litter. Pine-needle fragments were supported on a nylon mesh with 1 mm openings in a beaker of water and agitated gently at 20 oscillations per minute in an orbital shaking water bath for 24 hours at 20°C. Reproducible results could be obtained by this technique; 10 replicate 300 mg samples of litter from the F<sub>2</sub>-layer yielded a mean of 91.2 ± 6.1 (S.E.) nematodes. Yields from litter extracted by thermal misting attained 212 nematodes per g of litter.

##### 5. MONOXENIC CULTURES OF MYCOPHAGOUS NEMATODES

Monoxenic cultures of *A. avenae* obtained from all soil samples were established using a fungus as a food source growing on neutral Dox yeast (NDY) agar (Appendix 1), pH 5.5 with 100 ppm streptomycin and 10 ppm tetracycline, in 90 mm Petri plates. Cultures were normally started using two adult female specimens of the nematode. These were treated with 0.5% chlorhexidine gluconate, a bacteriocidal and fungicidal agent (Peacock, 1959), for 5 minutes and washed in sterile distilled water before being transferred by hand to a drop of sterile water on the surface of the agar. Plates were incubated at 25°C. Once cultures were established a single female from each was used to start new cultures and thus obtain nematodes of common descent for use in experiments.

A strain of *Rhizoctonia solani* obtained from Dr. J.M. Fisher was initially used as a food source to establish *A. avenae* from Mid-North

Wheatlands soil samples but this proved to be an unsuitable host for a number of isolates of the nematode. A strain of *Botrytis cinerea* isolated from a *Myoporum* sp. by Dr. J.H. Warcup proved to be a universally acceptable host for *A. avenae* isolates and was subsequently used to maintain all cultures of the nematode.

The same procedures were used to attempt culture of other possible mycophagous nematodes such as *Aphelenchoides* spp. and *Dorylaims*.

#### 6. KARYOTYPE DETERMINATION IN *APHELENCHUS AVENAE*

Meiotic chromosomes in *A. avenae* oocytes were stained with acetic orcein following a similar procedure to that of Triantaphyllou and Hirschmann (1966). Adult female *A. avenae* were smeared across clean microscope slides with the tip of a hypodermic needle and the cuticles removed. Slides were hydrolyzed in 1N HCl for 5-10 min., fixed in 1:3 acetic acid: ethanol for 20-40 min., stained in 2% orcein in 45% acetic acid for 20-30 min. and rinsed briefly in 45% acetic acid before mounting.

#### 7. SEPARATION OF POLYPEPTIDES IN NEMATODE HOMOGENATES BY POLYACRYLAMIDE GEL ELECTROPHORESIS

*A. avenae* were cultured in mass on *Botrytis cinerea* as a food source using the method of Evans (1970). The nematodes were concentrated into a small volume, suspended in cold 0.125 M Tris-HCl buffer, pH 6.8, spun down for several minutes in a bench centrifuge and the supernatant removed. Further buffer was then added equal to the wet weight of the nematodes. Nematodes were homogenized at 5°C with a teflon pestle, spun down for 10 min. at 12,800 g and the supernatant stored at -70°C until use.



## 8. SEPARATION OF LENGTH-CLASSES OF *APHELENCHUS AVENAE*

Attempts were made to separate mass cultures of *A. avenae* into uniform length-classes to compare polypeptide patterns of adult and larval stages by polyacrylamide gel electrophoresis. Laboratory sieves and polyamide monofilament cloth (Myers *et al.*, 1971) were found to be more convenient for this purpose than glass microbeads (Chow and Pasternak, 1969). Sieves were placed in containers partially filled with water and a suspension of nematodes added. After varying periods of time nematodes retained on sieves were separated from those having passed through and the lengths of ten randomly selected nematodes from each group measured from drawings made with a camera lucida (Appendix 2). Although useful separations of length-classes could be achieved by this technique, results varied with cultures of different ages.

## 9. GENERAL METHODS OF ISOLATION OF FUNGI FROM SOIL

Fungi were isolated from Williamstown soil samples using dilution and soil plate methods (Warcup, 1950) with neutral Dox yeast (NDY) agar, pH 5.5, containing 100 ppm streptomycin.

Dilution plates were prepared at a dilution of  $10^{-4}$ , incubated at 25°C and colonies sub-cultured onto NDY after 2-5 days. Soil plates were prepared by transferring about 5 mg of soil with a sterile spatula to a drop of sterile water in a Petri dish, dispersing the soil in the drop and pouring cooled but molten NDY into the dish. Soil plates were treated in the same manner as dilution plates.

Mycelial cultures of basidiomycetes were started from fruiting bodies by inoculation of 2% malt agar (Appendix 1) with small portions of cap or stipe tissue.

## 10. ISOLATION OF FUNGI FROM PLANT ROOTS

Washed wheat and *Pinus* roots were cut into 1 cm lengths, treated with either a solution containing 1000 ppm of both neomycin and streptomycin (30 min) or 0.5% sodium hypochlorite (1 min), washed in sterile water and plated onto NDY with 100 ppm streptomycin.

## 11. ISOLATION OF ACTINOMYCETES AND BACTERIA FROM SOIL

Actinomycetes and bacteria were isolated from Williamstown soil samples using dilution plates with 2% water agar, pH 7.2 (Lingappa and Lockwood, 1962). Dilution plates were prepared at a dilution of 1/5000 and incubated at 28°C for one week before counts were made of colonies and sub-cultures made. Actinomycetes were sub-cultured onto Krainsky's medium and bacteria onto nutrient agar (Appendix 1).

## 12. FEEDING TRIALS

Nematodes to be used in feeding trials were extracted from 2-week old cultures maintained at 25°C on *Botrytis cinerea* on NDY agar using Baermann funnels through 4 layers of facial tissue. This procedure removed much of the fungal debris and, provided cultures were kept in the dark to inhibit sporulation, fungal spores were rarely a problem. Since stock cultures were maintained on *B. cinerea* and nematodes used in feeding trials were subsequently discarded, each feeding trial represented a novel situation in that neither the nematodes nor their progenitors had been previously exposed (at least in the laboratory) to the test organism before.

Nematodes were treated with either 0.5% chlorhexidine gluconate for 2-10 min or a solution containing 1000 ppm benzyl-penicillin, 1000 pm streptomycin and 10 ppm tetracycline for 1 hour before washing in sterile

water. A drop of sterile water was placed on the surface of the agar of test plates and 5 adult female nematodes transferred to the drop by hand.

(a) Fungi

Fungi to be tested as food sources were grown at 25°C on NDY agar plates. Discs, 6 mm in diameter, were taken from the growing margin of 2 week-old cultures, or, in the case of slow-growing forms such as many of the basidiomycetes, from 4-6 week-old cultures. One disc was placed in the centre of the agar surface in each test plate which consisted of 9 cm plastic Petri dishes containing 10-12 ml of NDY agar, pH 5.5, with 100 ppm streptomycin and 10 ppm tetracycline.

(b) Actinomycetes

Actinomycetes were maintained on Krainsky's medium at 25°C and 9 cm plastic Petri dishes containing 10-12 ml of the same agar medium inoculated with actinomycetes by streaking with a sterile loop passed over the surface of the stock culture.

(c) Bacteria

Bacteria were tested as food sources of nematodes in the same manner as were actinomycetes, except that nutrient agar was used.

(d) Harvesting of Nematodes

Plates inoculated with nematodes and test organisms were incubated for 2 weeks in the dark at 25°C before harvesting. Plates were harvested by dicing the agar and placing on 2-ply facial tissue supported in a Petri dish of water by a coarse nylon mesh for 48 hours. An aliquot of the nematode suspension was examined under a dissecting microscope and the number of adults (of each sex) and larvae counted such that at least 100 individuals were counted per plate. The total number of nematodes per

plate was then calculated. If there were less than 100 nematodes per plate the total number was counted.

At least 4 replicate plates per test organism were used and in many cases this number was exceeded. However, plates were discarded if contaminated with extraneous bacteria or fungi.

### III. INTRASPECIFIC VARIATION AND ABUNDANCE OF APHELENCHUS AVENAE

Variation between populations of *A. avenae* and abundance of the nematode were studied firstly, in soil from a single type of habitat from a defined area - wheat-fields of the Mid-North Wheatlands Environmental Region - and secondly, in soil from a variety of habitats from different areas.

#### 1. ABUNDANCE OF *APHELENCHUS AVENAE* IN WHEAT-FIELDS OF THE MID-NORTH WHEATLANDS ENVIRONMENTAL REGION

*A. avenae* was found in soil from all 57 fields under wheat from the Mid-North Wheatlands Environmental Region. Mean population density was  $92.2 \pm 15.0$  (S.E.) per 50 ml soil (corresponding to  $349,242 \text{ m}^{-2}$  in the top 20 cm of soil) with numbers ranging from 4 to 520. It is clear that *A. avenae* comprises a numerically important part of the nematode fauna of South Australian wheat-field soils, a fact emphasized by a comparison with data from other sites. For instance, *A. avenae* ranged from  $3000\text{--}28000 \text{ m}^{-2}$  in the top 10 cm of soil from five New Zealand sites under pasture (Yeates, 1981). Under permanent pasture at the Waite Agricultural Research Institute, *A. avenae* was found at a mean population density of 12.9 per 50 ml soil.

*A. avenae* made up  $9.0 \pm 1.2$  (S.E.) % of total nematodes extracted from wheat-field soil. Relative abundance ranged from 0.4 to a massive 50.9% of total nematodes, the latter figure coming from soil first planted to wheat in the year of sampling on the edge of a brackish lagoon (see Black Springs Lagoon transect p. 31). Relative abundances were generally lower in the other wheat-fields which normally also had much longer cropping histories. *A. avenae* is known to be a significant component of the nematode fauna under other field crops; thus it made up 2-7% of total nematodes in soybean fields (Norton *et al.*, 1971). Relative abundance in wheat-field

soil was, however, much higher than that found in soils under pasture.

*A. avenae* made up only 1.5% of total nematodes under permanent pasture at the Waite Agricultural Research Institute, while Yeates (1981) found it comprised 0.3-3.5% of total nematodes in five New Zealand soils under grazed pasture.

Mean population density of nematodes from wheat-field soil was  $1105 \pm 127.3$  (S.E.) for 50 ml soil corresponding to  $4184\ 000\ m^{-2}$  in the top 20 cm of soil; a moderate value compared with other terrestrial ecosystems (Sohlenius, 1980) and exceeding that -  $638.4 \pm 83$  (S.E.) - calculated from data of Oostenbrink *et al.* (1956) for an equivalent amount of soil from 8 Dutch wheat-fields.

Washed wheat roots from several soil samples were treated with boiling lactophenol containing 0.1% cotton blue to stain nematodes but *A. avenae* were not detected in roots.

## 2. ASSOCIATIONS BETWEEN ABUNDANCE OF *APHELENCHUS AVENAE* AND ENVIRONMENTAL FACTORS IN MID-NORTH WHEATLANDS ENVIRONMENTAL REGION

Correlation and regression analyses were applied to data on abundance of *A. avenae* (no. per 50 ml soil and % of total nematodes) and the following environmental variables: EC 1:5 - electrical conductivity of 1:5 soil - water suspension in  $\mu S\ cm^{-1}$  at 25°C; ECe - electrical conductivity of soil-water suspension (EC 1:5) multiplied by a conversion factor (F) which varies with soil texture (Berstein, 1964). Conversion factors used were those listed in Heanes (1981) and soil texture classes of Chittleborough (1981) were assigned on the basis of % clay content; soil pH; % sand - soil fraction settling in 30 sec; P/E - mean annual precipitation/mean annual evaporation (mm) for the particular Environmental Association as listed by Laut *et al.* (1977); MA - mean altitude (m) of the particular Environmental Association as listed by Laut *et al.* (1977).

The resulting correlation matrix is presented in Table 3. At sites where *A. avenae* was abundant the nematode also tended to be relatively more abundant (as % of total nematodes), that is, the density of this nematode did not vary simply with the total population of nematodes. This suggests that some fields were more favourable than others for population development of *A. avenae*. Population densities of *A. avenae* tended to be higher in more alkaline soils and the nematode tended to be relatively more abundant in soils with higher levels of soluble salts (as reflected by higher E<sub>Ce</sub> and EC 1:5 values). This does not necessarily mean that *A. avenae* prefers soils of higher pH or that it is less sensitive to salinity than other nematodes of wheat-field soils; these factors may be influencing population densities indirectly. *A. avenae* tended to be relatively more abundant in sandier soils. Norton *et al.* (1971) also found *A. avenae* tended to be relatively more abundant in sandier soils compared with soils with higher clay contents from 40 soybean fields.

Soil pH was positively correlated with the level of soluble salts (EC 1:5 and, to a greater extent, with E<sub>Ce</sub>). E<sub>Ce</sub> (but not EC 1:5) was positively correlated with soil pH and, not surprisingly, % sand. A higher correlation was found between % total nematodes and E<sub>Ce</sub> than with EC 1:5. These results suggest conversion of data to E<sub>Ce</sub> is a useful step to take in ecological surveys of soil fauna. Mean altitude was negatively correlated with the precipitation/evaporation ratio and with soil pH.

Regression analyses were summarized in Table 4 and data are depicted in Figs. 3-8. Conclusions drawn were that *A. avenae* tends to be relatively more abundant in soils with higher levels of soluble salts (a relationship more clearly illustrated, once again, by the use of E<sub>Ce</sub>) and in sandier soils; and numerically more abundant in more alkaline soils.

TABLE 3: Correlation matrix (55 d.f.) between absolute and relative (% of total nematodes) abundance of *A. avenae* in 57 wheat-fields in the Mid-North Wheatlands Environmental Region and various environmental factors

	No.	% total	EC 1:5	pH	% sand	P/E	ECe	MA
No.	1.00							
% total	0.46*	1.00						
EC 1:5	0.25	0.31*	1.00					
pH	0.36*	0.18	0.52*	1.00				
% sand	-0.18	-0.26*	-0.03	-0.04	1.00			
P/E	-0.01	0.08	-0.09	0.07	0.04	1.00		
ECe	0.01	0.41*	0.83*	0.27*	0.38*	-0.01	1.00	
MA	0.03	0.18	0.18	-0.26*	-0.22	-0.36*	0.14	1.00

\*Correlation coefficient significant at 5% level.



TABLE 4: Summary of regression analysis and analysis of variance for absolute and relative (% of total nematodes) abundance of *A. avenae* in 57 wheat fields of Mid-North Wheatlands Environmental Region on various environmental variables

Regression	Regression Coefficient	F-value	% Variance accounted for
No. on EC 1:5	1.88	3.54	4.3
% total on EC 1:5	2.37*	5.64*	7.6
No. on ECe	0.07	0.01	R
% Total on ECe	3.30*	10.90*	15.0
No. on pH	2.89*	8.33*	11.6
% Total on pH	1.35	1.82	1.4
No. on % sand	-1.38	1.90	1.6
% Total on % sand	1.97*	3.89	4.9
No. on P/E	-0.08	0.01	R
% Total on P/E	0.57	0.32	R
No. on MA	0.21	0.05	R
% Total on MA	1.32	1.73	1.3

\* Significant at 5% level.

R - Residual variance exceeds variance of y-variate.

FIG. 3: Fitted regression lines for number of *A. avenae* per 50 ml soil (a) and as % of total nematodes (b) on electrical conductivity of 1:5 soil:water suspension from 57 wheat fields of Mid-North Wheatlands Environmental Region.

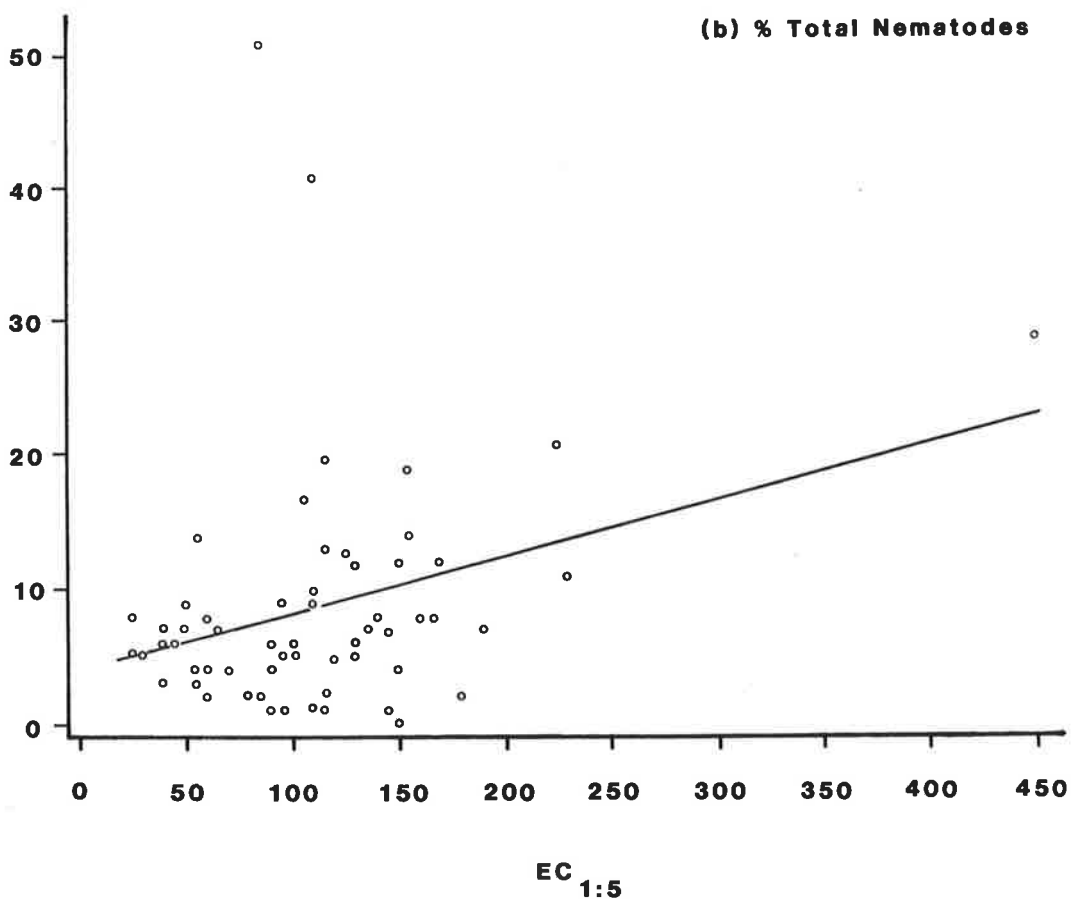
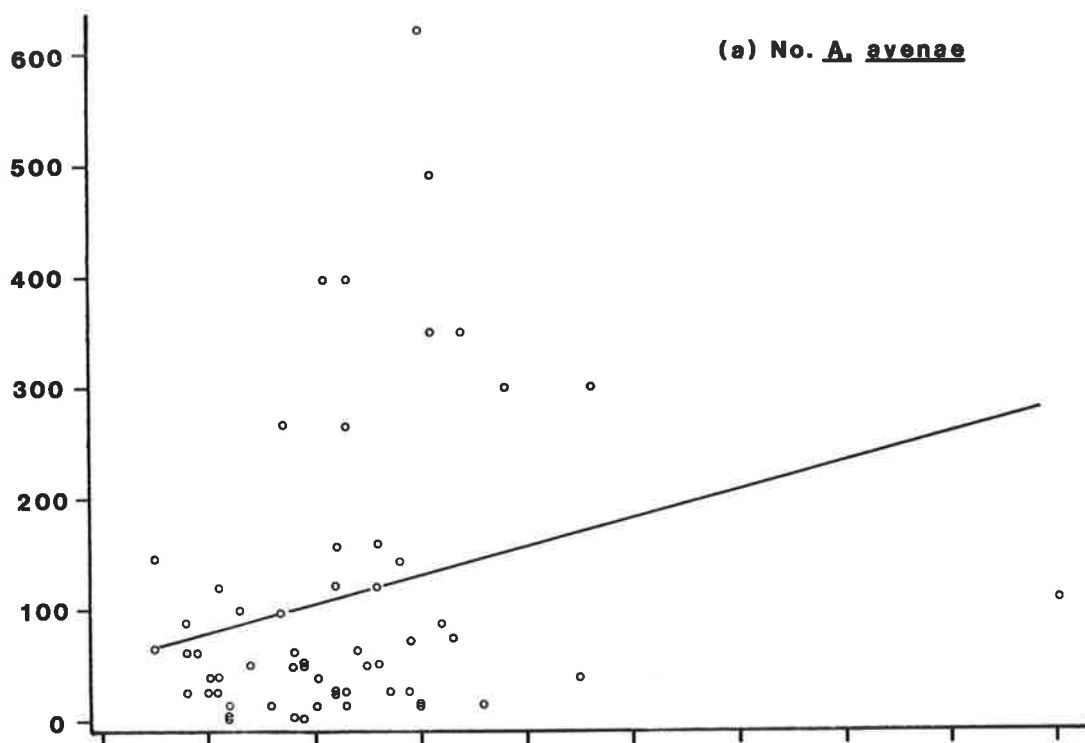


FIG. 4: Fitted regression lines for number of *A. avenae* per 50 ml soil (a) and as % of total nematodes (b) on electrical conductivity of soil-water suspension, modified to take account of variation arising from different soil textures (ECe) from 57 wheat fields of Mid-North Wheatlands Environmental Region.

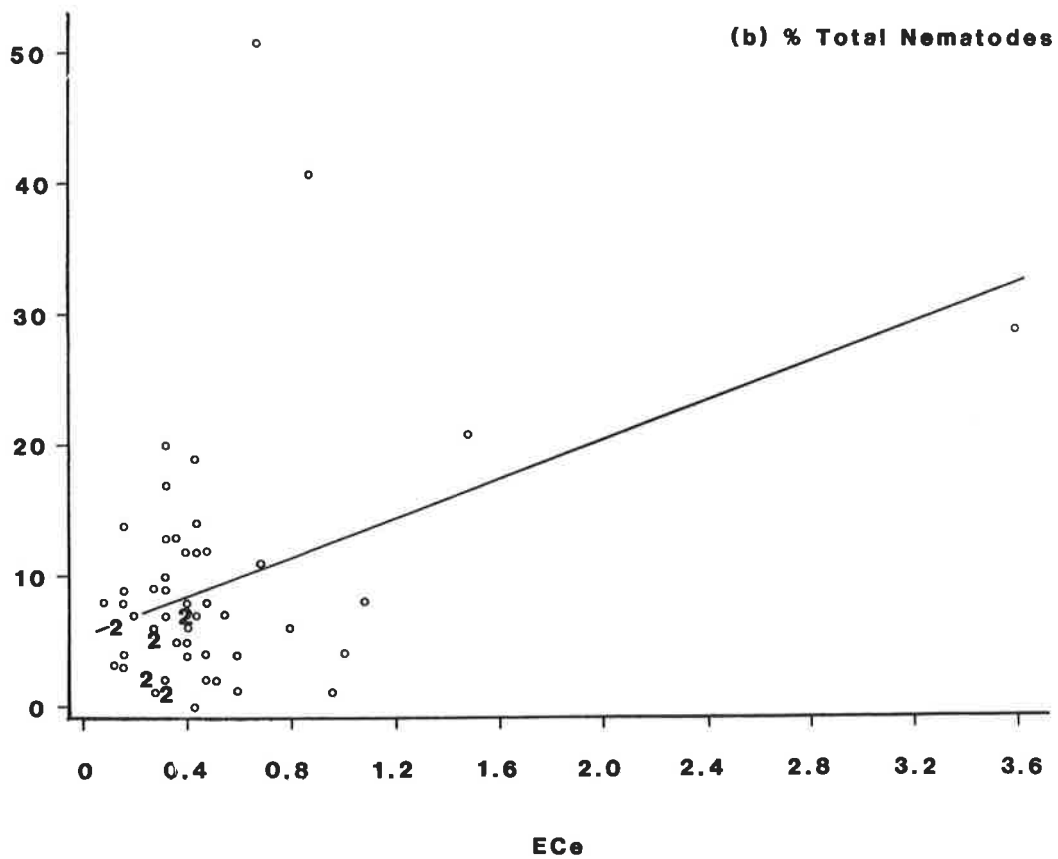
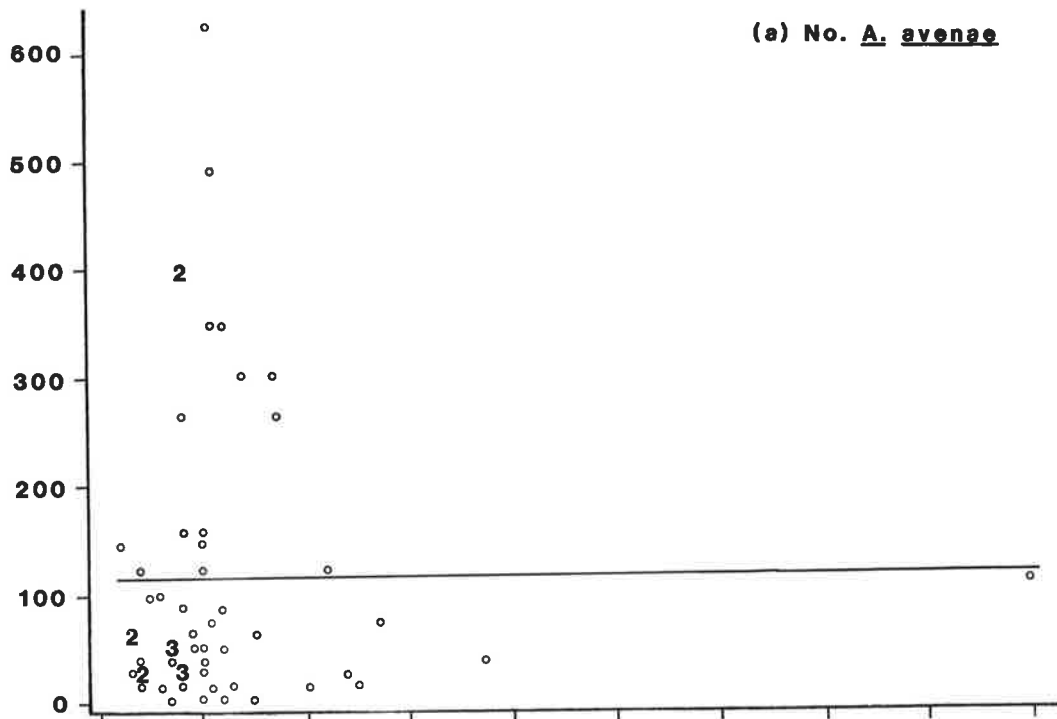


FIG. 5: Fitted regression lines for number of *A. avenae* per 50 ml soil (a) and as % of total nematodes (b) on soil pH for samples from 57 wheat fields of Mid-North Wheatlands Environmental Region.

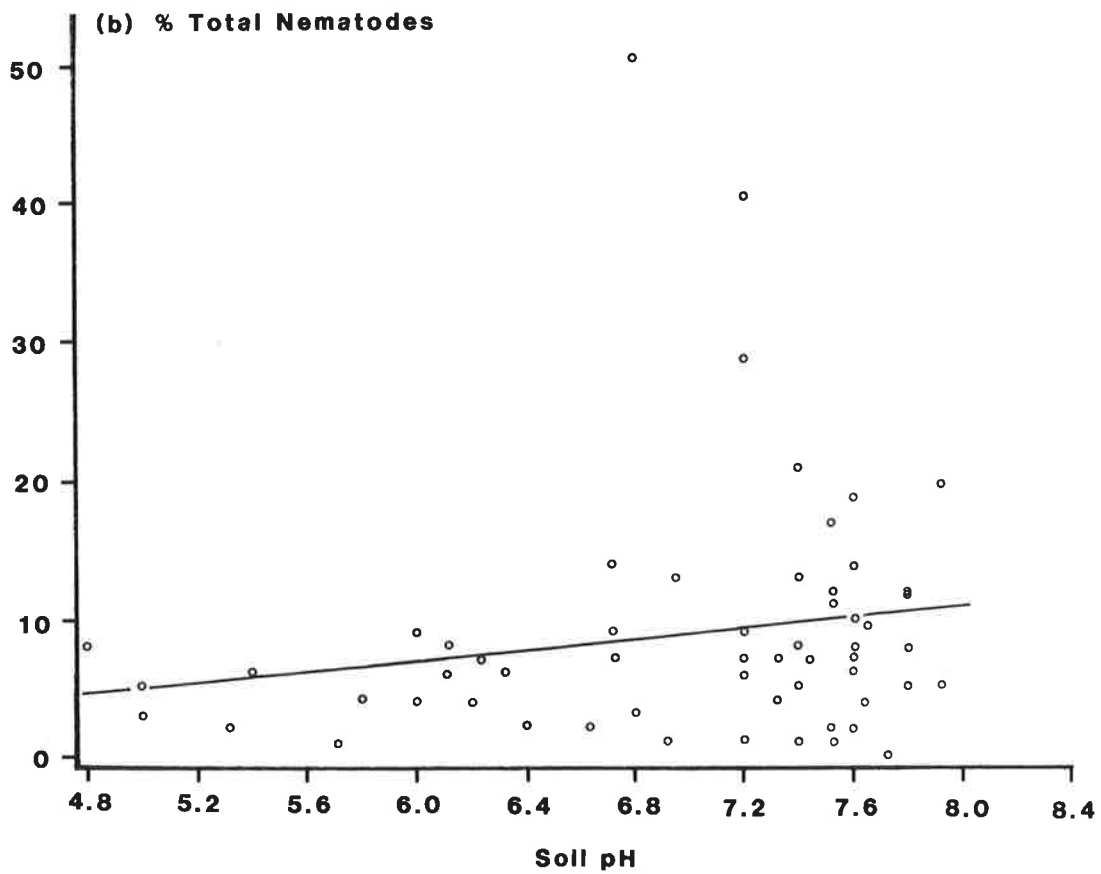
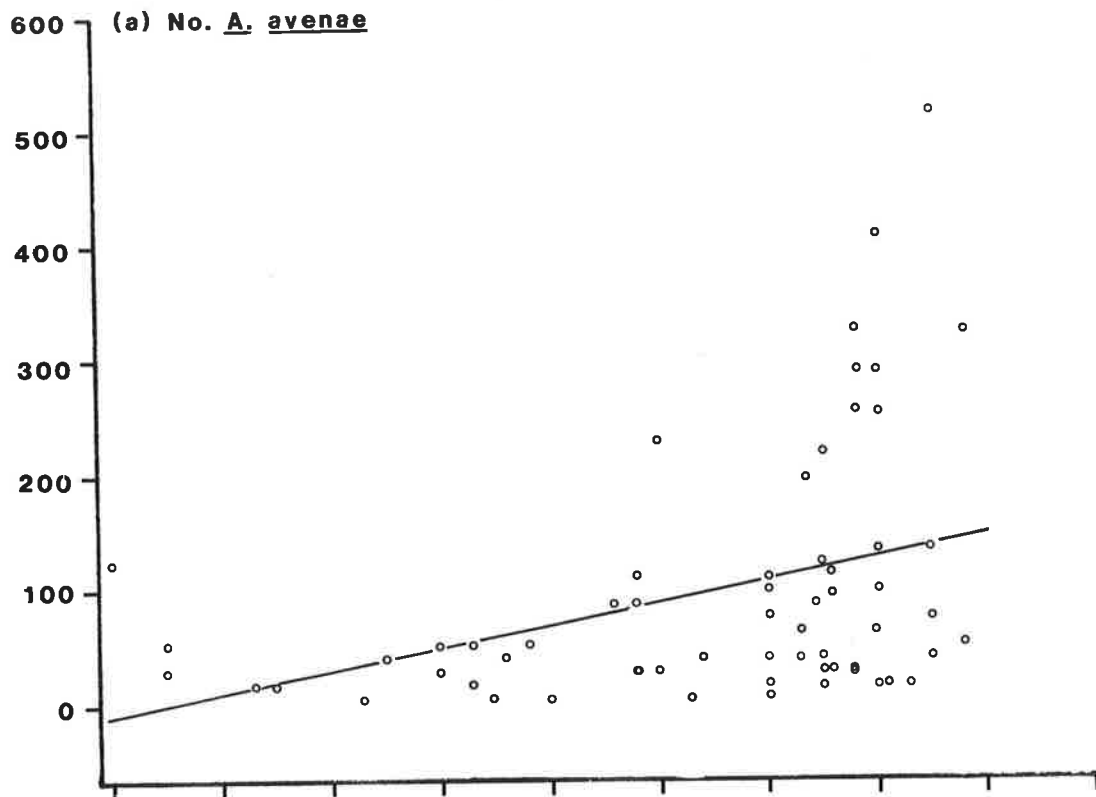


FIG 6: Fitted regression lines for number of *A. avenae* per 50 ml soil (a) and as % of total nematodes (b) on soil texture (% sand) from 57 wheat fields of Mid-North Wheatlands Environmental Region.



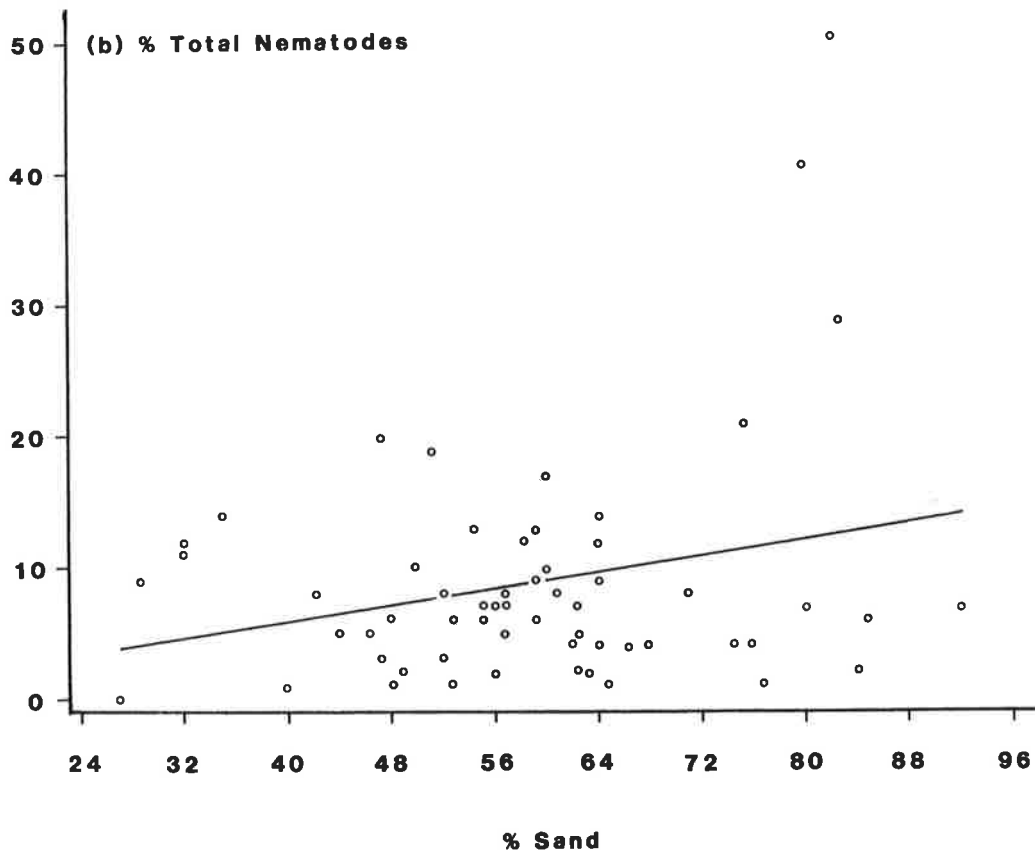
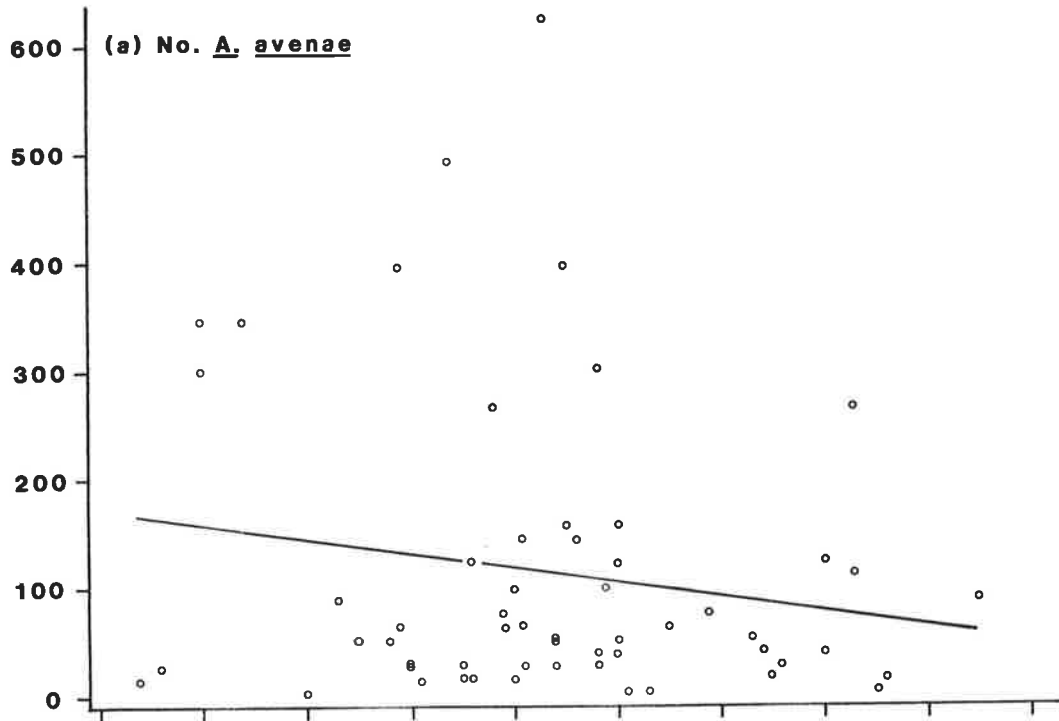
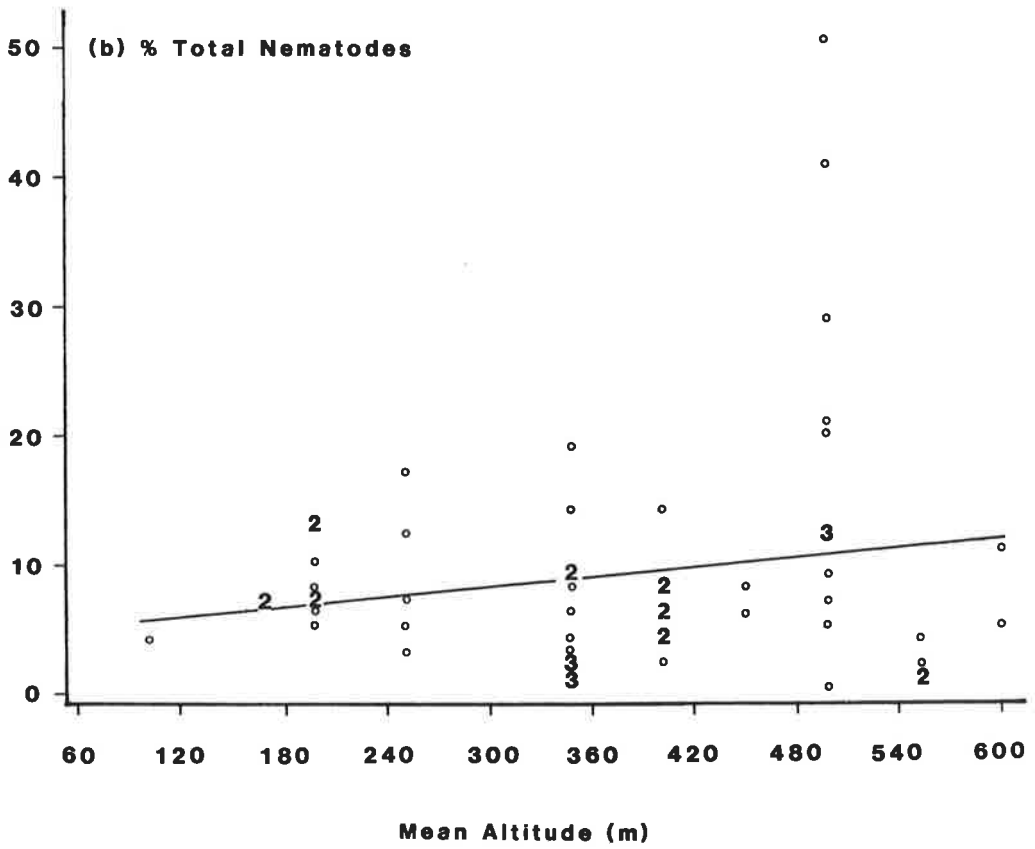
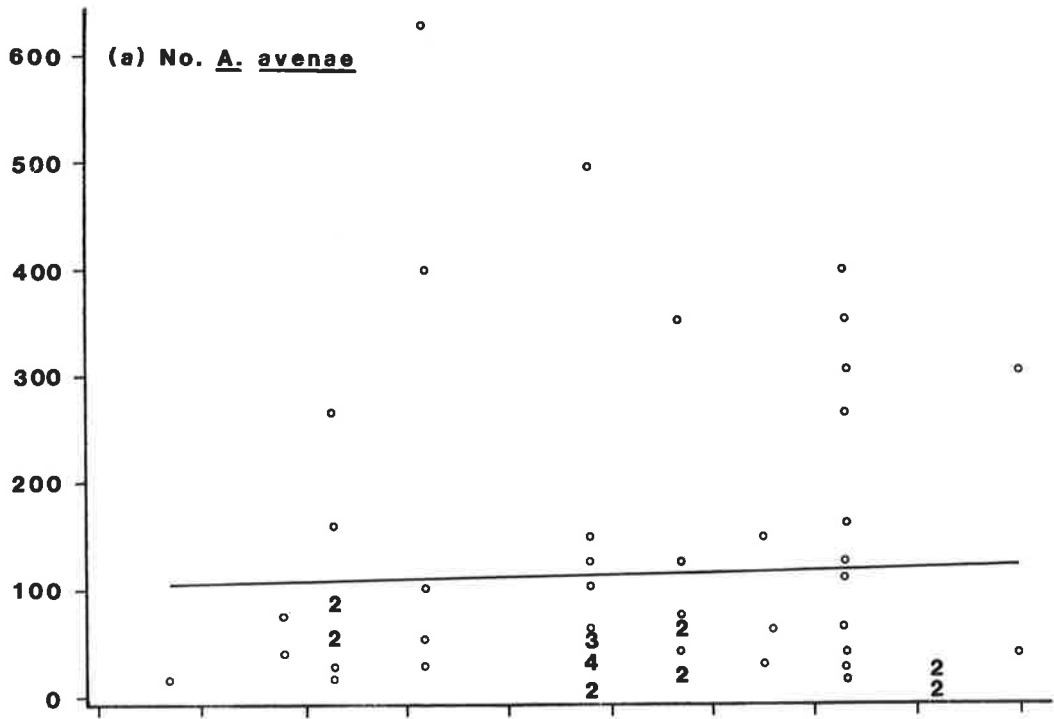


FIG. 7: Fitted regression lines for number of *A. avenae* per 50 ml soil (a) and as % of total nematodes (b) on ratio of precipitation over evaporation for 57 wheat fields from Mid-North Wheatlands Environmental Region.



FIG. 8: Fitted regression lines for number of *A. avenae* per 50 ml soil (a) and as % of total nematodes (b) on mean altitude for 57 wheat fields from Mid-North Wheatlands Environmental Region.



### 3. ABUNDANCE OF *APHELENCHUS AVENAE* IN SALINE SOILS

Since *A. avenae* was found to be relatively more abundant in wheat-field soils with higher levels of soluble salts the distribution of the nematode in saline soils was investigated by sampling along transects on the edge of several saline lagoons. Both lagoons are situated in the Hansen Environmental Association (3.3.11), 25 to 30 km east of Clare (33°50'S, 138°38'E).

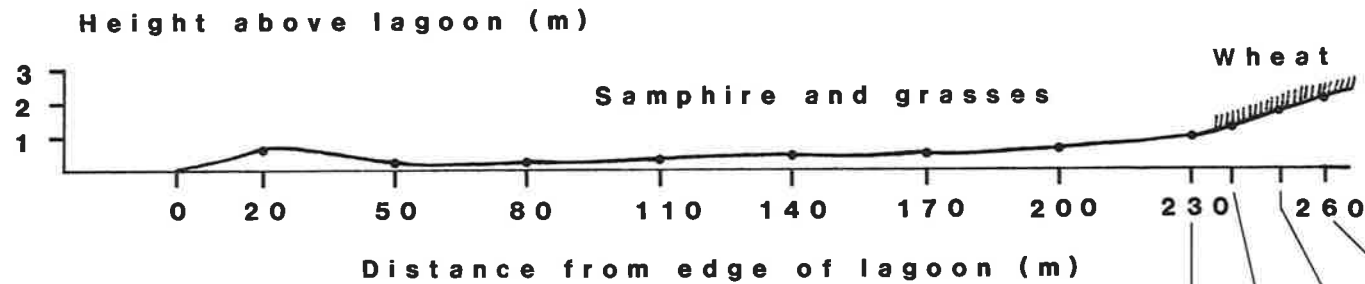
#### (a) Black Springs Lagoon Transect

Soil samples were taken along a transect from the edge of a field under wheat to the shores of the lagoon, 265 m distant (Fig. 9). Wheat (var. Halberd) had been planted for the first time during the year of sampling around the edge of the lagoon and occupied the first 30 m of the transect. The remainder of the transect was uncultivated and was covered by samphire and grasses. Soil samples were taken every 30 m (every 10 m under wheat) with an auger 75 mm in diameter to a depth of 145 mm. Three sub-samples were bulked per sample.

Soil surrounding the lagoon was highly saline; at a mere 65 m from the edge of the wheat-field, electrical conductivities reached levels sufficient to exclude agricultural crops entirely (Bower, 1963). On the edge of the wheat-field nearest the lagoon, EC 1:5 values reached  $500 \mu S cm^{-1}$ . Salinity of soil was influenced by topography; increasing in depressions and generally towards the lagoon, but decreasing again on a rim of higher ground near the edge of the lagoon. Soil pH followed a similar trend to EC 1:5 values; the more saline soils being higher in pH. The distribution of *A. avenae* was highly disjunct; the nematode being limited to the area under wheat, suggesting either a sensitivity to increasing salinity or a dependence

FIG 9: Abundance of nematodes and *A. avenae*,  
and soil characteristics along a tran-  
sect line at the edge of Black Springs  
Lagoon, South Australia.

**Black Springs Lagoon Transect**



	0	20	50	80	110	140	170	200	230	245	255	260
EC1:5 (micro s/cm)	290	8000	4500	4000	8000	17000	800	3400	450	85	110	
pH	7.6	7.8	7.8	7.7	7.8	8.0	7.5	7.7	7.2	6.8	7.2	
Nematodes/50 ml soil	646	20	28	125	54	4	67	30	310	440	251	
No. <i>A. avenae</i> /50 ml soil	0	0	0	0	0	0	0	0	91	224	103	
% Total nematodes	-	-	-	-	-	-	-	-	29	51	41	



upon food sources associated with wheat. That the latter is the more likely explanation is suggested by the absence of *A. avenae* from soil in the rim of high ground where salinity levels were well within the range found under wheat. Population density of *A. avenae* was very high in the soil under wheat and relative abundance was much higher than generally encountered in wheat-fields elsewhere. This may be related to the fact that wheat had been planted for the first time in this soil. The parthenogenetic mode of reproduction of *A. avenae* (or, at least, of the isolates examined in this locality) makes it ideally suited to colonizing a "new" habitat. Soil newly planted to wheat may be rapidly colonized by fungi associated with wheat and with soil fauna such as *A. avenae*. Rapid build-up of population levels may be possible while fields with longer cropping histories may harbour a greater diversity of mycophagous competitors.

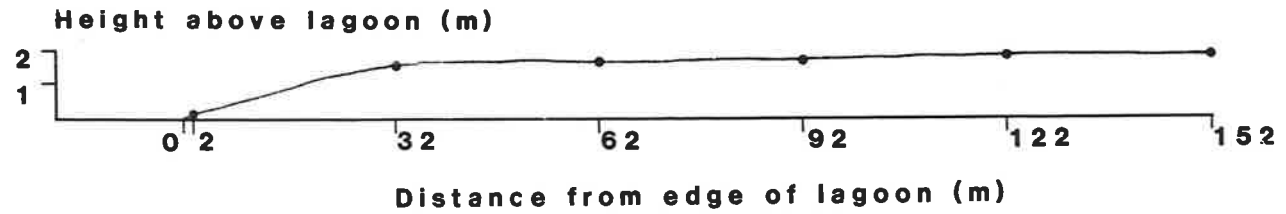
(b) Porter Lagoon Transect

A soil sample taken from under pasture at the edge of this lagoon yielded male *A. avenae* in numbers up to 18% of all adult *A. avenae* extracted. Since males had only rarely been isolated from soil during widespread sampling of wheat-fields, although seen regularly in laboratory cultures in small numbers (cultures established from wheat-field isolates were all found to be parthenogenetic), it was decided to investigate this phenomenon further. Soil samples were taken along a transect from near the edge of the lagoon at 30 m intervals to a point 150 m into a field of unseeded pasture containing rye grass, wild oats and other grasses (Fig. 10). Samples were taken with a spade, 153 mm wide and 225 mm deep.

Laboratory cultures of *A. avenae* from all samples were started using *B. cinerea* as a food source. All cultures were found to be parthenogenetic. Males were found only in soil near the edge of the lagoon and were

FIG. 10: Abundance of nematodes and *A. avenae*, and soil characteristics along a transect line at the edge of Porter Lagoon, South Australia.

Porter Lagoon Transect



EC1:5 (micro s/cm)	2400	310	210	200	160	230
pH	7.4	7.5	7.4	6.6	6.6	6.6
Nematodes / 50 ml soil	29.5#	476.5	648.5	299.5	377	351
<u>A. avenae</u> /50 ml soil	0.5#	17.5	4.5	9	18.5	2.5
% Total nematodes	1.7	3.7	0.7	3.0	4.9	0.7
Male <u>A. avenae</u> recorded	✓	-	-	-	-	-

# per 50g soil (for purposes of comparison, a g of soil can be considered

about equivalent to a ml of soil (Oostenbrink et al., 1956)).

not seen in samples from higher ground. Soil near the lagoon was highly saline, much more so than soil from Black Springs Lagoon from which *A. avenae* was found. Many factors, such as temperature, ethanol, carbon dioxide and fungi are known to induce the formation of males in various parthenogenetic strains of *A. avenae* in culture (Hansen, Buecher and Yarwood, 1972, 1973). It is possible that high salinity levels may operate in this way in field soils. However, since the soil from which males were found at Porter Lagoon was also saturated, it may be that some other factor such as carbon dioxide level was responsible. A further indication that the population was under stress in soil near the edge of the lagoon was the low density seen here. Population densities under pasture were generally lower than those seen under wheat.

#### 4. EFFECTS OF ENVIRONMENTAL VARIABLES ON POPULATION GROWTH OF *APHELENCHUS AVENAE* IN XENIC CULTURE

Since it appeared that certain environmental variables such as salinity and soil pH were influencing, directly or indirectly, distribution and abundance of *A. avenae* in the field, it was decided to assess the influence of these factors on population growth of *A. avenae* in xenic culture by modifying the culture medium. Changes to the culture medium will, of course, influence the host fungus in its growth rate and/or physiology, a mechanism by which the nematode is most likely to be affected in the soil.

The basic medium used was potato dextrose agar (Difco) containing 100 ppm streptomycin and 100 ppm vancomycin, amended with 1M NaOH to give a pH of 7.4. Nine cm Petri dishes containing 25 ml of culture medium were inoculated with an 11 mm disc of fungus taken with a cork-borer from agar cultures of either *Botrytis cinerea* or *Rhizoctonia solani*. Nematodes, 6 L3 per plate and surface - sterilized in 0.5% chlorhexidine gluconate for 5 min, were inoculated by hand onto plates. Plates were incubated for 3 weeks at

25°C unless otherwise indicated, the agar removed, diced and nematodes extracted and counted. There were 6 replicates per treatment. The maximum diameters of fungal colonies were measured after successive days to give an indication of growth rate. Although this method does not indicate the actual total production of mycelium it has the advantage of being non-destructive.

(a) Salinity

Potato dextrose agar (PDA) amended with 0.2% NaCl was used to examine the influence of salinity of the culture medium on population growth of the nematode. NaCl adversely affected the linear growth rate of both *Botrytis cinerea* and *Rhizoctonia solani*, during the later stages of growth of the former whilst only early in the mycelial growth of the latter (Table 5). Yield of nematodes was significantly reduced on NaCl-amended plates in the case of *Rhizoctonia solani*.

(b) pH

PDA (pH 5.6) and PDA amended with 1M NaOH to give a pH of 7.4 were used to determine influence of pH of the culture medium on population growth of *A. avenae*. Isolates 37 (from soil of pH 4.8) and 50 (from soil of pH 7.6), as well as an isolate from Brownhill Creek originally cultured by Evans (1968) and since maintained in culture by Dr. J.M. Fisher at the Waite Agricultural Research Institute, were used in these experiments.

The linear growth of *Botrytis cinerea* was significantly higher at the lower pH, as also was *Rhizoctonia solani* but only in the latter stages of growth (Table 6). Most fungi prefer acid conditions (Park, 1968) and further tests showed that the optimum pH for growth of the strain of

TABLE 5: Effect of 0.2% NaCl in agar medium upon radial growth of host fungi and numbers of nematodes produced

Host Fungus	<i>A. avenae</i> Isolate		PDA	PDA + NaCl
	Isolate #4		257142 ± 54381 (S.E.)	198479 ± 32295
<i>Botrytis cinerea</i>	Diameter	2 days	30.5 ± 1.5(S.E.)	31.2 ± 1.1
	Fungal	3 days	46.7 ± 2.8	43.0 ± 1.7
	Colony	4 days	62.2 ± 4.2	54.2 ± 2.8
	(mm)after	5 days	76.2 ± 4.1	64.0 ± 4.4
		6 days	82.8 ± 1.6	69.3 ± 4.2*
	Isolate # 7		84610 ± 12324	49276 ± 1291*
<i>Rhizoctonia solani</i>	Diameter	2 days	34.2 ± 0.5	30.3 ± 0.7*
	Fungal	3 days	44.8 ± 0.6	43.2 ± 0.6
	Colony(mm)	4 days	58.5 ± 1.7	56.1 ± 1.0
	after	5 days	67.9 ± 2.0	65.7 ± 1.7
		6 days	77.2 ± 1.9	75.3 ± 2.3

\*Pairs of means for two media significantly different at 5% level.

TABLE 6: Effect of pH of agar medium upon radial growth of host fungi and numbers of nematodes produced

Host Fungus	<i>A. avenae</i> Isolate	PDA pH 5.6	PDA pH 7.4
	Isolate #37	210558 + 70273 (S.E.)	206518 ± 19080
<i>Botrytis cinerea</i>	Diameter 2 days	30.8 ± 1.7(S.E.)	22.5 ± 0.8*
	Fungal 3 days	55.3 ± 2.1	30.8 ± 1.0*
	Colony(mm) 4 days after	81.3 ± 2.3	45.2 ± 3.9*
	Isolate #50	283057 ± 41435	413985 ± 57580
	Diameter 2 days	35.5 ± 1.8	22.3 ± 1.7*
	Fungal 3 days	56.2 ± 2.1	31.5 ± 2.4*
	Colony(mm) 4 days after	81.0 ± 1.7	45.2 ± 3.9*
	Brownhill Creek Isolate	-	443242 ± 24169
<i>Rhizoctonia solani</i>	Brownhill Creek Isolate	285761 ± 45946	58646 ± 3170*
	Diameter 2 days	29.7 ± 0.3	30.7 ± 0.3
	Fungal 3 days	42.7 ± 0.3	43.8 ± 0.3*
	Colony(mm) 4 days after	67.7 ± 2.1	58.3 ± 0.5*
	5 days	76.2 ± 1.1	70.5 ± 0.4*

\* Means at different pH's significantly different at 5% level.

*Rhizoctonia* used in these experiments was on the acid side (Table 7).

In the case of the Brownhill Creek isolate on *Rhizoctonia solani*, significantly more nematodes were produced in the more acid medium. No significant differences between nematode yields could be demonstrated on *Botrytis cinerea* at the two pH levels despite the marked influence of pH on growth of the fungus. Intraspecific variation was suggested by the significantly higher yields ( $P = 0.05$ ) of the Brownhill Creek and #50 isolates on *Botrytis cinerea* at pH 7.4 compared with isolate #37.

(c) Temperature

The temperature response of isolates 13 (taken from latitude 34°29'S) and 57 (latitude 32°47'S) on *Botrytis cinerea* were compared at 20° and 28°C.

Raising the temperature to 28°C had an adverse effect on the linear growth of *Botrytis cinerea* but total production of *A. avenae* was significantly higher at the higher temperature (Table 8). There were no significant differences in total production of nematodes of both isolates at either temperature, but the sex ratio was markedly higher in isolate 57, especially at 28°C where males outnumbered females. It is interesting that despite the abundance of temperature-induced males in this isolate at 28°C, the total production of nematodes was not apparently affected. Fisher (1972) found that males from an amphimictic population reduced the number of eggs laid by parthenogenetic *A. avenae* females.

5. TEMPERATURE-INDUCED MALES IN PARTHENOGENETIC ISOLATES OF *APHELENCHUS AVENAE*

Different isolates of *A. avenae* from the Mid-North Wheatlands Environmental Region responded very differently to increased incubation temperatures, some producing an abundance of males while in others the sex



TABLE 7: Mean radial growth of *Rhizoctonia solani* on NDY/6 at 25°C on agar media of differing pH (n = 12)

Diameter of Fungal Colony (mm)	pH 5.3	pH 7.0
After 2 days	31.8 ± 0.8(S.E.)	28.7 ± 0.4*
3 days	48.0 ± 0.5	45.7 ± 0.4*
4 days	63.8 ± 0.4	62.6 ± 0.3*

\* Pairs of means at different pH's significantly different at 5% level.

TABLE 8: Effect of incubation temperature on radial growth of *Botrytis cinerea* and numbers of nematodes produced

<i>A. avenae</i> Isolate	Diameter Fungal Colony (mm)	20°C	28°C
		14737 ± 5968(S.E.)	246043 ± 36372*
13	After 2 days	23.0 ± 1.5 (S.E.)	24.3 ± 0.9
	3 days	35.5 ± 2.2	35.0 ± 1.6
	4 days	53.8 ± 3.1	47.8 ± 1.8*
	5 days	79.5 ± 2.1	62.2 ± 1.6*
	Sex ratio**	~0%	15.0%
		12544 ± 3036	256681 ± 10203*
57	After 2 days	23.3 ± 1.9	22.8 ± 0.5
	3 days	36.7 ± 2.5	32.8 ± 1.2*
	4 days	54.5 ± 3.1	46.5 ± 1.2*
	5 days	79.2 ± 1.7	59.3 ± 1.4*
	Sex ratio	0.4%	57.5%

\*Pairs of means at the two temperatures significantly different at the 5% level.

\*\*Sex ratio = No. adult males/No. adults of both sexes x 100%. No males recorded in isolate 13 at 20°C amongst over 400 adult females counted.

ratio remained essentially stable (Table 9). This response to temperature amongst the different isolates did not appear to follow any pattern, geographic or otherwise, arguing against a direct adaptive function in relation to temperature for this trait. This fact was emphasized by the very different responses from two isolates originating from a pine-forest near Williamstown; the P-isolate showing almost complete sex reversal at 30°C, a temperature which elicited only a marginal increase in frequency of males in the S-isolate (Table 9). The sex ratio tended to increase with age of the culture; thus, the stimulus for production of males may have been associated with overcrowding, shortage of food or "staling" products from the fungus.

Some organisms, such as certain aphids, rotifers and cladocerans exhibit cyclical parthenogenesis (Maynard Smith, 1978) in which the sexual and asexual phases have different ecological roles; the sexual phase commonly occurring during periods of adverse environmental conditions. This, however, is not the case in *A. avenae*. Although temperature-induced males may inseminate both parthenogenetic females - particularly of their own population - and amphimictic females, their spermatozoa are non-functional and actual fertilization has not been observed (Fisher and Triantaphyllou, 1976). Poinar and Hansen (1983) suggested that differences in chromosome number between populations (Triantaphyllou and Fisher, 1976) may have contributed to sperm incompatibility observed between temperature-induced males of a Californian population and amphimictic females of a Western Australian population. However, Fisher and Triantaphyllou (1976) observed the same phenomenon between amphimictic males and parthenogenetic females of an Australian population having the same number of chromosomes.

TABLE 9: Sex ratio of *A. avenae* isolates in monoxenic culture on *Botrytis cinerea* at different temperatures (sex ratio = No. adult males/No. of adults of both sexes x 100%).

<i>A. avenae</i> Isolate	20°C	25°C	28°C	30°C	Ambient Temperature**
13 (3 wk)*	~0%		15.0%		
16 (4 wk) (6 wk)		~0%			7.4%
19 (4 wk) (6 wk)		~0%			3.8%
37 (3 wk)					~0%
42 (3 wk) (4 wk) (6 wk)		10.9% 13.8%			38.7%
50 (2 wk)		0.2%		0.6%	
52 (3 wk)					42.5%
57 (3 wk) (7 wk)	0.4%	1.0%	57.5%		43.8%
W (2 wk)		0.4%		<0.04%	
P (2 wk)		0.4%		89.3%	
S (2 wk)		0.2%		0.5%	

\* Age of culture in weeks in brackets. Williamstown isolates of *A. avenae* denoted by W (wheat-field soil isolate); P (pine-forest soil isolate) and S (isolate from specimen obtained from sporocarp of *Suillus luteus* from pine-forest).

\*\* Cultures kept in a non-refrigerated incubator set to 25°C but exposed to temperatures above 30°C for several days during a heat-wave.

6. AMPHIMICTIC AND PARTHENOGENETIC POPULATIONS  
OF *APHLELENCHUS AVENAE*

*A. avenae* occurs in both amphimictic and parthenogenetic populations in Australia (Evans, 1968; Evans and Fisher, 1970) and Fisher (1972) reported different egg-laying responses by females of an amphimictic and parthenogenetic population to males of the amphimictic population. Males reduced the number of eggs deposited by parthenogenetic females while an increase in density of males sometimes increased and, at other times, decreased the number of eggs laid by amphimictic females. All other factors being equal, the reproductive rate of a parthenogenetic strain which does not produce males should be twice that of a corresponding sexual strain (Maynard Smith, 1978). It is of interest to determine whether, in *A. avenae*, all other factors are in fact equal, and whether the adverse effect of males noted by Fisher on the egg-laying of parthenogenetic females could at least partially explain why the amphimictic population is not selectively displaced by parthenogenetic *A. avenae* in nature. The continued existence of sexual reproduction in nature and the observed rarity of parthenogenesis is of course normally explained by the limited ability of parthenogenetic forms to evolve and their consequent high rate of extinction (Maynard Smith, 1978).

(a) Interactions between amphimictic and parthenogenetic populations of *A. avenae* in monoxenic culture

Petri plates containing PDA (25 ml per 9 cm plate) pH 5.6 with 100 ppm streptomycin and 100 ppm vancomycin were inoculated with *Botrytis cinerea* as a food source and with adult *A. avenae* of the Western Australian (WA) and Brownhill Creek (BHC) isolates, surface-sterilized for 5 min in 0.5% chlorhexidine gluconate. Initial densities of males and females were: 1) 20 WA ♂ + 20 WA ♀ + 20 BHC ♀; 2) 6 WA ♂ + 6 WA ♀ + 6 BHC ♀, and 3) WA ♂ + 6 WA ♀ + 6 BHC ♀. Some plates containing PDA with 0.2% NaCl

were also used in this experiment. There were 6 replicates per treatment and plates were harvested after 3 or 5 weeks incubation at 25°C.

Total production of nematodes after 5 weeks was always significantly higher ( $P = 0.05$ ) than that after 3 weeks but no significant differences in sex ratio were detected over this time (Table 10). Although sex ratios declined from those at the start of the experiments, no further decline was seen from 3 to 5 weeks and, in fact, mean sex ratios increased over this period of time except in the case of plates where amphimictic females initially outnumbered males 2:1. By about 2 weeks plates had been denuded of aerial mycelium but it is not known if an actual food shortage existed such that amphimictic and parthenogenetic forms were in competition.

The failure to observe a decline in the sex ratio between the two harvests suggests that the amphimictic form was not selectively displaced by the parthenogenetic form under the conditions used. If the trend towards an increased sex ratio with time is real then the opposite may in fact occur. It seems likely that an inhibitory effect of amphimictic males on the egg-laying of parthenogenetic females may be involved in this phenomenon. Whether these results reflect the situation as it occurs in nature is not known, but they suggest a basis by which the amphimictic population could maintain itself in the presence of parthenogenetic competitors. Fisher (1972) suggested that the amphimictic population in Western Australia may be geographically isolated by the Nullabor Plain, however, there is no evidence that parthenogenetic populations do not occur in Western Australia.

(b) Rate of egg-laying by amphimictic  
and parthenogenetic females

Rate of egg-laying was determined by washing nematodes from monoxenic cultures on *Botrytis cinerea* with sterile water and transferring

TABLE 10: Total production of nematodes on *Botrytis cinerea* with different initial densities of parthenogenetic females (BHC ♀) and amphimictic males (WA ♂) and females (WA ♀) at 25°C. Mean diameters of fungal colonies combine data from plates harvested at both 3 and 5 weeks. Sex ratios represent numbers of adult males as % of total adults (parthenogenetic and amphimictic females could not be distinguished morphologically)

	3 week harvest		5 week harvest	
	PDA	NaCl - PDA	PDA	NaCl - PDA
20 WA ♂ : 20 WA ♀ : 20 BHC ♀	242250 ± 10329(S.E.)	-	330188 ± 27781	336375 ± 18355
Mean Sex Ratio (%)	10.1 ± 1.3(S.E.)	-	16.4 ± 4.1	10.7 ± 2.0
Mean diameter fungal colony (mm)				
after 2 days	45.8 ± 1.1(S.E.)			
after 3 days	85.8 ± 0.2(S.E.)			
6 WA ♂ : 6 WA ♀ : 6 BHC ♀	211546 ± 20111	208667 ± 12054	325333 ± 44336	410625 ± 28135
Mean Sex Ratio (%)	15.1 ± 4.2	11.5 ± 2.5	25.3 ± 5.7	19.1 ± 5.2
Mean diameter fungal colony (mm)				
after 2 days	47.5 ± 0.7	-		
after 3 days	85.8 ± 0.2	65.6 ± 1.5		
3 WA ♂ : 6 WA ♀ : 6 BHC ♀	232683 ± 16670	-	287500 ± 11369	-
Mean Sex Ratio (%)	13.0 ± 4.1	-	10.3 ± 1.6	-
Mean diameter fungal colony (mm)				
after 2 days	47.0 ± 0.8			
after 3 days	85.8 ± 0.3			

adult females to glass wells containing sterile water (5-10 ♀ per well) stored in an incubator and counting number of eggs laid. Alternatively, nematodes were placed on the surface of agar (NDY/6, pH 7 containing 100 ppm vancomycin and 100 ppm neomycin) in 35 mm plastic Petri dishes.

Mean rate of egg-laying by amphimictic (WA) females and parthenogenetic (BHC) females was not significantly different, either in sterile water at 16° or 25°C (Fig. 11c) or on agar at 25°C (Fig. 11b). At 30°C, however, BHC females had a significantly higher rate of egg-laying than WA females (Fig. 11c). Analysis of variance of the data summarized in Fig. 11c using a split-plot design (Table 11) indicated that incubation temperature, number of hours incubation and the interaction between these two terms were significant sources of variation.

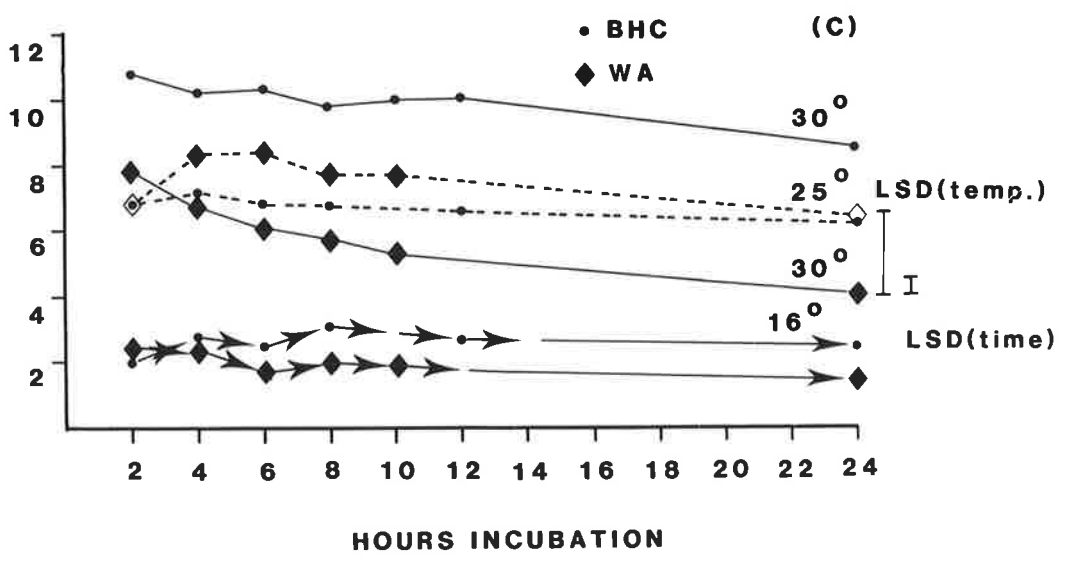
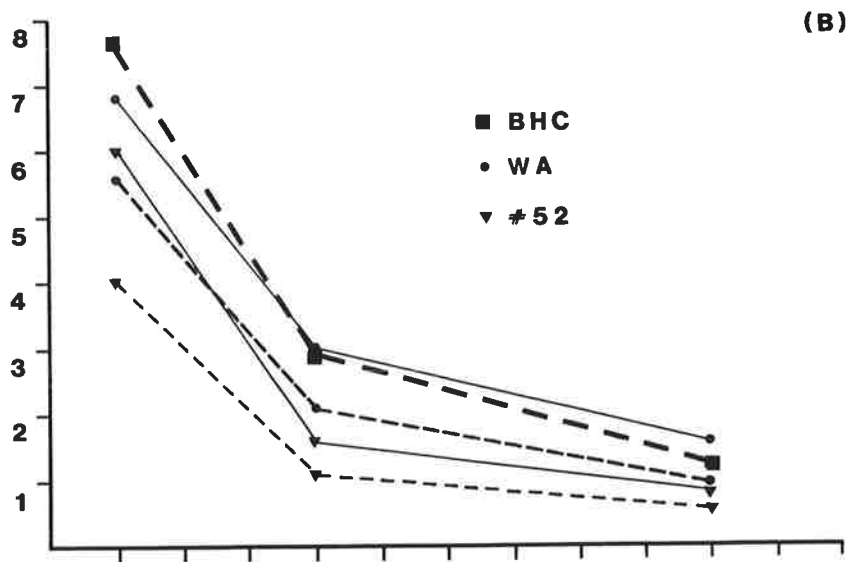
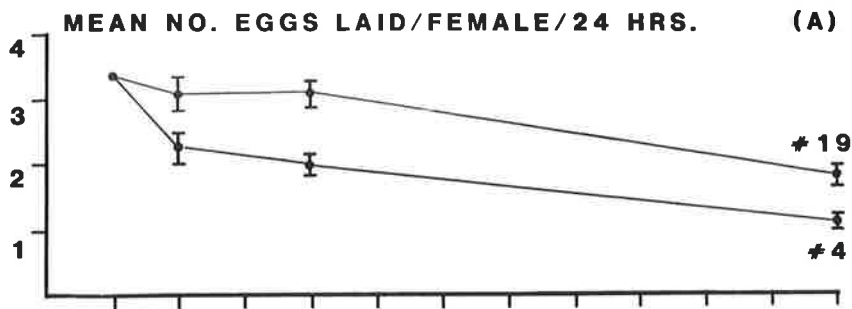
On agar, amphimictic males at a ratio of one male to one female did not significantly depress the rate of egg-laying of amphimictic females (Fig. 11b). To determine whether heat-induced males in a parthenogenetic isolate had any effect, two males of isolate #52 were placed with five females of the same isolate on each of six Petri dishes containing NDY/6, pH 7. Rate of egg-laying was not significantly depressed by the addition of this number of males although the mean rate was significantly below that of either BHC females or WA females in the absence of males.

Several other parthenogenetic isolates tested (Fig. 11a) were found to have rates of egg-laying significantly less than those of either #52 or BHC isolates, suggesting intraspecific variation in this character. After 2 hours incubation at 25°C, females of isolate 19 had a significantly higher rate of egg-laying than those of isolate 4 (Fig. 11a).



FIG.11: Mean rate of egg-laying by adult female *A. avenae* reared on *Botrytis cinerea*.

- (a) Mean rates of egg-laying of isolates 4 and 19 in sterile water at 25°C. Bars represent standard errors of means.
- (b) Mean rates of egg-laying of isolates from Brownhill Creek (parthenogenetic, with heat-induced males rare), Western Australia (amphimictic) and of isolate 52 (parthenogenetic, with heat-induced males common) at 25°C on agar. BHC females (represented by squares) incubated without males present, WA females (circles) incubated both with (WA) males in ratio 1 ♂ : 1 ♀ (circles connected by broken lines) and without (circles connected by unbroken lines), and isolate 52 females (triangles) incubated with heat-induced males in ratio 2 ♂ : 5 ♀ (triangles connected by broken lines) and without (triangles connected by unbroken lines).
- (c) Mean rate of egg-laying of females of Brownhill Creek (BHC) and Western Australian (WA) isolates at 16° (arrowed lines), 25° (broken lines) and 30°C (unbroken lines) in sterile water. Data was analyzed according to a split-plot design and least significant differences (at 5% level) are given for comparing pairs of means at both differing temperatures and times.



HOURS INCUBATION

TABLE 11: Analysis of variance for rate of egg-laying by females of the parthenogenetic Brownhill Creek population and the amphimictic Western Australian population at three different temperatures (data summarized graphically in Fig. 9c). Since successive measurements were not independent, the data was analyzed using a split-plot design

Source of variation	df	Sum of squares	Mean square	F
Stratum	5	589.41		
<u>Main plot analysis:</u>				
Nematode isolate	1	62.66	62.66	1.34 N.S.
Temperature	2	1148.70	574.35	12.26 **
Nema x Temp	2	155.25	77.62	1.66 N.S.
Residual	25	1170.94	46.84	
Total	30	2537.55		
<u>Sub-plot analysis:</u>				
Time	4	47.49	11.87	10.04 **
Time x Nema	4	7.84	1.96	1.66 N.S.
Time x Temp	8	44.30	5.54	4.68 **
Time x Nema x Temp	8	11.02	1.38	1.17 N.S.
Residual	120	141.90	1.18	
Total	144	252.55		
Grand total	179	3379.51		

\*\* Significant at  $P = 0.01$ ; N.S. = Non-significant.

(c) Hatching of eggs of amphimictic and parthenogenetic isolates

Eggs of the amphimictic (WA) isolate had a higher initial rate of hatch than the parthenogenetic (BHC) isolate (Table 12a) at 25°C in sterile water. A much higher % of eggs of the WA isolate failed to hatch at 30°C than at 25°C, while this increase in temperature failed to affect hatch of BHC eggs (Table 12b). This factor, along with the observation made earlier that an increase in temperature from 25 to 30°C increased the rate of egg-laying by BHC females but decreased it in WA females (Fig. 9c), suggests that the BHC isolate is better adapted to higher temperatures than the WA isolate. It is also interesting that males are still rare in BHC cultures grown at 30°C, such that egg-laying would not be inhibited at this temperature by males. Yet parthenogenetic isolates obtained from localities further north than the BHC isolate and subjected to higher temperatures in the field exhibit marked induction of males at this temperature. Hatching of eggs of both isolates was markedly inhibited at 16°C (Table 12).

It is concluded that, in many important parameters such as rate of egg-laying and hatching of eggs, differences exist between the amphimictic and parthenogenetic populations of *A. avenae* that influence the so-called "two-fold advantage of parthenogenesis" (Maynard Smith, 1978).

## 7. *APHELENCHUS AVENAE* FROM OTHER HABITATS

To study variation between populations of *A. avenae* from other habitats, both natural and agricultural, soil was collected from various locations over a wide area. Nematodes were extracted using a modified Baermann method (see Materials and Methods) unless otherwise indicated.

TABLE 12a: % of eggs hatching with time at 25°C in sterile water of Brownhill Creek (BHC - 596 eggs) and Western Australian (WA - 344 eggs) isolates

	Hours Incubation at 25°C					
	48	72	78	96	120	192
BHC	1.7	-	81.2	92.0	92.0	92.0
WA	20.1	87.8*	84.0	90.1	91.3	-

\* For 156 eggs only examined.

TABLE 12b: % of eggs not hatching after 192 hours incubation at 16°, 25° and 30°C of Brownhill Creek (BHC) and Western Australian (WA) isolates

	Incubation Temperature (°C)		
	16	25	30
BHC (No. eggs)	46.2 (158)	8.0 (596)	7.4 (256)
WA (No. eggs)	32.4 (71)	8.7 (344)	22.3 (121)

(a) Dangerous Reef, South Australia (34°49'S, 136°12'E)

This reef, situated in Spencer Gulf, consists of a series of low-lying rocks, the main one of which possesses a thin covering of guano-rich soil supporting a sparse vegetation dominated by *Atriplex muelleri* Benth.. Soil samples were collected here on 28 October 1980, and a nematode, thought to be *A. avenae*, was found at a mean density of 4.8 per 50 ml soil (n = 4 samples), representing 2.8% of total nematodes. Males were common and no morphological differences were detected between the nematode and *A. avenae* from other populations except that a greater variability in the shape of the female tail was observed. Females usually had the blunt tail typical of *A. avenae* but some tended towards a more conical shape. The isolate was cultured readily on *Botrytis cinerea* and the ability of the isolate to reproduce asexually was tested by inoculating 35 mm Petri plates containing PDA seeded with *B. cinerea* with one L2 or L3 larva per plate. Plates were maintained at 25°C and after 5 weeks nematodes were extracted. There was no evidence of reproduction although single adult nematodes were still alive; it was surmised that males and females were necessary for reproduction.

The only other amphimictic population of *A. avenae* known from Australia is in Western Australia (Evans, 1968) and it has been suggested that this population is geographically isolated by the Nullabor Plain (Fisher, 1972). The Dangerous Reef population is, of course, geographically isolated and it is tempting to speculate that this distribution may represent amphimictic ancestral populations occupying relictual habitats. It has been commonly observed that some parthenogenetic animals are more widespread in distribution than their sexual progenitors (Cuellar, 1977).

(b) Soil from under permanent pasture, Waite Agricultural Research Institute, collected 23 January 1981

Two samples consisting of 3 cores each were taken with a 75 mm auger to a depth of 140 mm from a field plot of permanent pasture. A total of 700 ml of soil was processed by the centrifugal-flotation technique (Jenkins, 1964), and *A. avenae* was found at a mean density of 12.9 per 50 ml soil, representing 1.5% of total nematodes. No male *A. avenae* were seen and the sex ratio of a monoxenic culture suggested that the population was parthenogenetic.

(c) Snowy Mountains, New South Wales (soil samples collected by Prof. H.R. Wallace during the southern winter in early May, 1981)

In soil from under open heath near Kiandra (35°23'S, 148°30'E) and under pasture near Cooma (36°15'S, 149°07'E) *A. avenae* made up approximately 2.5 and 0.2% respectively of total nematodes extracted. The sex ratio seen in monoxenic cultures of these isolates suggested that the populations were parthenogenetic.

(d) Hay Plains, New South Wales (soil collected by Prof. H.R. Wallace in early May 1981)

*A. avenae* made up approximately 0.3% of nematodes extracted from soil near Hay (34°31'S, 144°31'E). The sex ratio of a monoxenic culture suggested that the population was parthenogenetic.

(e) Macquarie Island (54°29'S, 158°58'E)

Soil samples were collected from this sub-Antarctic island during the southern spring on 28 October 1981, by Messrs. Montgomery and Cronin of the Tasmanian National Parks and Wildlife Service. Samples were taken from under *Poa* grasses (*P. foliosa* and *P. annua*), *Stilbocarpa polaris*

(Araliaceae) and a moss (*Azorella* sp.) to a depth of 10 cm from the northern end of the island between North Head and the southern end of the Isthmus.

*A. avenae* was not recovered from any of the soil samples and it is interesting that Bunt (1954) failed to record this nematode from 42 different localities sampled on Macquarie Island 30 years prior to this study. It may be that conditions on this island are too harsh for *A. avenae* to survive or that the nematode has not yet reached there.

Large numbers of larvae of a cyst nematode were found in two of the samples. This nematode was identified as *Punctodera matadorensis* Mulvey & Stone, 1976 by Dr. J.M. Fisher; recorded here for the first time in the Southern Hemisphere. It was previously described from natural grasses in Canada (Mulvey & Stone, 1976) and is probably parasitic upon *Poa* grass on Macquarie Island. Bunt (1954) makes no mention of any nematode which might fit the description of *P. matadorensis* in his comprehensive survey of nematodes on Macquarie Island. A possible early source of introduction of the nematode to Macquarie Island was through the many North American sealing vessels which called there shortly after its discovery in 1810.

Total nematodes recovered from Macquarie Island soil ranged from 7 to 947 per 100 ml soil, much lower than those typically recovered from temperate soils. It is curious that Bunt (1954) recorded much higher nematode populations; ranging from 5000 to 17000 per 100 g soil. His method of extraction differed in that much smaller (10 g) samples of soil were extracted using water heated to 40°C and this may have contributed to the wide discrepancy in results.

#### 8. INTRASPECIFIC VARIATION IN KARYOTYPE IN *APHELENCHUS AVENAE*

The haploid chromosome number of *A. avenae* has been reported as either  $n = 8$  or  $n = 9$  for several populations studied by Triantaphyllou and



TABLE 13: Nematodes extracted from soil under various types of vegetation on Macquarie Island.  
 Counts represent means of 2 replicate 50 ml-samples; figures in brackets give % of total  
 nematodes extracted

Sample No.	1	2	3	4	5	6
Associated vegetation*	P.f.	P.f.	S.p.	P.a.	P.f.	A.
Stylet-bearing nematodes						
<i>Punctodera matadorensis</i>	17.5 (34.3)	-	-	-	-	321 (80.3)
<i>Aphelenchoides</i> sp.	13.5 (26.5)	-	-	-	-	-
<i>Tylenchus</i> spp. ( <i>sensu lato</i> )	-	10 (20.8)	0.5 (14.3)	8.5 (4.3)	246 (52.0)	-
Nematodes without stylets (several spp.)	20 (39.2)	38 (79.2)	3 (85.7)	191.5 (95.7)	227.5 (48.0)	79 (19.7)

\*P.f. = *Poa foliosa*; S.p. = *Stilbocarpa polaris*; P.a. = *Poa annua*; A. = *Azorella* sp.

Fisher (1976), and it was decided to determine the karyotype of a number of isolates from the Mid-North Wheatlands Environmental Region to determine the extent of intraspecific variation in this character.

As noted by Triantaphyllou and Fisher (1976), metaphase chromosomes in oogonia were rarely observed and determination of the somatic chromosome number was often difficult due to fusion of chromosomes. When bivalents could be distinguished, 8 or 9 could be seen (Fig. 12). Triantaphyllou and Fisher (1976) reported a haploid chromosome number of  $n = 8$  for the Brownhill Creek population (B in Fig. 13). There appeared to be a pattern in the distribution of the 8 and 9 chromosome populations (Fig. 13); those with 9 chromosomes being distributed north of about  $34^{\circ}\text{S}$  and those with 8 chromosomes to the south.

#### 9. VARIATION IN PROTEIN PATTERNS BETWEEN *APHELENCHUS AVENAE* POPULATIONS

Denatured proteins from homogenates of various *A. avenae* isolates, including a series of isolates derived from points along a transect at Porter Lagoon, were separated by electrophoresis on polyacrylamide gels. Protein patterns of all isolates, including the amphimictic isolate from Dangerous Reef, shared many bands of similar mobility (Fig. 14a). However, differences were noticed in protein patterns between isolates, particularly amongst the less mobile bands or proteins of higher molecular weight. Some differences could even be observed in protein patterns of the various isolates from Porter Lagoon.

Evans (1971) also observed differences in protein patterns and enzyme polymorphisms in populations of *A. avenae*. However, as in the present study, analyses were made on mixtures of nematodes in different developmental stages. Enzyme patterns have been shown to vary between stages in several nematodes (see Hussey, 1979). Hussey (1979) suggested that some

TABLE 14: Number of counts of meiotic oocyte chromosomes stained with acetic orcein made in determining karyotype of *A. avenae* isolates from Mid-North Wheatlands Environmental Region

<i>A. avenae</i> isolate	Haploid chromosome number	No. counts made
8	8	2
11	8	1
W	8	27
44	9	20
50	9	2
52	9	3
54	9	9
57	9	3

FIG. 12: Meiotic chromosomes (metaphase) from oocyte of *A. avenae* isolate #50, stained with acetic orcein. The bar represents 5  $\mu\text{m}$ .

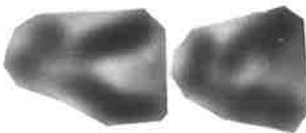
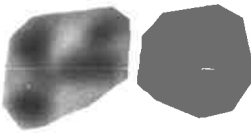
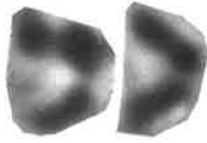


FIG. 13: Distribution of populations of *A. avenae* with  $n = 8$  (diamonds) or 9 (circles) haploid chromosome number.

W = population from Williamstown wheat-field,  
B = Brownhill Creek population studied by Triantaphyllou  
and Fisher (1976).

Numbers denote isolates from Mid-North Wheatlands  
Environmental Region as depicted in Fig. 2.

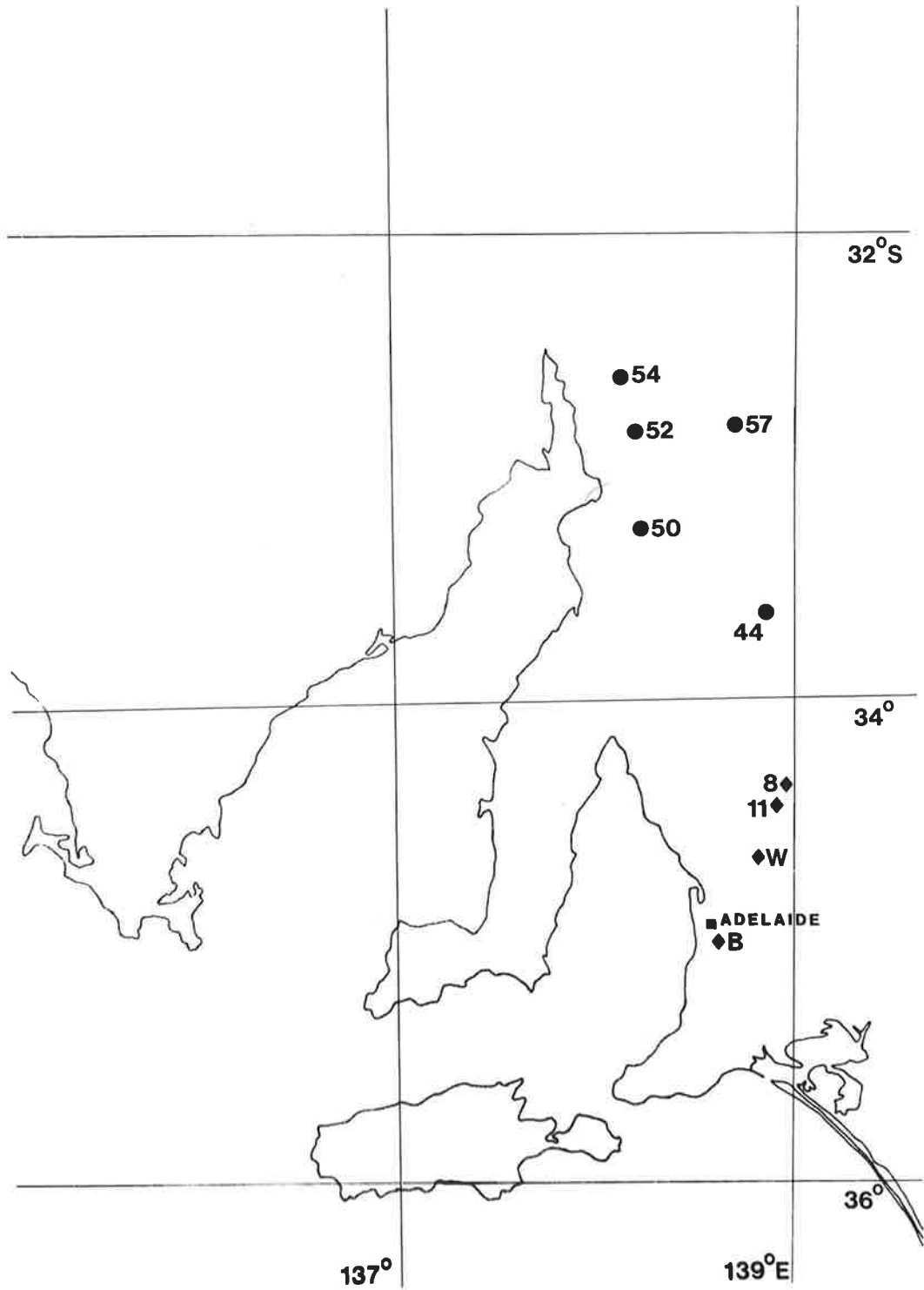
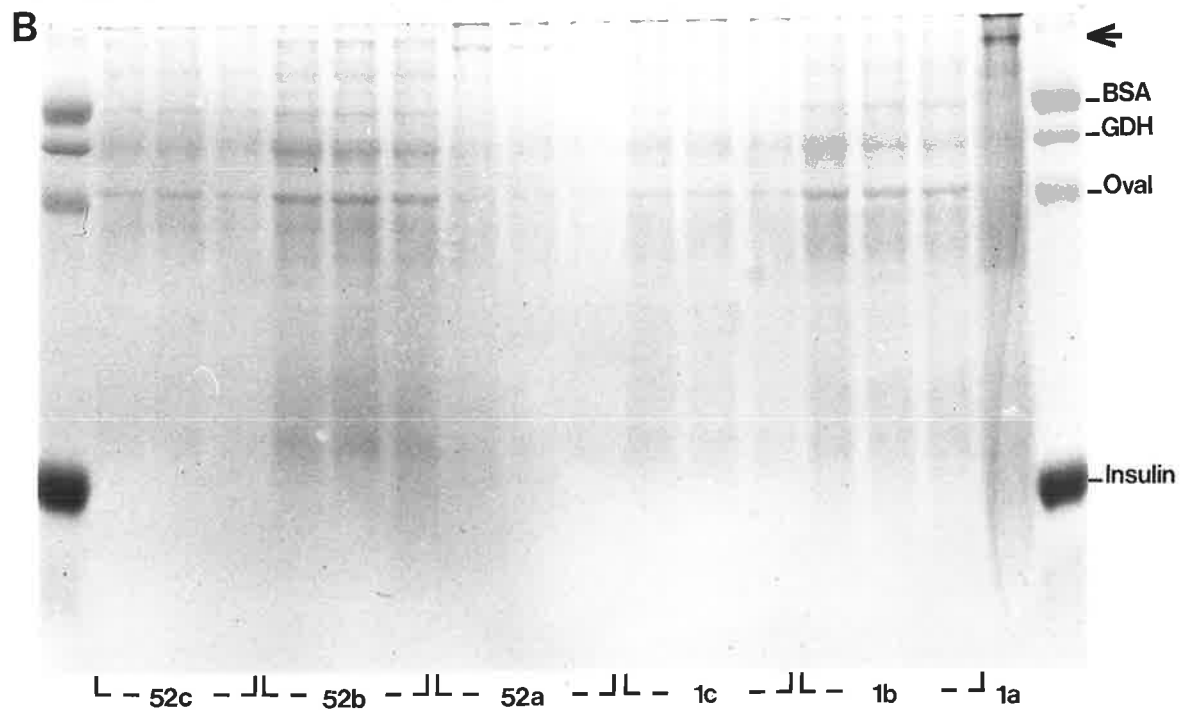
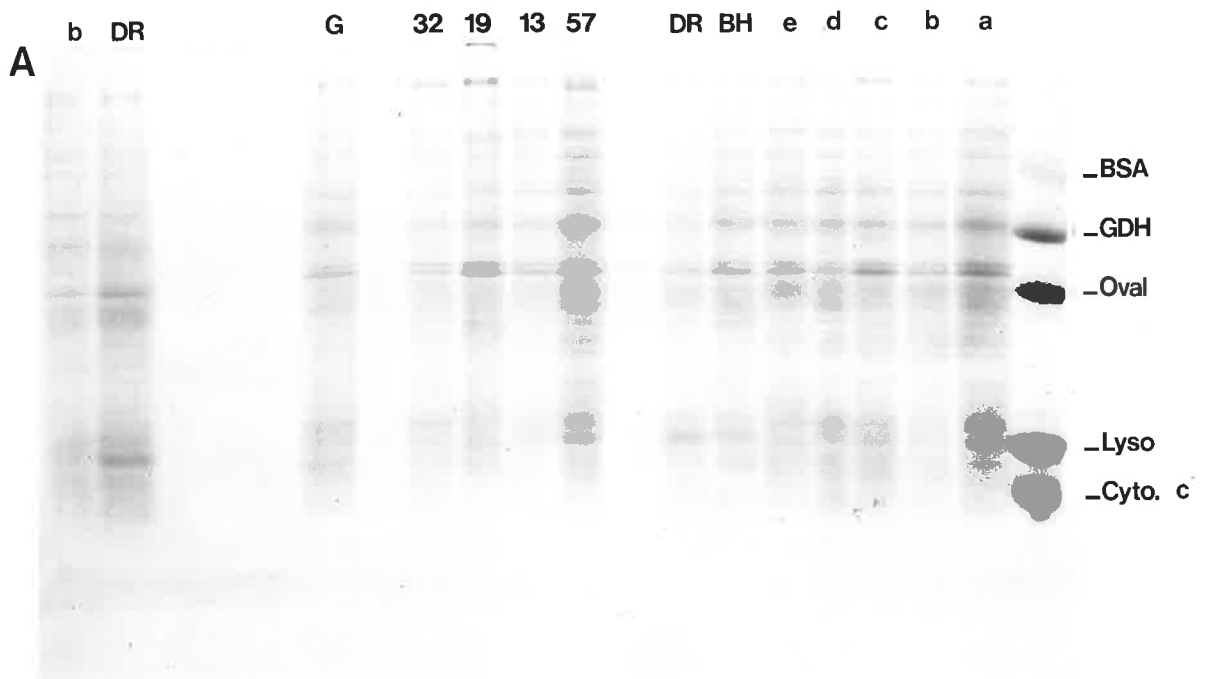


FIG. 14: Denatured proteins from homogenates of *A. avenae* isolates separated by electrophoresis on polyacrylamide gels and stained with Coomassie brilliant blue. The following molecular weight markers were used: bovine serum albumin (BSA) - 68,000; glutamate dehydrogenase (GDH) - 53,000; ovalbumin (Oval) - 43,000; lysozyme (Lyso) - 14,300; cytochrome C (Cyto-C) - 12,300, and insulin - 5,700.

*A. avenae* isolates:-

- (a) Isolates from transect at Porter Lagoon (see Fig.10) derived from soil taken at 32(a), 62(b), 92(c), 122(d) and 152 m(e) respectively from the edge of the lagoon; BH = Brownhill Creek isolate; DH = Dangerous Reef (amphimictic) isolate; and G = isolate from wheat-field from Hundred of Gilbert. Numbered isolates refer to those from wheat-fields of Mid-North Wheatlands Environmental Region.
- (b) Isolates 1 and 52 separated by sieving into groups a (those nematodes passing through a 45  $\mu\text{m}$ -mesh sieve), b (those retained on both 45 and 355  $\mu\text{m}$ -mesh sieves) and c (those retained on 45  $\mu\text{m}$ -mesh sieve but passing through 355  $\mu\text{m}$ -mesh sieve).





of the variability reported in enzyme patterns of nematodes may have stemmed from the use of mixtures of stages. To determine whether this factor may have been responsible for some of the variability in protein patterns reported in this study, length-classes of several *A. avenae* isolates were separated on sieves of different mesh-sizes and proteins separated by electrophoresis (Fig. 14b). Nematodes from culture jars were placed on a 45  $\mu\text{m}$ -mesh sieve for one minute and those retained on the sieve were placed on a 355  $\mu\text{m}$ -mesh sieve, also for one minute. Thus, nematodes were separated into three groups; those having passed through the 45  $\mu\text{m}$ -mesh sieve (mean length of ten nematodes :  $490 \pm 56 \mu\text{m}$  S.E.), those having been retained on the 45  $\mu\text{m}$ -mesh sieve but passing through the 355  $\mu\text{m}$ -mesh sieve ( $679 \pm 58 \mu\text{m}$ ) and those having been retained on both sieves ( $888 \pm 43 \mu\text{m}$ ). There appeared to be differences in protein patterns between the first and last of these groups and these differences were, as before, seen amongst the bands of lower mobility (marked by an arrow-head in Fig. 14b). It was concluded that mixed developmental stages could contribute to variability in protein patterns and that improved methods of separating stages would be needed before electrophoresis could be used with confidence to study intra-specific variation in *A. avenae*.

IV. THE ASSOCIATION BETWEEN SOIL MYCOFLORA AND ABUNDANCE OF  
APHELENCHUS AVENAE IN A WHEAT-FIELD AND PINE-FOREST

Although correlations between abundance of *A. avenae* and certain physico-chemical properties of soil had been found in a survey of wheat-fields, laboratory studies suggested that these factors were more likely to influence population density through their effects on host fungi. To study the influence of the soil mycoflora on abundance of *A. avenae*, these components of the soil biota were compared in two contrasting habitats; a wheat-field and a pine-forest.

1. SOIL CHARACTERISTICS OF THE STUDY SITES

The forest soil was more acidic than the wheat-field soil (Table 15), as would be expected for a mor soil (Griffin, 1972). The higher moisture content of the forest soil was also anticipated as a result of the shading and cover provided by the canopy and surface litter. However, soil texture differed markedly between the two sites; the forest soil being classed as a sandy loam and the wheat-field soil a clay loam on the basis of international particle-size distributions (Table 15). This may suggest a fundamental difference in basal materials between the two sites.

In the wheat-field, a gully ran in an east-westerly direction through quadrats E and I and soil here was noticeably heavier than in the rest of the field. Wheat growing in this gully was stunted compared with plants in the surrounding soil (Fig. 15).

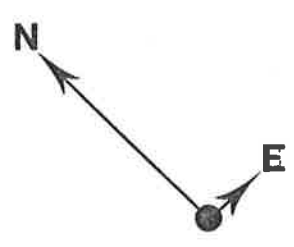
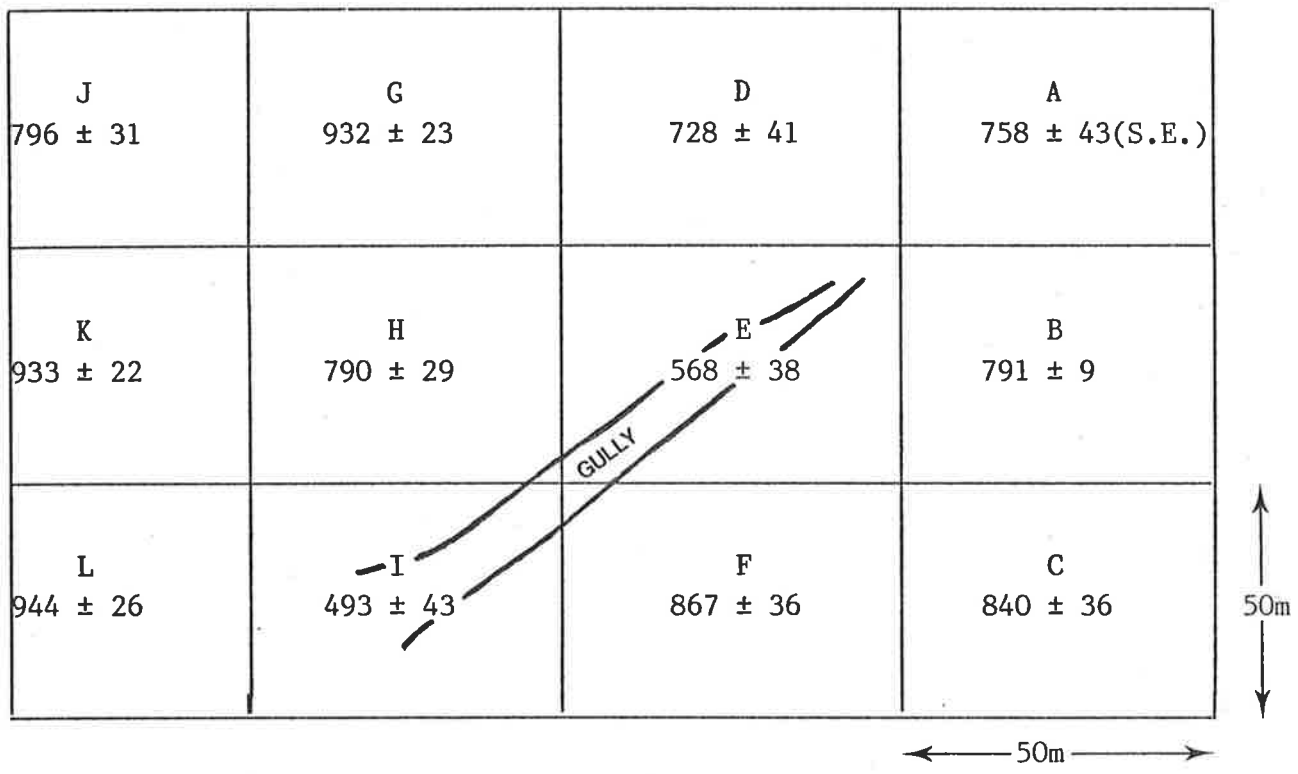
The forest floor was covered by pine-needle litter and where litter had accumulated to a sufficient depth the different layers described by Hesselman (1926) and Kubiena (1953) could be discerned. In places mosses and liverworts formed thick mats and patches of grass occurred also

TABLE 15: Soil characteristics of the Williamstown wheat-field and pine-forest

	Mean pH (n = 12)	Mean % moisture ( n = 24)	Mean EC 1:5 $\mu$ Siemens $\text{cm}^{-1}$ (n = 24)	% Sand	% Silt	% Clay
Wheat-field	5.93*	5.95*	286	48.5	24.8	26.7
Pine-forest	5.17	7.30	251	85.2	4.9	9.9

\*Means for wheat-field and pine-forest soils significantly different at 5% level.

FIG. 15: Quadrats of Williamstown wheat-field showing mean heights (from tip to point of attachment of grain,  $n = 6$ ) of wheat plants at time of soil sampling.



where a break in the canopy permitted sunlight to penetrate to the forest floor. Soursofs (*Oxalis pes-caprae* L.) were also common, as were the fruiting bodies of higher fungi. Basidiomycete hyphae, especially *Amphinema byssoides*, formed almost a continuous mat in the pine litter. The ground covers at the locations sampled in the 12 quadrats are given below:

- Quadrat A: Almost entirely pine litter with sparse moss and  
*Oxalis pes-caprae*
- Quadrat B: Thick moss mat with pine litter and numerous sporocarps of *Thelephora terrestris*
- Quadrat C: As for A
- Quadrat D: As for A
- Quadrat E: Pine litter with sparse moss
- Quadrat F: Pine litter with sparse moss and sporocarps of  
*Hebeloma* sp.
- Quadrat G: Pine litter, sparse moss and *O. pes-caprae*, and  
abundant sporocarps of *Hebeloma* sp.
- Quadrat H: Pine litter with sparse moss, *O. pes-caprae* and sporocarps of *Hebeloma* sp. and *Thelephora terrestris*
- Quadrat I: Almost entirely pine litter
- Quadrat J: Pine litter with sparse moss and *O. pes-caprae*
- Quadrat K: Pine litter with sparse moss, liverworts and sporocarps of *Hebeloma* sp.
- Quadrat L: Pine litter with sparse moss and *O. pes-caprae*.

## 2. ABUNDANCE OF *APHELENCHUS AVENAE* IN WHEAT-FIELD AND PINE-FOREST SOILS

Although nematodes were more abundant in the forest soil, *A. avenae* was significantly more abundant in the wheat-field soil (Table 16). *A. avenae* comprised over 10% of all nematodes extracted from wheat-field soil compared with only 0.25% from pine-forest soil. A correlate to its low

TABLE 16: Abundance of nematodes and *A. avenae* in Williamstown wheat-field and pine-forest soils (per 50 ml soil, n = 24)

	Mean total nematodes	Mean No. <i>A. avenae</i> (% total nematodes)
Wheat-field soil	113.8***	11.4*** (10.04)
Pine-forest soil	201.1	0.5 (0.25)

\*\*\* Means for wheat-field and pine-forest soils significantly different at 0.1% level.



abundance in the forest soil was the observed patchiness of distribution there (Poisson indices of dispersion 30.1 for the wheat-field and 56.1 for the pine-forest with a critical level of 35.2 at  $P = 0.05$ ). *A. avenae* was recovered from only 25% of soil samples from the forest compared with 96% from the wheat-field. Higher numbers of *A. avenae* than had previously been seen in the pine-forest were found in several soil samples, subsequently taken from areas where sunlight penetrated the canopy and allowed a denser growth of grasses.

*Aphelenchoides* spp., a genus known to include fungivores (Franklin, 1978) were also found in both pine-forest and wheat-field soils at densities of 2.9 and 3.3 per 50 ml soil respectively ( $n = 12$  samples). Thus *A. avenae* was more abundant than total *Aphelenchoides* in wheat-field soil while the reverse was true of forest soil. Many of the *Aphelenchoides* spp. found were reared successfully on *Botrytis cinerea*, indicating at least partial mycophagy.

### 3. ABUNDANCE OF NEMATODES IN FOREST LITTER

*A. avenae* was not found in forest litter extracted by the modified Minderman (1956) technique (Table 17d), but its presence was revealed when larger (10 g) samples were processed using a thermal misting apparatus. In contrast, an *Aphelenchoides* sp. with a tufted mucro which appeared to be closest to *A. coffeae* (Zimmerman, 1898) Filipjev, 1934 (after key of Fortuner, 1970), occurred in all samples of all litter layers by either method. *Aphelenchoides* spp. were most abundant in the F-layers where fungal mycelium was also dense, a fact reflected in the high moisture content of these layers (Table 17a). These nematodes multiplied readily on fungal cultures suggesting at least some degree of mycophagy. Thermal misting (of 10 g litter samples) yielded *Aphelenchoides* in numbers up to 80 per g of litter

TABLE 17a: Abundance of *A. avenae* and *Aphelenchoides* in pine-forest litter (per g litter, means of n = 12 samples)

Litter	Mean % moisture	Mean total nematodes	Mean no. <i>A. avenae</i>	Mean no. <i>Aphelenchoides</i> sp. (% total nematodes)
L	48.8	7.2	—*	0.8 (11.5)
F <sub>1</sub>	63.3	23.1	—	3.6 (15.6)
F <sub>2</sub>	70.6	65.3	—	2.2 (3.4)
H,	53.8	59.4	—	1.4 (2.4)

\*Dash indicates not recorded at level of sampling intensity used.

TABLE 17b: Nematodes in pine-forest litter other than *A. avenae* and *Aphelenchoides*. Mean number nematodes per g litter. Figures in brackets represent % of total nematodes extracted

Litter-layer	Rhabditids	Dorylaimids	Mononchids	Tylenchids*	Others
L	6.2 (84.6%)	-	-	0.3 (3.9%)	-
F <sub>1</sub>	18.8 (81.9)	0.3 (1.2)	-	-	0.3 (1.2)
F <sub>2</sub>	55.0 (84.4)	3.3 (0.9)	-	0.5 (0.1)	4.7 (1.2)
H	51.7 (86.8)	1.7 (2.8)	0.3 (0.5)	-	4.5 (7.5)

\*Other than *Aphelenchoides*.

from the F<sub>1</sub>-layer and comprising 75% of total nematodes extracted, much higher relative numbers than those from 300 mg samples extracted with the Minderman technique.

Nematodes were most abundant in the lower litter-layers (F<sub>2</sub> and H), while *Aphelenchoides* were most abundant in the F-layers and reached their highest relative abundance in the upper litter-layers (F<sub>1</sub> and L) (Table 17a). Rhabditids were the most numerous nematodes found in litter and reached their highest densities in the lower litter-layers (Table 17b). Rhabditids were also numerous in forest soil, especially *Acrobeles*.

#### 4. NEMATODES FROM FUNGAL SPOROCARPS COLLECTED FROM THE FOREST FLOOR

Sporocarps of 17 species of higher fungi were collected from the forest floor, among them representatives of the genera *Clitocybe*, *Collybia*, *Galerina*, *Hebeloma*, *Laccaria*, *Rhizopogon*, *Suillus* and *Thelephora*. Nematodes were extracted from sporocarp tissue (free of any adhering soil particles) of several of these spp. The most commonly encountered mycophagous nematode was an *Aphelenchoides* sp. with a tufted mucro, apparently the same as that found in litter. This nematode was recovered from fructifications of 5 out of 6 basidiomycete spp. examined, while *A. avenae* was found in only 2 of these species. In *Suillus luteus*, *Aphelenchoides* and *A. avenae* were recovered in numbers up to 3.9 and 3.2 per g fresh weight fructification respectively. Corresponding densities in *Rhizopogon luteolus* reached 2.2 and 0.95 per g respectively. Desiccated nematodes of both species were commonly recovered from fructifications, suggesting that exploitation of these food sources may depend on favourably moist conditions. Rhabditid nematodes were very common on sporocarps. *Ditylenchus* was rarely recorded. *Tylenchus sensu lato* was not recorded from sporocarps although it was common in one sample containing mycelium from the stalks of several

*Suillus* caps and adhering soil. *Tylenchus* comprised 11.9% of nematodes extracted from this sample, with the remainder consisting of *Aphelenchoides* - 9.7%, Doryaimids - 3.8% and Rhabditids - 74.6%).

The ability of *A. avenae* to feed on sporocarp tissue was tested on tissue of a *Coprinus comatus* cap, selected because thin sections allowed some passage of light whilst observations were made through a microscope. Thin sections of tissue from the top of the cap and fragments of gill tissue were placed in drops of water on a microscope slide. Adult female *A. avenae*, surface-sterilized in 0.5% chlorhexidine gluconate, of an isolate from the pine-forest soil kept in monoxenic culture, were placed on the surface of the sporocarp tissue, a coverslip applied and the nematodes observed at 420 x magnification. Feeding was observed on both cap and gill tissue; the nematodes punctured cell walls with their stylets and evacuation of cell protoplasm was accompanied by pulsation of the valve plates in the median oesophageal bulb. Feeding was not observed on thin sections of stalk tissue, perhaps because cell walls here appeared to be thicker. Surface-sterilized *A. avenae* were also placed in drops of water on the gills of upturned caps of 5 species collected from the forest and incubated in sealed plastic jars at 25°C. However, multiplication did not occur possibly because caps soon decayed or dried out.

##### 5. USE OF FUNGAL BAITS TO TRAP MYCOPHAGOUS NEMATODES IN PINE-FOREST LITTER AND SOIL

To determine whether potential competitors of *A. avenae* other than *Aphelenchoides* spp. were present in the pine-forest, fungal baits in porous bags were used to attract mycophagous nematodes in litter and soil. Bags, about 2 cm x 2 cm, were made of either silk, with pores up to about 90 x 50  $\mu\text{m}$ , or cotton with pores up to about 950 x 700  $\mu\text{m}$  and were closed on three sides. Two *Penicillium* isolates (P86B and P94B) from pine-forest

soil were grown on a 2% solution of malt extract (Difco) in McCartney bottles, the mat of mycelium removed, washed well under running water and placed in a bag. The opening to the bag was sealed with high vacuum grease and bags containing mycelium were transported to the forest in a container of water to ensure they did not dry out. Two sites were selected at random for depositing the baits, one site for each fungus. At each site a bag of each type was placed at the following levels through the litter and soil:

- (a) In the middle of the L litter-layer.
- (b) At the interface between the F<sub>1</sub> and F<sub>2</sub> litter-layers.
- (c) Between the bottom of the H litter-layer and the top of the mineral soil, and
- (d) At 10 cm below the top of the mineral soil.

The disturbed soil and litter was replaced over the baits and left for 48 hours before removal and extraction of nematodes from mycelium by the modified Baermann technique.

No nematodes were recovered from baits placed below the surface of the mineral soil but nematodes were recovered from mycelium which had been placed in the litter, albeit in small numbers. The *Aphelenchoides* sp. with the tufted mucro previously encountered was most commonly recovered, followed by bacteria-feeding Rhabditid nematodes. No *A. avenae* were isolated by this method. Other mycophagous nematodes may have been present but not detected by this technique. The small numbers of nematodes found suggested that the technique was inefficient. Different results may have followed from more extensive sampling or from the use of different baits.

## 6. FUNGI FOUND IN WHEAT-FIELD AND PINE-FOREST SOILS

### (a) Isolation of fungi from soil on dilution and soil plates

Fungal colonies isolated from dilution plates (325 and 468 from wheat-field and pine-forest soils respectively) and soil plates (252 and 234 respectively) were classified according to their morphology on NDY; 166 morphological groups were identified from the wheat-field and 121 from the pine-forest. Lactophenol cotton-blue or acid fuschin mounts were then prepared and isolates identified where possible to at least generic level (see Appendices 3-4) using the keys of Domsch *et al.* (1980) and Barron (1968). Fungal cultures failing to fruit on NDY were classified as "sterile cultures". A small number of colonies sub-cultured from dilution plates (1.85% of wheat-field soil colonies and 0.21% of pine-forest soil colonies examined) and soil plates (0.79% and 0.43% respectively) failed to grow on NDY and were discarded.

Comparison of the fungal genera and species groups most commonly isolated (comprising more than 1% of either total dilution plate or soil plate cultures) from wheat-field and pine-forest soils revealed markedly different mycofloras in these two soils (Table 18). *Penicillium* dominated the fungi isolated by both methods from forest soil, whereas a more diverse mycoflora was revealed in wheat-field soil with many genera, commonly recorded from this soil, being either rare (*Aspergillus* and *Fusarium*) or not recorded at all (*Acremonium*, *Broomella*, *Cladosporium*, *Myrothecium* and *Torulomyces*) from the forest soil. Sterile fungi and Mucorales were more prominently represented in wheat-field soil compared with pine-forest soil while *Trichoderma* was more frequently recorded from the latter soil. *Penicillium* isolates were classified according to subgenus after the key of Pitt (1979) and a significant association between habitat and incidence of

TABLE 18: Frequency of fungal genera and species groups commonly isolated from wheat-field and pine-forest soils by dilution and soil plate methods as % of total colonies isolated

	Dilution plates		Soil plates	
	Wheat-field	Pine-forest	Wheat-field	Pine-forest
	%	%	%	%
<i>Penicillium</i>	33.2	90.2	34.1	86.3
<i>Aspergillus</i>	12.0	0.4	9.5	0.4
Mucorales				
(a) Total	6.5	1.7	11.5	3.4
(b) <i>Mortierella</i>	3.7	1.5	6.4	3.0
(c) <i>Mucor</i>	0.6	0.2	3.2	0.4
<i>Fusarium</i>	14.5	1.1	15.1	3.9
<i>Broomella</i>	10.2	- *	6.8	-
<i>Cladosporium</i>	0.6	-	3.2	-
<i>Myrothecium</i>	1.9	-	-	-
<i>Paecilomyces</i>	1.9	1.5	0.4	0.9
<i>Torulomyces</i>	1.5	-	1.6	-
<i>Acremonium</i>	0.9	-	1.6	-
<i>Trichoderma</i>	1.5	1.9	0.4	2.1
Sterile cultures	8.0	0.6	9.5	1.7
<b>Total</b>	<b>92.7</b>	<b>97.4</b>	<b>93.7</b>	<b>98.7</b>

\*Dash indicates fungi not recorded at level of sampling intensity used.



sub-genera was found (Table 19), indicating that the two habitats differed in composition of this component of their mycofloras.

Many genera with a frequency of <1% on dilution or soil plates from wheat-field soil (*Absidia*, *Actinomucor*, *Conidiobolus*, *Coniothyrium*, *Cunninghamella*, *Doratomyces*, *Gliocladium*, *Gongronella*, *Graphium*, *Metarrhizium*, *Phialocephala*, *Rhizopus*, *Stachybotrys*, *Ulocladium* and several unidentified Coelomycetes) were not recorded from pine-forest soil, but very few genera (*Botrytis*, *Verticillium*) were recorded only from the latter. Similarly, many individual species were recorded solely from wheat-field soil whereas few species, other than some *Penicillium* spp, were found only in forest soil (Appendices 3-4). More *Trichoderma* spp. were recorded from pine-forest soil (*T. hamatum*, *T. harzianum* and *T. koningii*) than from wheat-field soil (*T. harzianum* and *T. viride*).

Mean propagule counts per g soil of fungal genera and species groups commonly recorded from dilution plates of wheat-field and pine-forest soils are presented in Fig. 16. No significant differences result from converting propagule counts to per unit dry soil (Table 20). Fungal propagules were far more numerous in the forest soil but belonged predominantly to *Penicillium* spp. whereas other genera, apart from *Trichoderma*, made up greater proportions of the total propagule count in wheat-field soil. Propagules of *Fusarium* and sterile fungi were significantly more abundant in wheat-field soil.

(b) Selective isolation of specific fungi from soil

Certain fungi are only rarely isolated if at all from dilution or soil plates, hence selective isolation methods were used to augment the picture obtained of the mycoflora by the former methods.

TABLE 19: Isolates of *Penicillium* from wheat-field and pine-forest soils classified according to subgenus (after Pitt, 1979)

	Subgenus				Total
	<i>Aspergilloides</i>	<i>Penicillium</i> <i>Biverticillium</i>	<i>Furcatum</i>		
			Section		
			<i>Furcatum</i>	<i>Divaricatum</i>	
Wheat-field	19*	17	24	11	71
Pine-forest	39	10	16	23	88
Total	58	27	40	34	159

\*There is a significant ( $P < 0.01$ ) association between habitat and incidence of subgenera ( $\chi^2 = 12.9$  with 3 d.f.).

FIG. 16: Mean propagule counts per g soil of common genera and species groups of fungi from wheat-field (shaded bars) and pine-forest (open bars) soils. Pairs of means marked with a star are significantly different at the 2% level or better.

FUNGAL PROPAGULES  $\times 10^3$  /g SOIL

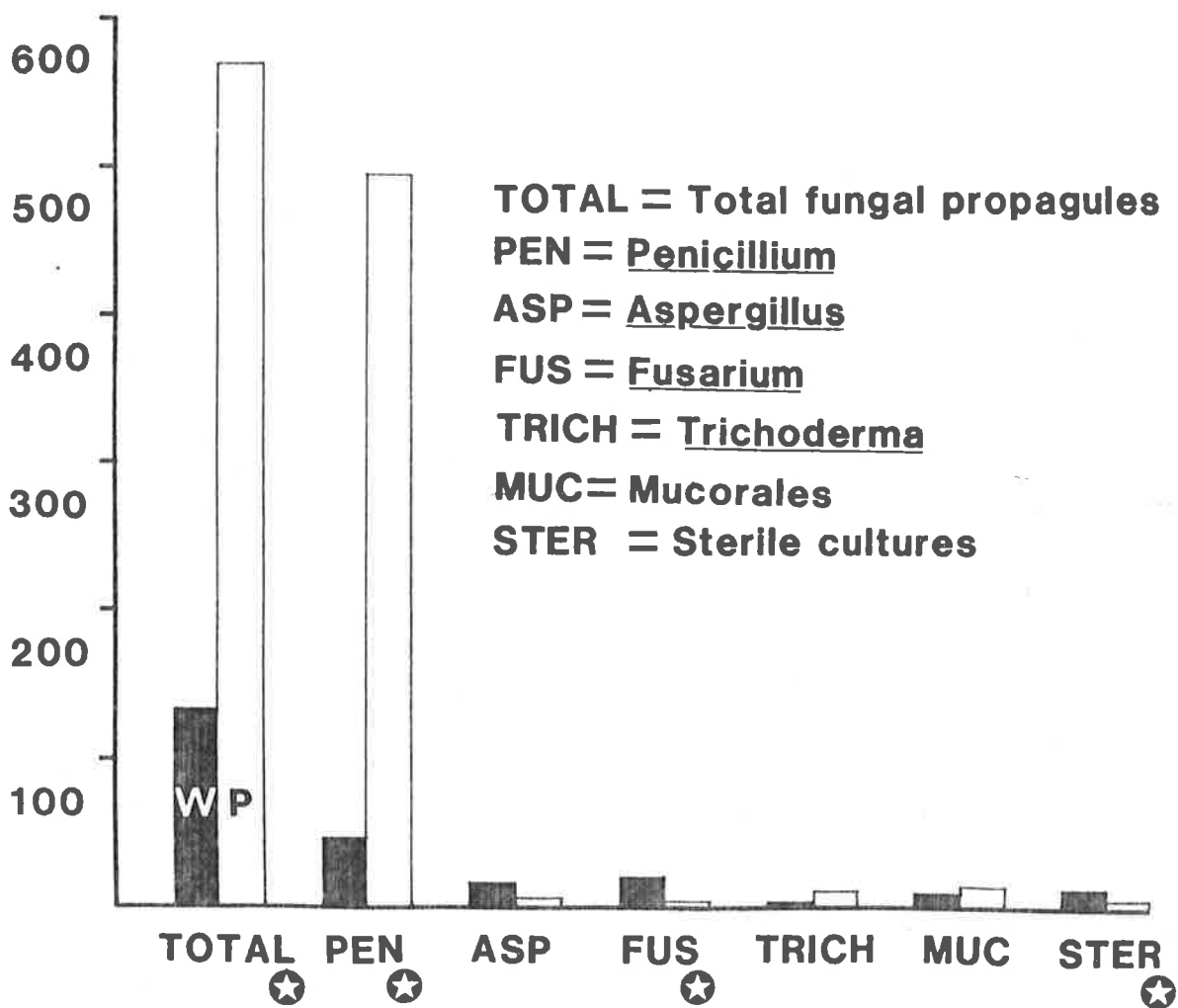


TABLE 20: Mean propagule counts per g soil (a) and per g dry soil (b) of common genera and species groups of fungi from wheat-field and pine-forest soils (n = 24 for total fungal propagules, n = 12 for genera and species groups)

Propagules x 10 <sup>4</sup>		Wheat-field soil	Pine-forest soil
Total fungal	(a)	13.33 ± 1.20(S.E.)	55.58 ± 6.76***
	(b)	14.14 ± 1.30	59.75 ± 7.30***
<i>Penicillium</i>	(a)	4.50 ± 0.85	49.46 ± 7.78***
	(b)	4.81 ± 0.93	53.40 ± 8.45***
<i>Aspergillus</i>	(a)	1.63 ± 0.41	0.50 ± 0.42
	(b)	1.73 ± 0.43	0.54 ± 0.45
<i>Fusarium</i>	(a)	1.96 ± 0.33	0.26 ± 0.14***
	(b)	2.08 ± 0.35	0.28 ± 0.15***
<i>Trichoderma</i>	(a)	0.21 ± 0.13	0.97 ± 0.40
	(b)	0.23 ± 0.14	1.05 ± 0.44
Mucorales	(a)	0.88 ± 0.18	1.35 ± 0.83
	(b)	0.92 ± 0.18	1.46 ± 0.90
Sterile cultures	(a)	1.08 ± 0.20	0.34 ± 0.19**
	(b)	1.16 ± 0.22	0.36 ± 0.20**

Pairs of means for wheat-field and pine-forest soils significantly different at P = 0.02 (\*\*) or P = 0.001 (\*\*\*).

(1) *Rhizoctonia solani sensu lato*

(i) Using the selective medium of Ko and Hora (1971):- soil from Quadrat I of the wheat-field (15 x 2 g sub-samples) was mixed into a paste with sterile distilled water and applied in clumps (10 per 2 g sub-sample per plate) to Petri plates containing the selective medium. These were incubated at 28°C for 24 hours and then those hyphae showing the greatest linear growth (greater than 5 mm) away from the edges of the soil clumps were sub-cultured onto NDY for subsequent identification. *Rhizoctonia* was not found using this method, an unidentified hyphomycete being the most common fungus isolated followed by several sterile hyaline fungi.

A modification of Ko and Hora's medium was used incorporating selective agents in place of "Dexon" to suppress the growth of other fungi. The base medium of Ko and Hora, minus "Dexon" and with streptomycin increased to 100 ppm, was amended with "Hymexazol" (50 ppm) and "Ridomil" (10 ppm) and tested as before with 15 x 2 g sub-samples of wheat-field soil. After 24 hours at 28°C, maximum growth away from clumps was lower than on the unmodified medium, ranging from 1.5 to 6 mm compared with 3-9 mm. *Rhizoctonia* was not isolated but the medium did prove selective for *Actinomucor*, 63% of isolates from hyphae 5 mm in length or more after 24 hours belonging to this genus.

Subsequently, *Rhizoctonia* was readily isolated from organic debris particles in wheat-field soil. Ko and Hora (1971) claimed that the dilution plate method was unsuitable for estimating abundance of *R. solani* due to the low population level of this fungus in most field soils and recommended their selective method for this purpose

because of its sensitivity. The failure of the method here may have to do with variability amongst isolates; for example, temperature requirements of *R. solani* isolates are very variable (Domsch et al., 1980) yet the above isolation method relies upon a high rate of growth at 28°C.

(ii) Baiting:- the "infected host method" of Davey and Papavizas (1962) was tested for isolation of *Rhizoctonia* from wheat-field soil. Soil from Quadrats G and J was placed in dishes, 60 mm deep, watered freely with distilled water and covered with about 80 surface-sterilized wheat (var. Halberd) seeds (5 min in 4% sodium hypochlorite followed by rinsing in sterile water). The seeds were then covered in a thin layer of soil, the dishes sealed in plastic bags and stored in darkness (to stress the seedlings) at 25°C for 7-10 days. The root systems of 35(G) and 50(J) plants were examined for lesions and 138 and 179 root segments respectively plated onto NDY (with 100 ppm streptomycin and 10 ppm tetracycline) after surface sterilization in 0.5% sodium hypochlorite (30 sec) and washing in sterile water.

*Rhizoctonia* was not detected by this method suggesting that pathogenic isolates of this fungus were not common in the soil examined.

(iii) From the organic debris fraction of soil:- Boosalis and Scharen (1959) considered that this fraction was the natural habitat of *Rhizoctonia* in soil and, indeed, the fungus was readily isolated from wheat-field soil using a modification of their method.

Soil (100 g) from wheat-field Quadrat K and pine-forest Quadrat I was suspended in 2.5L of water in a bucket, stirred vigorously and allowed to settle for 30 sec before the supernatant was passed through

a 250  $\mu\text{m}$ -mesh sieve. This procedure was repeated 6 times and the residue on the sieve washed thoroughly for one minute under running water whilst agitating the sieve. The organic debris was further cleared of soil particles by rubbing with a rubber pipette bulb on the end of a pencil under running water for another minute. Petri plates with 2% water agar pH 7.2 were cleared of excess moisture by evaporation in a laminar flow and then spotted (4 spots per plate) with 2 drops of a solution containing 1% of both streptomycin and neomycin. After the antibiotic solution had been absorbed by the agar, individual organic particles blotted dry on sterile filter paper, were placed, one per spot, onto plates. Some 104 and 100 organic debris particles from wheat-field and pine-forest soils respectively were plated and incubated at 25°C. Hyphal tips were sub-cultured up to 9 days later onto NDY/6 for subsequent identification, although *Rhizoctonia* could be immediately recognized by its distinctive characteristics.

Those fungi which remained sterile in culture, especially the dark forms, constituted the most commonly isolated group of fungi from wheat-field organic debris (Table 21). Warcup (1957) obtained a similar result using the hyphal isolation method on wheat-field soil, and it is interesting that many of the hyphae isolated by this method are found to be attached to humus particles (Warcup, 1955). *Fusarium* was also more commonly isolated from organic debris particles than with dilution and soil plate analyses of wheat-field soil; *Penicillium* on the other hand was much reduced in frequency. A possible *Fusidium* sp., not otherwise isolated, was common on organic debris particles and a number of other fungi (for example, *Rhizoctonia* and *Humicola*) were isolated only from organic debris. It appeared that the organic fraction was characterized by a different array of fungi to that found



TABLE 21: Frequency of fungi isolated from washed, organic debris particles as % of total (wheat-field soil n = 116, pine-forest soil n = 55)

Wheat-field	%	Pine-forest	%
<i>Fusarium</i> spp.	26.7	<i>Penicillium</i> spp.	47.6
<i>Fusidium</i> sp.?	14.6	<i>Trichoderma</i> spp.	19.0
<i>Aspergillus</i> spp.	6.9	<i>Fusarium</i> spp.	14.3
<i>Humicola</i> sp.	2.6	<i>Mortierella</i> spp.	9.5
<i>Mortierella</i> sp.	1.7	<i>Paecilomyces</i> sp.	2.4
<i>Paecilomyces lilacinus</i>	1.7	Dark sterile cultures	4.8
<i>Rhizoctonia solani</i>	1.7	Hyaline sterile cultures	2.4
<i>Chaetoceratostoma</i> sp.?	0.9		
<i>Chaetomium indicum</i>	0.9		
<i>Gliocladium solani</i>	0.9		
<i>Penicillium</i> sp.	0.9		
Unidentified Coelomycete	0.9		
Dark sterile cultures	28.4		
Hyaline sterile cultures	11.2		
Total	100.0%	Total	100.0%

for soil generally by dilution and soil plate analyses.

Fungi were both more frequently isolated from organic debris particles from wheat-field soil than from pine-forest soil (116 isolations compared with 55) and in greater diversity (Table 21). As in dilution and soil plate analyses of forest soil, *Penicillium* was the most commonly isolated genus from organic debris. *Trichoderma* and *Fusarium* exhibited an increased frequency of isolation by this method. The nature of organic debris in forest soil, being high in phenolics, may dictate a more specialized and limited array of fungi associated with it than in the wheat-field.

(2) *Pythium* spp.

*Pythium* spp. were rarely found on soil and dilution plates hence the dilution plate technique combined with a selective medium based on the methods of Vaartaja and Bumbieris (1964) and Tsao and Ocana (1969) was used to study their incidence further. The medium consisted of cornmeal agar (Difco) with "Pimaricin" (10 ppm), pentachloronitrobenzene (100 ppm), vancomycin (200 ppm) and (optional) rose bengal (50 ppm). Five 10 g sub-samples of soil from Quadrats I and K of the wheat-field and Quadrats C and I of the pine-forest were serially diluted in sterile distilled water and two dilution plates at a final dilution of 1/100 prepared per sub-sample. Wheat-field sample I and pine-forest sample C were processed using the medium containing rose bengal and due to inhibition of growth, colonies were not counted until after 4 days at 25°C compared with 2 days for the other 2 samples on the medium without rose bengal (Table 22). The first five colonies encountered growing across each plate were sub-cultured onto cornmeal agar (with and without  $\beta$ -sitosterol at 0.01 g per litre medium) for subsequent identification, giving a total of 50 cultures per sample.

TABLE 22: Mean number of fungal propagules per g of soil growing on *Pythium* selective medium with and without rose bengal after 4 and 2 days incubation respectively at 25°C. Means for wheat-field and pine-forest soils on medium with rose bengal significantly different at  $P = 0.001$ ; on medium without rose bengal at  $P = 0.02$ .

Selective medium	Soil sample	Propagules x $10^2$ per g soil	Propagules x $10^2$ per g dry soil
Selective medium with rose bengal	Wheat-field Sample I	30.30 ± 2.23(S.E.)	30.83 ± 2.28
	Pine-forest Sample C	11.30 ± 0.79	12.00 ± 0.84
Selective medium without rose bengal	Wheat-field Sample K	23.60 ± 1.97	24.81 ± 2.07
	Pine-forest Sample I	16.40 ± 1.71	17.77 ± 1.86

Mean numbers of fungal propagules per g soil that grew on the selective medium were significantly higher in wheat-field soil compared to pine-forest soil. Although a greater proportion of these propagules on the medium with rose bengal were taken up by *Mortierella* spp. in wheat-field soil (Table 23), *Pythium* propagules were still more abundant than in forest soil. A greater proportion of colonies sub-cultured from the selective medium with rose bengal failed to grow on cornmeal agar compared with that from the medium alone.

A greater diversity of *Pythium* spp. was isolated from wheat-field soil than from pine-forest soil (Table 23). Species lacking oogonia and sporangia on cornmeal agar (although sometimes possessing thick walled chlamydospores) dominated both soil types but were the only ones isolated from forest soil at all. *Pythium acanthicum* was subsequently isolated from pine-forest soil using pine-needles as bait and *P. dissotocum* was isolated from soil on a *Phytophthora* selective medium.

Four isolates of these non-fertile species from both habitats and which were very similar in appearance were tested for rate of growth on cornmeal agar (with 100 ppm vancomycin) at 25°C. Mean linear growth of the pine-forest isolates over 24 hours was 12.1 mm (range 11.5-12.5 mm) compared with 9.1 mm (range 8-10.5 mm) for the wheat-field isolates; suggesting that different species were involved. To induce formation of oogonia and sporangia, agar blocks of the fungi were transferred to water and fungi sub-cultured onto lima bean agar (with and without  $\beta$ -sitosterol) and cornmeal agar (Difco) with fresh grated carrot (15 g/L), soil (1 g/L) and  $\beta$ -sitosterol (0.01 g/L) (Middleton, 1943). Oogonia failed to appear and the isolates (probably heterothallic) were assigned to the *Pythium afertile* species group after Middleton's classification.

TABLE 23: Identity of fungi sub-cultured from *Pythium* selective media onto cornmeal agar from wheat-field and pine-forest soils (% of total cultures examined)

Selective Medium	With rose bengal		Without rose bengal	
	Wheat-field I	Pine-forest C	Wheat-field K	Pine-forest I
<i>Pythium</i>				
<i>acanthicum</i>	2	-	2	-
<i>afertile</i>	20	48	62	86
<i>irregulare</i>	6	-	-	-
<i>monospermum</i>	2	-	-	-
<i>paroecandrum</i>	6	-	30	-
Other fungi*	42	30	4	2
Fungi failing to grow	22	22	2	12
Total	100%	100%	100%	100%

\*Predominantly *Mortierella* spp.

*A. avenae* isolates earlier obtained from a number of wheat-field soils failed to reproduce upon an isolate of *Pythium ultimum* (supplied by Mr. M. Bumbieris), as was also found for the Brownhill Creek population by Evans (1968). It was therefore of interest to determine whether this fungus was present in soil at the study sites, and the selective method of Stanghellini and Hancock (1970) was used for this purpose. Soil (1 g) from wheat-field Quadrats I and K and pine-forest Quadrats C and I was shaken in 99 ml sterile distilled water for 2 min on a test-tube shaker, 1 ml withdrawn from 2 cm below the meniscus and placed drop by drop on the margin of 3-day old water agar (2%) in Petri plates. The suspension was agitated for a further 2 min and another 1 ml sample applied to other plates in the same manner. Plates were incubated at 24°C for 18-24 hours at which time any hyphae growing away from the margins of the drops were sub-cultured onto cornmeal agar for subsequent identification. Only 9 out of 179 drops produced hyphae and none of these was *Pythium ultimum*. It was concluded that this fungus was rare if present at all in the soil examined.

(3) *Phytophthora* spp.

Selective methods used to study the incidence of *Phytophthora* spp. included pear baiting, and pine-needle baiting (Dance *et al.*, 1975) combined with the selective medium of Tsao and Guy (1977). This selective medium was also used directly with soil, as recommended by Tsao and Guy (1977).

About 200 g of soil from Quadrats I and C of the forest and Quadrats I and K of the wheat-field was placed in 120 mm-diameter dishes, a clean pear placed on top and rainwater added to several cm above the soil. Lesions on pears were sub-cultured onto cornmeal agar (Difco) over the course of a week. *Phytophthora* spp. were not detected by this method.

In using pine-needles as bait, soil (from forest Quadrats I, B and C and wheat-field Quadrats I and K) was added to dishes as before and covered with either distilled water, autoclaved peptone/soil extract (1 g Bacto-peptone incubated for 2 days with 100 g soil and extracted in 1 L water) or autoclaved pond water/distilled water (1:2). The basal portions of fresh *Pinus radiata* needles (6 per dish) were then floated on the surface of the liquid and dishes incubated at room temperature or 16°C for 3 days. Needles were then surface-sterilized for 30 sec in 0.5% sodium hypochlorite, washed 3 times in sterile distilled water and plated onto the *Phytophthora* selective medium of Tsao and Guy (1977). Plates were incubated at 25°C and fungi sub-cultured onto cornmeal or lima bean agar up to one week later. *Phytophthora* spp. were not isolated by this method and it was concluded that, if present, they were rare in these soils. Of 92 fungi isolated from pine-needles exposed to pine-forest soil, all but one (a *Trichoderma* sp.) proved to be *Pythium acanthicum*, a fungus not isolated from this soil previously on *Pythium* selective media.

Soil (100 mg) from forest Quadrat C and wheat-field Quadrat I was sprinkled evenly over the surface of the selective medium (Tsao and Guy, 1977) in Petri dishes (10 mg of soil per dish) and incubated at 25°C in the dark. Colonies were counted after 72 hours and the first 5 colonies encountered per plate (giving a total of 50 per sample) sub-cultured onto cornmeal agar for identification. After 72 hours the wheat-field soil had produced  $109.2 \pm 4.7$  (S.E.) colonies per plate compared with only  $18.7 \pm 2.1$  (S.E.) for the pine-forest soil. No *Phytophthora* spp. were recovered by this method; the most commonly isolated fungi from forest soil were *Mortierella* spp. (58%) and *Pythium afertile* spp. (36%), while *Gongronella butleri* (68%) and *Mortierella* spp. (20%) were most commonly isolated from wheat-field soil.

(4) Botrytis spp.

A *Botrytis* sp. had been isolated from pine-forest soil from dilution plates and the selective medium of Kritzman and Netzer (1978) was used to further study its incidence.

Soil (10 g) from forest Quadrat C and wheat-field Quadrat I was serially diluted in sterile distilled water to give 5 plates at a final dilution of 1/100 and one plate at a dilution of 1/10 per sample. Plates were poured with the selective medium and after 48 hours at 25°C brown-pigmented colonies were counted and sub-cultured onto NDY for identification.

Most colonies so isolated proved to be *Fusarium*, *Phoma* or *Stemphylium* spp.. No *Botrytis* spp. were isolated, suggesting that they were rare in these soils.

7. FUNGI ISOLATED FROM ROOTS OF WHEAT AND *PINUS*

*Penicillium* and *Trichoderma* spp. were the dominant fungi isolated from *Pinus* roots, while wheat roots yielded a greater diversity of fungi (Tables 24, 25). The composition of the mycoflora from *Pinus* roots was generally similar to that found for organic debris from pine-forest soil but differed from that of soil. In particular, the frequency of *Trichoderma* (especially *T. koningii*) was higher than that for soil. Wheat roots exhibited a higher incidence of dark sterile fungi and a reduced frequency of *Penicillium* and *Aspergillus* spp. compared with soil. Data for sodium hypochlorite-treated wheat roots are not given (Table 25) due to destruction of cultures by mites. Of 20 cultures not destroyed, 75% were of dark sterile fungi, suggesting a similar picture to that found for streptomycin-treated roots.



TABLE 24: Frequency of fungi isolated from streptomycin-treated root segments as % of total (wheat n = 80, *Pinus* n = 95)

Wheat roots		<i>Pinus</i> roots	
	%		%
<i>Paecilomyces lilacinus</i>	7.5	<i>Penicillium</i> spp.	52.6
<i>Aspergillus</i> spp.	3.8	<i>Trichoderma koningii</i>	29.5
<i>Penicillium</i> spp.	3.8	<i>T. harzianum</i>	2.1
<i>Phoma</i> spp.	3.8	<i>Mortierella</i> spp.	6.3
<i>Mortierella</i> spp.	3.8	Hyaline sterile cultures	8.4
<i>Phialocephala</i> sp.	2.5	Dark sterile cultures	1.1
<i>Fusarium roseum</i> sp. gr.	2.5		
<i>F. solani</i>	1.2		
<i>Fusarium</i> sp.	1.2		
<i>Broomella</i> sp.	1.2		
<i>Cochliobolus sativus</i>	1.2		
<i>Myrothecium verrucaria</i>	1.2		
<i>Periconia macrospinoso</i>	1.2		
Hyaline sterile cultures	3.8		
Dark sterile cultures	61.3		
Total	100.0%	Total	100.0%

TABLE 25: Frequency of fungi isolated from sodium hypochlorite-treated *Pinus* roots (n = 54)

<i>Pinus</i> roots	
<i>Penicillium</i> spp.	35.2
<i>Trichoderma</i>	
<i>koningii</i>	24.1
<i>harzianum</i>	5.5
<i>Pythium</i> spp.	13.0
<i>Fusarium</i> spp.	9.3
<i>Mortierella</i> spp.	9.3
<i>Phoma eupyrena</i>	3.6
Total	100.0%

## 8. ACTINOMYCETES AND BACTERIA IN WHEAT-FIELD AND PINE-FOREST SOILS

Actinomycetes and bacteria were extracted from soil samples from wheat-field Quadrat I and pine-forest Quadrat C using a dilution plate technique (Lingappa and Lockwood, 1962). Total available propagules of both actinomycetes and bacteria were significantly higher in wheat-field soil compared with pine-forest soil (Table 26). This may be related in part to the preference by these organisms for neutral-alkaline conditions (Griffin, 1972).

TABLE 26: Mean numbers of actinomycete and bacterial propagules per g of wheat-field and pine-forest soil (n = 5). Numbers in brackets represent mean numbers of propagules per g of dry soil

	Wheat-field soil	Pine-forest soil
Actinomycetes	10 460 000*** (10 641 960***)	812 000 (862 510)
Bacteria	8 796 000*** (8 948 170***)	2 062 000 (2 190 260)

\*\*\*Means for wheat-field and pine-forest soils significantly different at 0.01% level.

V. SOIL FUNGI, BACTERIA AND ACTINOMYCETES AS FOOD  
RESOURCES OF APHELENCHUS AVENAE AND OTHER NEMATODES

It had been established that *A. avenae* was significantly less abundant in the pine-forest soil compared with the wheat-field soil and that these two soils possessed different mycofloras. To test the hypothesis that this difference in abundance may have resulted from differences in the suitability of these mycofloras as food sources of *A. avenae*, feeding trials between the nematode and fungi from the two soils were conducted. To determine whether differences existed in rates of reproduction achieved on given fungi by *A. avenae* from the wheat-field and pine-forest populations, isolates of both populations were tested on fungi from both soils. Several isolates from the same nematode population were also tested to determine the level of intra-population variability in reproduction rates on different fungi. Lastly, other presumed mycophagous nematodes from wheat-field and pine-forest soils were tested on fungi also obtained from these soils to determine if resource overlap in food occurred between them and *A. avenae*. Such resource overlap would be a pre-requisite to establishing the existence of competition for food between these nematodes; a possible alternative hypothesis in explaining differences in abundance of *A. avenae* between the two sites. In this regard it should be noted that the nematodes tested here are most certainly not the only mycophagous organisms (or, indeed, nematodes) present in these soils.

Tikyani and Khera (1969) reported that an isolate of *A. avenae* failed to reproduce on the bacterium *Bacillus megaterium* but was "cultivated on an unidentified bacterium when transferred in masses". Feeding trials between *A. avenae* and various cultures of soil bacteria were conducted to determine whether bacteria could be significant food resources for this nematode. Although actinomycetes are abundant in soil they have not previously been tested as food sources for *A. avenae*.

Fungi, bacteria and actinomycetes isolated from wheat-field and pine-forest soils were tested as food sources for *A. avenae* and other nematodes isolated from the same soils and maintained in monoxenic culture on *Botrytis cinerea*.

#### 1. BACTERIA AND ACTINOMYCETES

Three randomly selected bacterial isolates from each soil, four actinomycetes from wheat-field soil and three actinomycetes from pine-forest soil were tested as food sources for *A. avenae* isolated from soil of Quadrat A of both sites.

*A. avenae* failed to reproduce above those numbers on uninoculated agar when placed on cultures of bacteria from either soil (Table 27). It was concluded that *A. avenae* was not able to utilize these bacteria as food sources.

Actinomycetes on which *A. avenae* were tested were also found to be unsuitable as hosts, and although several yielded numbers slightly in excess of controls (Table 27) they were very small when compared with yields achieved on fungal hosts, which may exceed hundreds of thousands. Numbers of adults recovered from plates did not exceed the initial inoculum and the small increases in numbers presumably resulted from hatching of eggs laid by females. Females transferred from a suitable fungal host will lay a small number of eggs without any further feeding, however, the larvae which hatch from these eggs require a suitable food source to reach maturity.

It therefore appeared unlikely that *A. avenae* utilized bacteria or actinomycetes as food sources in the soils tested.

TABLE 27: Mean numbers of *A. avenae* (wheat-field and pine-forest cultures) reared on cultures of bacteria and actinomycetes from wheat-field and pine-forest soils. Controls refer to plates inoculated with nematodes alone

	Nutrient agar				Krainsky's medium				
	<u>Control</u>	<u>Bacteria</u>			<u>Control</u>	<u>Actinomycetes</u>			
		1	2	3		1	2	3	4
Wheat-field	0.8	1.0	0.6	1.0	1.8	7.4	4.0	2.4	2.0
Pine-forest	0.8	0	0.8	0	0.4	2.8	7.2	3.4	-

## 2. FUNGI

(a) Description of Nematode Isolates Tested on Fungi

Various isolates of *A. avenae* and *Aphelenchoides* spp. originating from the two sites were used in feeding trials with fungi from pine-forest and wheat-field soils, viz.:

*A. avenae* -

(1) Pine-forest

- (i) isolate from pine-forest soil, Quadrat A.
- (ii) isolate from stalk of *Suillus luteus* sporocarp from pine-forest.

(2) Wheat-field

- (i) isolate from wheat-field soil, Quadrat A.

*Aphelenchoides* spp. -

(1) Pine-forest

- (i) isolate from L-litter layer of pine-forest, appearing to be closest to *A. coffeae* (Zimmerman, 1898) Filipjev, 1934 (after key of Fortuner, 1970). Adult females  $734.5 \pm 22.7 \mu\text{m}$  (S.E.) in length (mean 20 specimens, range 508-874  $\mu\text{m}$ ), with four incisures in lateral field, oocytes in a single row and bearing a tufted mucro on the tip of the tail. (All measurements from specimens from two-week old cultures on *Botrytis cinerea* incubated at 25°C). Males not seen; asexual reproduction (apparently by parthenogenesis) confirmed by starting cultures with single second-stage larvae.
- (ii) isolate from fruiting body of *Rhizopogon luteolus*, apparently belonging to the same species as the isolate from L-litter layer.



(iii) isolate from pine-forest soil, Quadrat C. Adult females  $597 \pm 15.4 \mu\text{m}$  (S.E.) in length (mean 10 specimens, range 543-689  $\mu\text{m}$ ), with three incisures in the lateral field, and bearing a simple mucro on the tip of the tail. Stylet 11-12  $\mu\text{m}$  in length. Sex ratio of males to females 1:19, asexual reproduction (apparently by parthenogenesis) confirmed. Adult males  $568.8 \pm 16.5 \mu\text{m}$  (S.E.) in length (mean 10 specimens, range 441-639  $\mu\text{m}$ ), curved ventrally when killed by heat, spicules paired, cuniform, with prominent apex and rostrum and measuring  $20.7 \pm 0.7 \mu\text{m}$  (S.E.) around the arc of the dorsal limb (mean of 5 specimens, range 18.6-22.6  $\mu\text{m}$ ). Mail tail without bursa or gubernaculum but with three pairs of papillae. Tail of female more bluntly rounded than depicted for *A. composticola* by Franklin (1957) and also differing from this species by the presence of annules in the head region.

(2) Wheat-field

- (i) isolate from wheat-field soil, Quadrat B, and similar in appearance to the isolate from L-litter layer of the pine-forest. Adult females  $696.5 \pm 14.9 \mu\text{m}$  (S.E.) in length (mean 21 specimens, range 568-804  $\mu\text{m}$ ) and bearing a tufted mucro on the tip of the tail. Males absent, asexual reproduction (parthenogenetic) confirmed.
- (ii) isolate from wheat-field soil, Quadrat H. Adult females  $597.5 \pm 15.4 \mu\text{m}$  (S.E.) in length (mean 20 specimens, range 499-739  $\mu\text{m}$ ), with three incisures in lateral field and tail without a mucro. Males absent, asexual reproduction (parthenogenetic) confirmed.

In these feeding trials "Hibitane" (chlorhexidine gluconate), an agent that is both bacteriocidal and fungicidal, was at first used to free nematodes of surface contaminants prior to inoculation. Peacock (1959) recommended an immersion time of 15 min for treating *Meloidogyne* egg-masses. In the feeding trials an immersion time of 10 min was initially used and was subsequently decreased to 5 min and then to 2 min. Although no immediate mortality resulted from treatment with "Hibitane", toxic effects became evident when nematodes were observed over several days. Consequently, this agent was replaced by a mixture of antibiotics (penicillin, streptomycin and tetracycline) which was found to give better survival of nematodes and reduce variability in the results, but to lead to more plates being discarded due to contamination by fungi. The two sets of results (from nematodes treated with "Hibitane" or antibiotics) are analyzed separately, data from the latter being subjected to an analysis of variance.

(b) Results of Feeding Trials on Fungi

Evans (1968) found that the population (on a log. scale) of *A. avenae* reared on a fungus in culture followed a sigmoid curve at temperatures between 20°C and 30°C. From several fungi he tested, highest numbers of nematodes were produced on an isolate of *Rhizoctonia solani* and after fourteen days at 25°C the population was nearing the end of the log. phase of growth and was approaching a plateau. A similar result was indicated in the present study on *Botrytis cinerea*, the fungus used to maintain cultures of the nematode. Given a suitable host fungus, the conditions used in this study - fourteen days incubation at 25°C - were sufficient to allow build-up of large nematode populations. However, Evans (1968) pointed out that interpretation of results showing different populations on different host fungi at a single harvest was difficult unless it is known at which phase in the growth curve the harvest is made. In this study, fungal groups

involving many isolates rather than individual fungi were compared.

Control plates in which nematodes were inoculated onto agar without fungi did not support any population increase. Production of the various isolates of *A. avenae* and *Aphelenchoides* spp. on genera and species groups of fungi from wheat-field and pine-forest soils are summarized in Tables 28a and 29a respectively. A log transformation [ $y = \log_e (\text{total nematodes} + 1)$ ] was applied to this data and analyses of variance performed both for individual fungal genera (Appendices 5a-v and 6a-1) and for the data overall (Tables 28b and 29b).

Variation in numbers of nematodes produced between both nematode isolates and fungal groups was highly significant ( $P = 0.001$ ; Tables 28b and 29b). These two variables were clearly not independent, as the interaction between them was found to be highly significant ( $P = 0.001$ ; Tables 28b and 29b). It was concluded that the nematode isolates differed in their rate of population growth on fungi, that numbers of a given nematode isolate produced on some fungal groups were higher than on others, and that the suitability of different fungal groups as food-hosts tended to follow a consistent pattern across nematode isolates (that is, fungi that were "poor" food-hosts for one nematode isolate tended to be poor hosts for other isolates).

Overall, the *A. avenae* isolates produced higher numbers on fungi from both habitats compared with any of the *Aphelenchoides* isolates (Figs. 17-18, Tables 28a and 29a). Since all of the *Aphelenchoides* isolates used were smaller in body length than *A. avenae* and probably smaller in volume, the above finding suggests that the rate of assimilation of fungal protoplasm and conversion to biomass was considerably higher in *A. avenae*. This may indicate a higher degree of specialization towards mycophagy by *A. avenae*, it being possible that some or all of the *Aphelenchoides* spp. were only facultative fungal feeders. Yields from *Aphelenchoides* isolates C (pine-

TABLE 28a: Table of means for production of nematodes/<sup>on</sup> fungal genera and species groups from wheat-field soil. *A. avenae* isolates: P - pine-forest soil; W - wheat-field soil. *Aphelenchoides* isolates: pine-forest - L (litter); C (soil); wheat-field soil - B, H.

Fungi	<i>A. avenae</i>		<i>Aphelenchoides</i> isolates				Total
	P	W	L	C	B	H	
<i>Penicillium</i>	1942.9	5965.7	-	3.8	1068.0	364.6	2642.7
<i>Fusarium</i>	10216.7	16599.9	2621.8	1432.6	2803.1	528.6	8077.9
<i>Trichoderma</i>	1305.0	2447.3	5775.0	2602.5	7177.5	-	3457.4
<i>Mortierella</i>	12135.0	12843.9	-	-	9143.3	1305.0	10825.6
Mucorales*	7023.8	16577.0	1236.3	1035.0	1271.7	257.5	6409.7
<i>Aspergillus</i>	73.6	320.0	-	-	51.5	332.5	121.6
<i>Metarrhizium</i>	3312.5	8375.0	-	-	-	-	5843.8
<i>Cladosporium</i>	1485.0	6800.0	-	-	-	-	4142.5
<i>Paecilomyces</i>	3960.0	17812.5	-	-	-	-	11875.7
<i>Acremonium</i>	630.0	31968.8	-	0.5	1.5	201.3	9665.7
<i>Gliocladium</i>	265.0	662.5	-	15.5	0.5	232.5	235.2
<i>Graphium</i>	8925.0	17375.0	-	1176.0	205.0	-	7740.9
<i>Broomella</i>	9875.0	32500.0	1267.3	335.0	865.3	12.5	7655.0
<i>Phoma</i>	11929.4	24861.5	1507.1	939.0	1240.0	1085.0	10286.5
<i>Cochliobolus</i>	10000.0	25375.0	-	-	-	-	20250.0
<i>Myrothecium</i>	1509.7	4691.9	-	5.4	693.7	190.0	1610.7
<i>Torulomyces</i>	9410.0	30531.3	-	-	-	-	19970.6
<i>Doratomyces</i>	485.0	14093.8	-	-	75.0	2100.0	6169.5
<i>Coniothyrium</i>	1940.0	2730.0	-	-	69.8	-	1867.4
<i>Periconia</i>	15458.3	20562.5	-	-	-	-	17500.0
<i>Ulocladium</i>	9266.7	12481.3	29.5	-	-	19.0	6728.6
Sterile fungi	6966.4	16324.6	7990.8	229.0	5827.5	542.5	7255.7
Total	7462.8	14237.6	3609.2	595.8	2636.2	511.2	6777.5

\*Other than *Mortierella* (*Mucor*, *Gongronella* and *Cunninghamella*).

TABLE 28b: Analysis of variance of data as summarized in Table 28a. Before analysis a log. transformation ( $y = \log_e (\text{total no. nematodes} + 1)$ ) was applied to the data. Significance levels for variance ratios:  $P = 0.001$  (\*\*\*) ;  $P = 0.01$  (\*\*);  $P = 0.05$  (\*); N.S. = non-significant.

Source of variation	df	Sum of squares	Mean square	F
Fungal genera	21	1942.90	92.52	35.58 ***
Nematode isolates	5	3267.47	653.50	251.29 ***
Interaction	66	1066.51	16.16	6.21 ***
Residual	1111	2889.17	2.60	
Total	1203	9166.05		

TABLE 29a: Table of means for production of nematodes on fungal genera and species groups from pine-forest soil. Nematode isolates as given in legend to Table 28a, except for *A. avenae* isolate S from sporocarp of *Suillus luteus* collected in pine-forest

Fungi	<i>A. avenae</i>			<i>Aphelenchoides</i> isolates				Total
	P	W	S	L	C	B	H	
<i>Penicillium</i>	1982.5	10536.3	15117.7	2523.8	12.6	1.0	-	4474.8
<i>Paecilomyces</i>	3110.3	14012.5	-	1600.0	3247.5	285.0	-	6411.5
<i>Trichoderma</i>	13337.5	17109.4	-	915.0	864.4	1311.3	-	9906.8
<i>Mortierella</i>	12277.3	14168.8	15657.1	-	1170.6	2924.0	-	9303.7
<i>Mucor</i>	7400.0	4808.8	-	-	-	-	-	6620.0
<i>Fusarium</i>	4668.2	16357.6	9500.0	1631.1	366.6	2553.8	-	7888.4
<i>Verticillium</i>	3481.5	8609.1	-	2370.0	2.1	4287.5	-	4220.6
<i>Pestalotia</i>	7946.3	28041.7	-	15600.0	877.5	-	27.5	13171.2
<i>Botrytis</i>	20550.0	39625.0	-	-	-	-	13.0	26265.9
<i>Chaetomium</i>	6425.0	8366.7	-	-	-	-	-	7257.1
Sterile fungi	5164.5	8085.2	7708.3	1031.2	237.9	22877.5	-	6899.1
P16*	4245.6	11612.5	15675.0	-	140.0	-	-	7554.5
Total	4286.6	14170.3	13874.4	2731.2	372.3	6767.0	20.3	6770.3

\*Unknown Hyphomycete bearing conidia.

TABLE 29b: Analysis of variance of data as summarized in Table 29a. Before analysis a log. transformation ( $y = \log_e (\text{total no. nematodes} + 1)$ ) was applied to the data.

Source of variation	df	Sum of squares	Mean square	F
Fungal genera	11	751.46	68.31	25.45 ***
Nematode isolates	6	3082.11	513.69	191.38 ***
Interaction	36	383.77	10.66	3.97 ***
Residual	728	1954.01	2.68	
Total	781	6171.35		

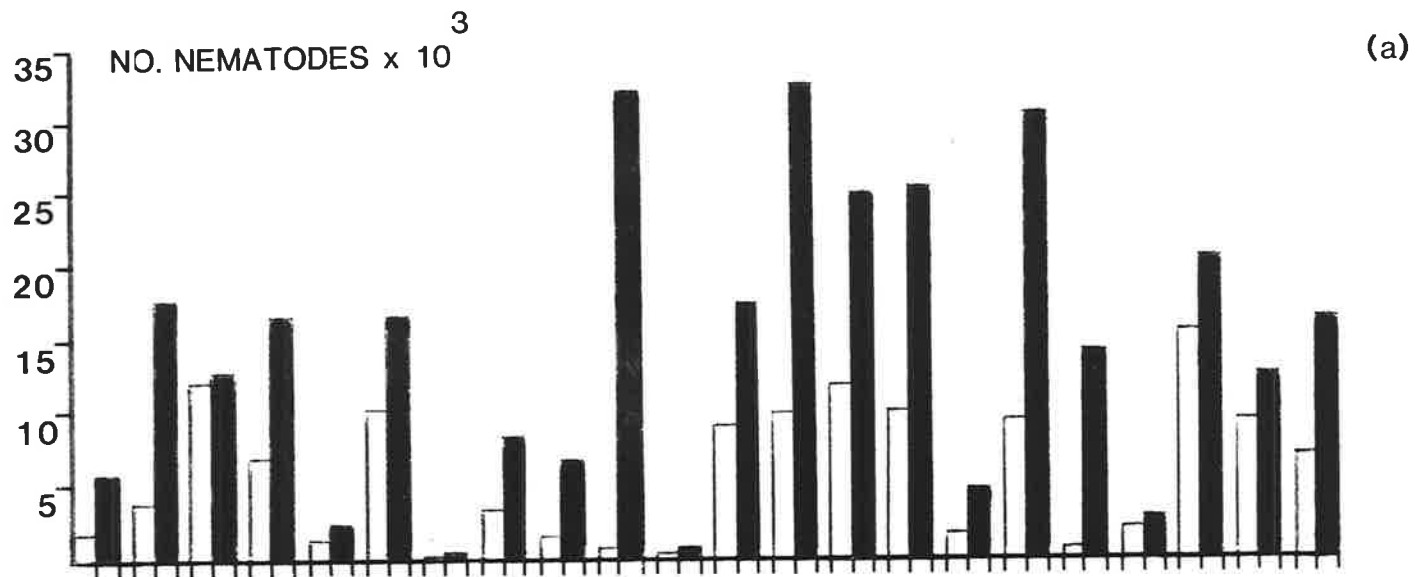
\*\*\* F-value significant at  $P = 0.001$ .

FIG. 17: Mean production of antibiotic-treated nematodes on genera and species groups of fungi from wheat-field soil.

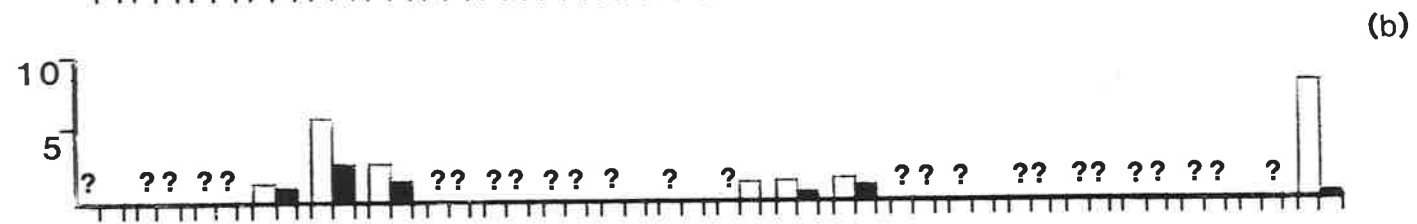
- (a) *A. avenae*:  
 shaded bars - wheat-field soil isolate;  
 unshaded bars - pine-forest soil isolate.
- (b) Pine-forest *Aphelenchoides* isolates:  
 shaded bars - isolate C from soil;  
 unshaded bars - isolate L from L-litter layer.
- (c) Wheat-field *Aphelenchoides* isolates:  
 shaded bars - isolate H from soil;  
 unshaded bars - isolate B from soil.  
 (question marks indicate isolates not tested on those fungal groups).

PEN = *Penicillium*; PAE = *Paecilomyces*; MOR = *Mortierella*; MUC = Mucorales other than *Mortierella* (*Mucor*, *Gongronella* and *Cunninghamella*); TRI = *Trichoderma*; FUS = *Fusarium*; ASP = *Aspergillus*, MET = *Metarrhizium*; CLA = *Cladosporium*; ACR = *Acremonium*; GLI = *Gliocladium*; GRA = *Graphium*; BRO = *Broomella*; PHO = *Phoma*; COC = *Cochliobolus*; MYR = *Myrothecium*; TOR = *Torulomyces*; DOR = *Doratomyces*; CON = *Coniothyrium*; PER = *Periconia*; ULO = *Ulocladium*; STE = sterile cultures.

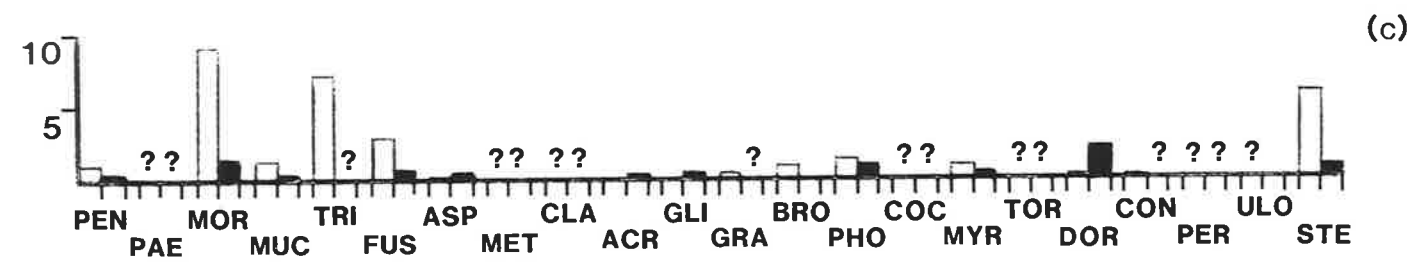




(a)



(b)

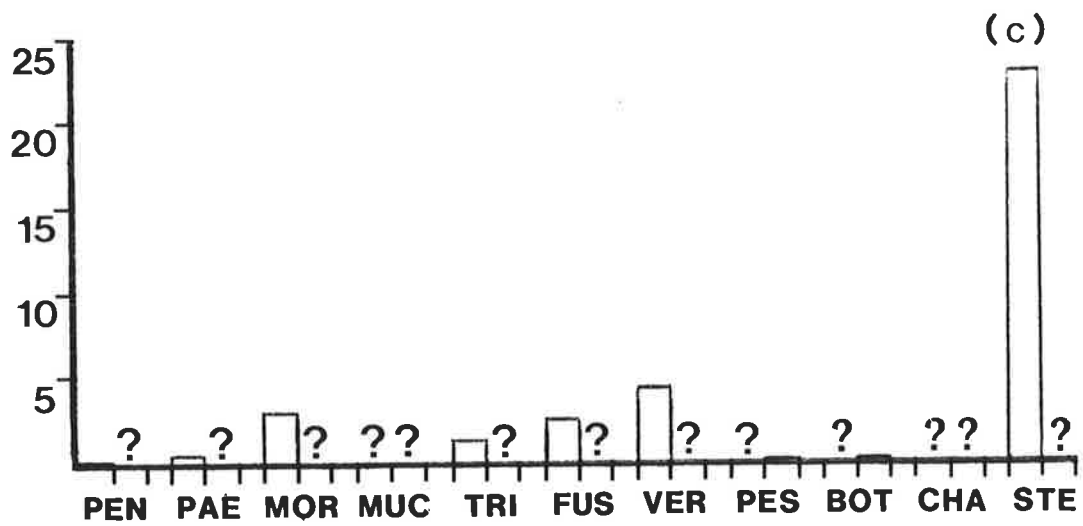
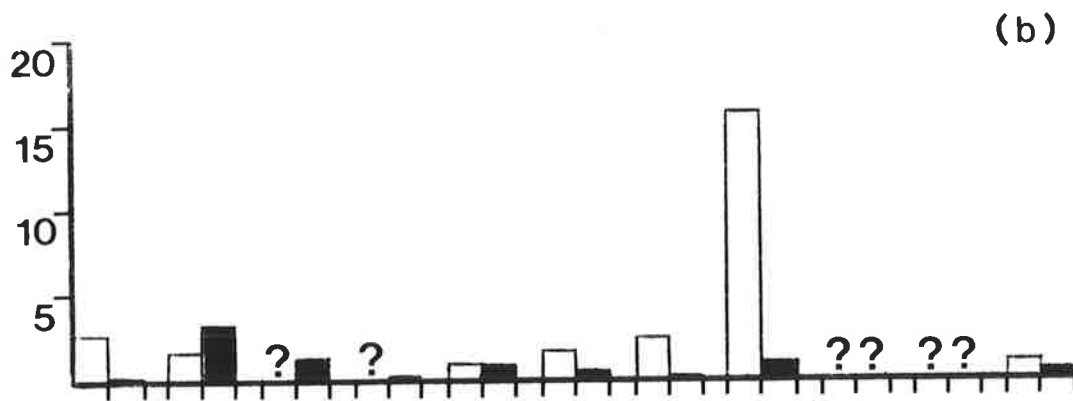
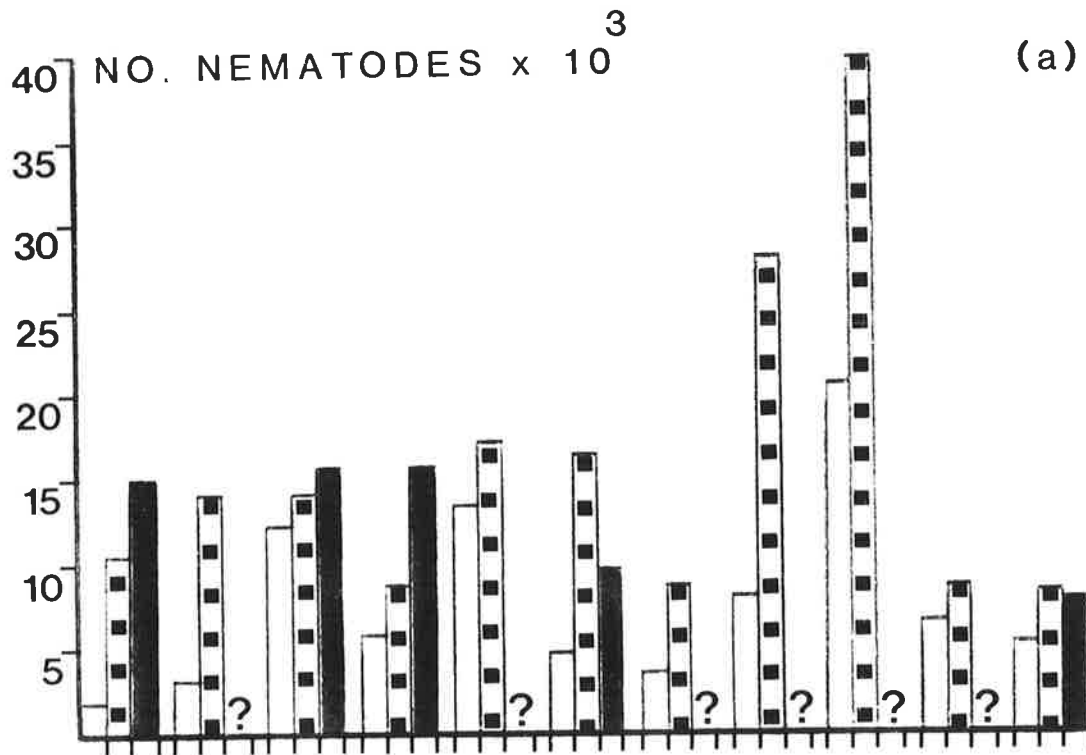


(c)

FIG. 18: Mean production of antibiotic-treated nematodes on genera and species groups of fungi from pine-forest soil.

- (a) *A. avenae*:  
 shaded bars - isolate from *Suillus luteus* sporocarp from pine-forest;  
 partially shaded bars - isolate from wheat-field soil;  
 unshaded bars - isolate from pine-forest soil.
- (b) Pine-forest *Aphelenchoides* isolates:  
 shaded bars - isolate C from soil;  
 unshaded bars - isolate L from L-litter layer.
- (c) Wheat-field *Aphelenchoides* isolates:  
 shaded bars - isolate H from soil;  
 unshaded bars - isolate B from soil.

PEN = *Penicillium*; PAE = *Paecilomyces*; MOR = *Mortierella*; MUC = *Mucor*; TRI = *Trichoderma*;  
 FUS = *Fusarium*; VER = *Verticillium*; PES = *Pestalotia*;  
 BOT = *Botrytis*; CHA = *Chaetomium*; STE = sterile cultures; Pl6 = unknown Hyphomycete bearing conidia.



forest) and H (wheat-field) were particularly low compared with *A. avenae*. Yields of isolates L (pine-forest) and B (wheat-field) were similar for many groups of fungi. The similarity in morphology between these two *Aphelenchoides* isolates was noted earlier. Overall yields of *Aphelenchoides* isolates from the pine-forest were not markedly different from those from the wheat-field.

The *A. avenae* isolate from wheat-field soil generally yielded higher mean populations on fungal genera and species groups from both wheat-field and pine-forest soils compared with the isolate from pine-forest soil (Figs. 17a, 18a, and 19-20; Tables 28a and 29a). However, it became apparent that this result could not be used to account for the higher density of *A. avenae* in wheat-field soil compared with pine-forest soil. Thus, it was found that isolates of *A. avenae* from the same habitat could vary as much in their rates of population growth in monoxenic culture as between isolates from different habitats. This was demonstrated by a pine-forest isolate of *A. avenae* originating from a *Suillus luteus* sporocarp which, like the wheat-field soil isolate, also produced higher populations than the pine-forest soil isolate (Fig. 18a). It appeared that within the 3-hectare area of forest sampled there co-existed *A. avenae* with substantially differing intrinsic rates of population growth in monoxenic culture. This further suggested an underlying genetic diversity, possibly in the form of distinct (parthenogenetic) clones. There is no reason to believe that this situation would not apply equally to the wheat-field or any other habitat.

Mean production of *A. avenae* isolates over the range of fungi tested from wheat-field soil was not markedly different from that of pine-forest soil (see total means Tables 28a and 29a), although the total mean for the pine-forest isolate was somewhat higher on the wheat-field fungi. However, different fungal genera and species groups varied markedly in their capacities to support population growth of *A. avenae* and, as was noted

FIG. 19: Mean numbers of pine-forest soil (shaded bars) and wheat-field soil (unshaded bars) isolates of *A. avenae* produced on some common fungal genera and species groups from both wheat-field and pine-forest soils.

Means for genera were obtained by averaging means for the individual isolates belonging to a genus and were determined for both nematodes treated with chlorhexidine gluconate (a) and antibiotics (b) prior to inoculation.

Number of isolates tested are given below the bars.

PEN = *Penicillium*; ASP = *Aspergillus*; MUC = Mucorales; STER = sterile cultures.

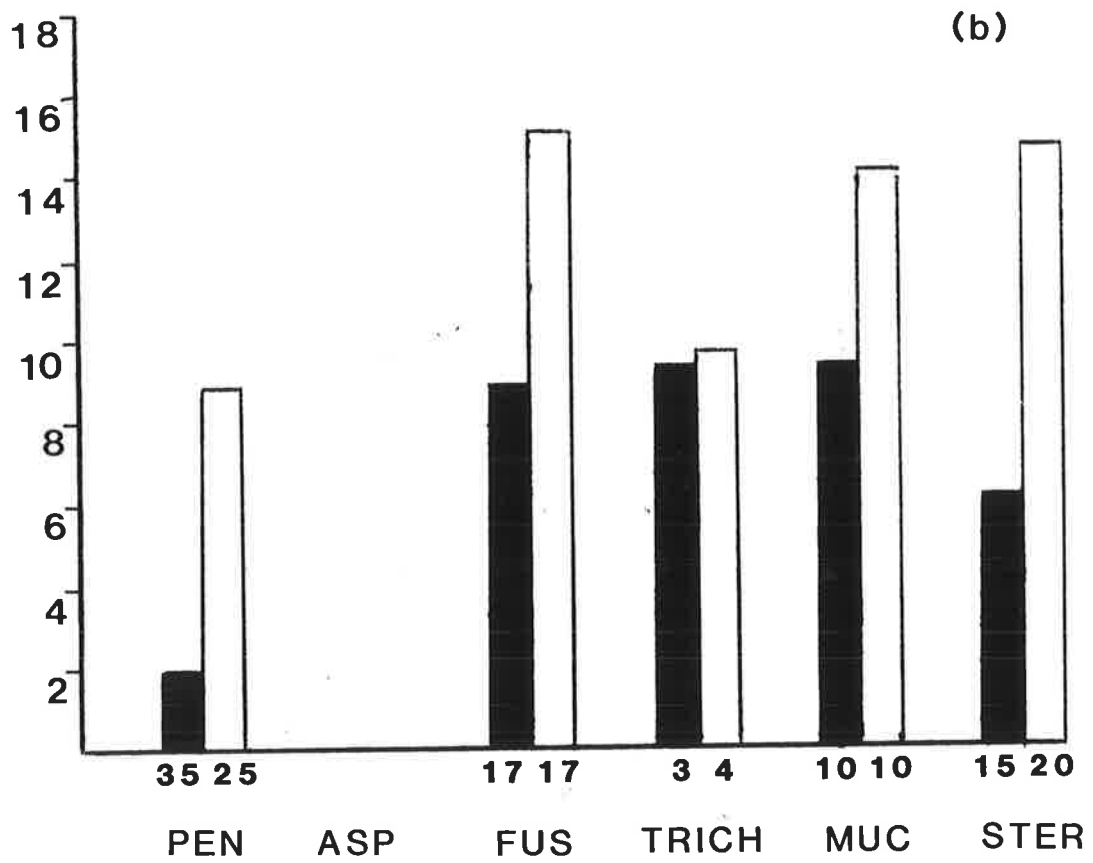
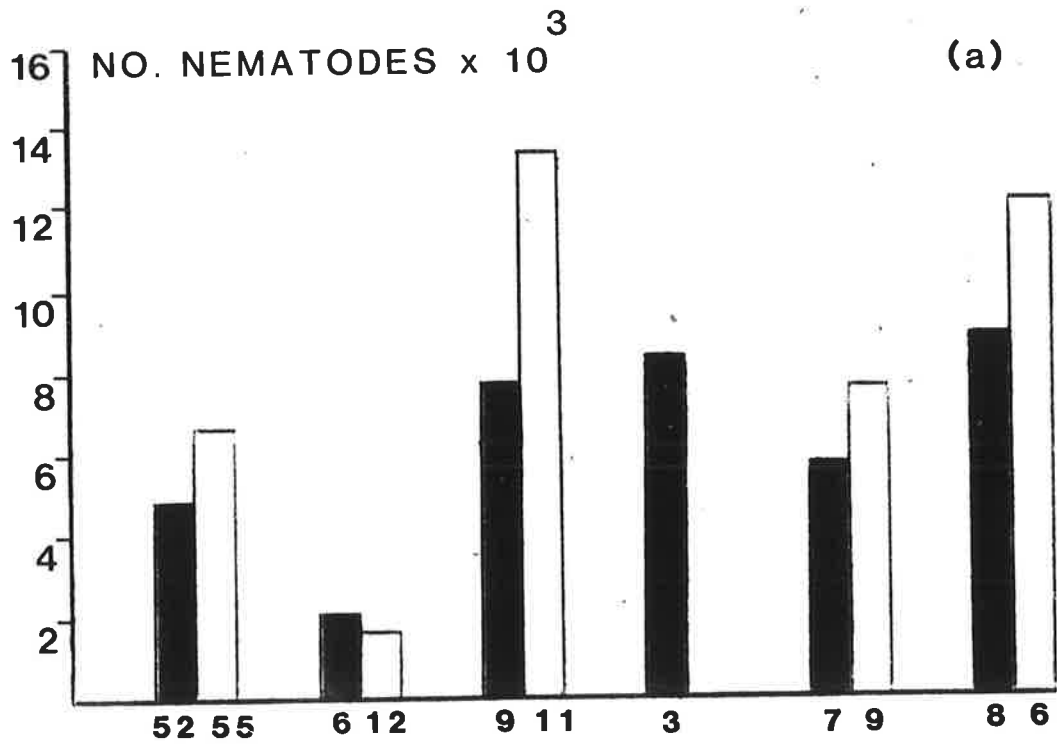
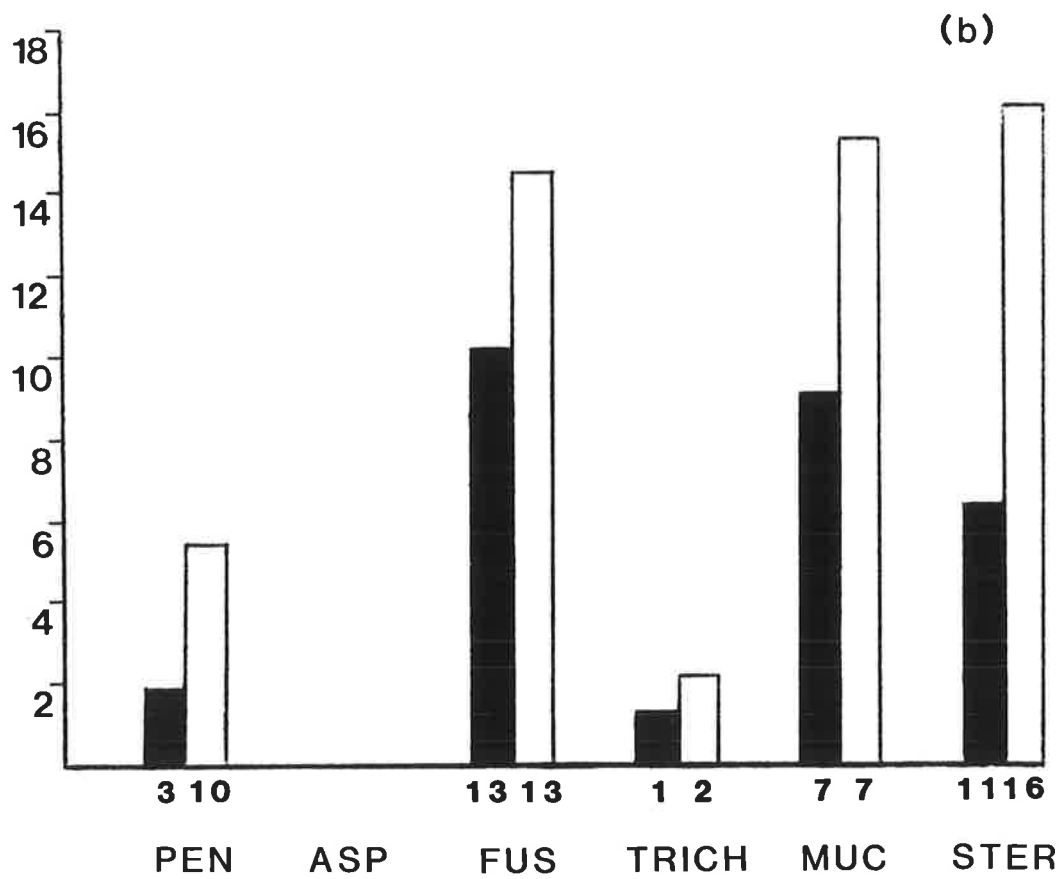
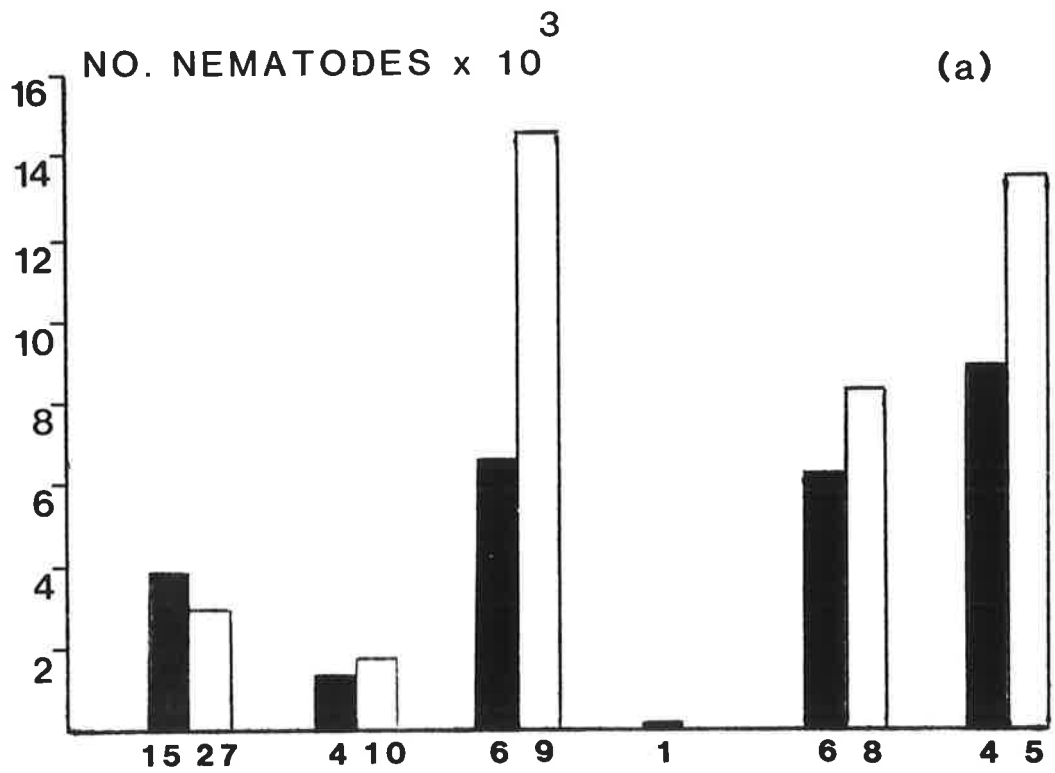


FIG. 20: Mean numbers of pine-forest soil (shaded bars) and wheat-field soil (unshaded bars) isolates of *A. avenae* on common fungal genera and species groups from wheat-field soil. (Nematodes treated with chlorhexidine gluconate (a) and antibiotics (b)).





earlier, the wheat-field and pine-forest soils differed in the frequency with which many of these genera could be isolated by various methods. In particular, it appeared that *Penicillium* spp. were especially abundant in pine-forest soil compared with wheat-field soil. Mean rate of reproduction of *A. avenae* and, indeed, of all the nematodes on *Penicillium* isolates was comparatively low (Tables 28a and 29a). It appeared that *Penicillium* spp. were generally poor food-hosts of *A. avenae*. Other fungi which fell into this category were *Aspergillus*, *Gliocladium*, *Coniothyrium* and *Myrothecium*.

In contrast, those fungal groups which were isolated in significantly greater frequency from wheat-field soil - *Fusarium* and sterile fungi - tended to be more productive food-hosts of *A. avenae*. Other "good" hosts of the nematode included:

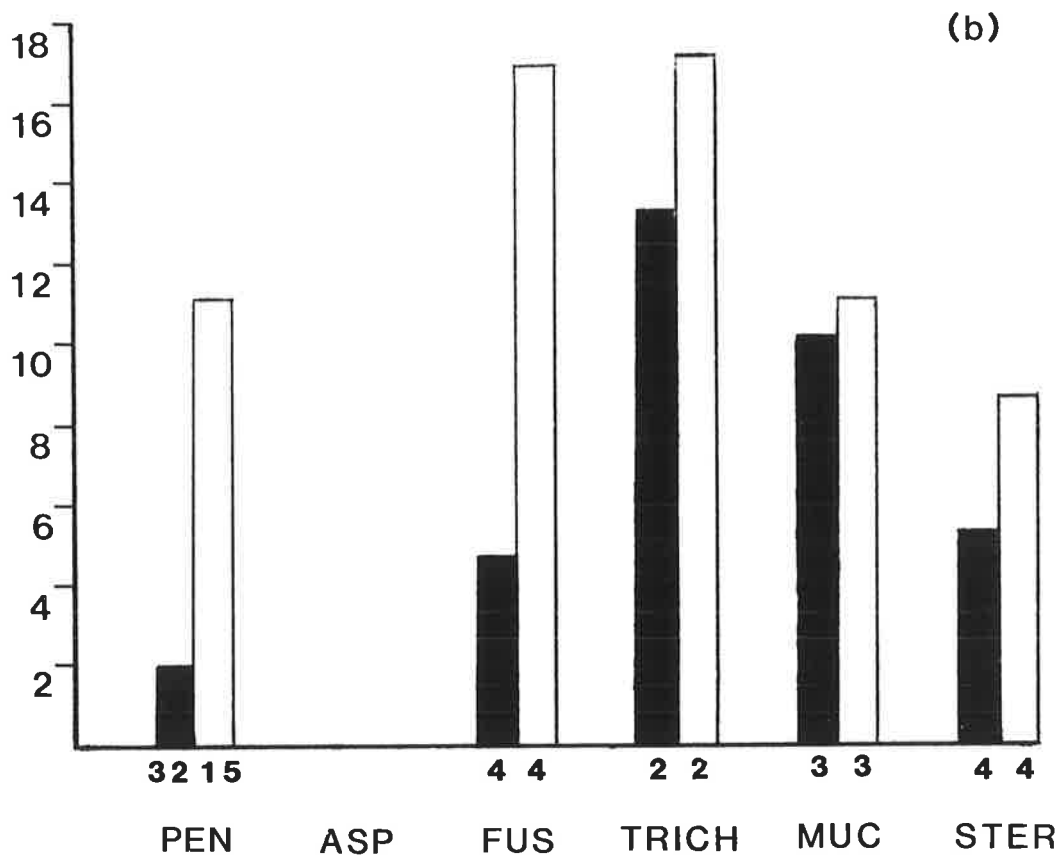
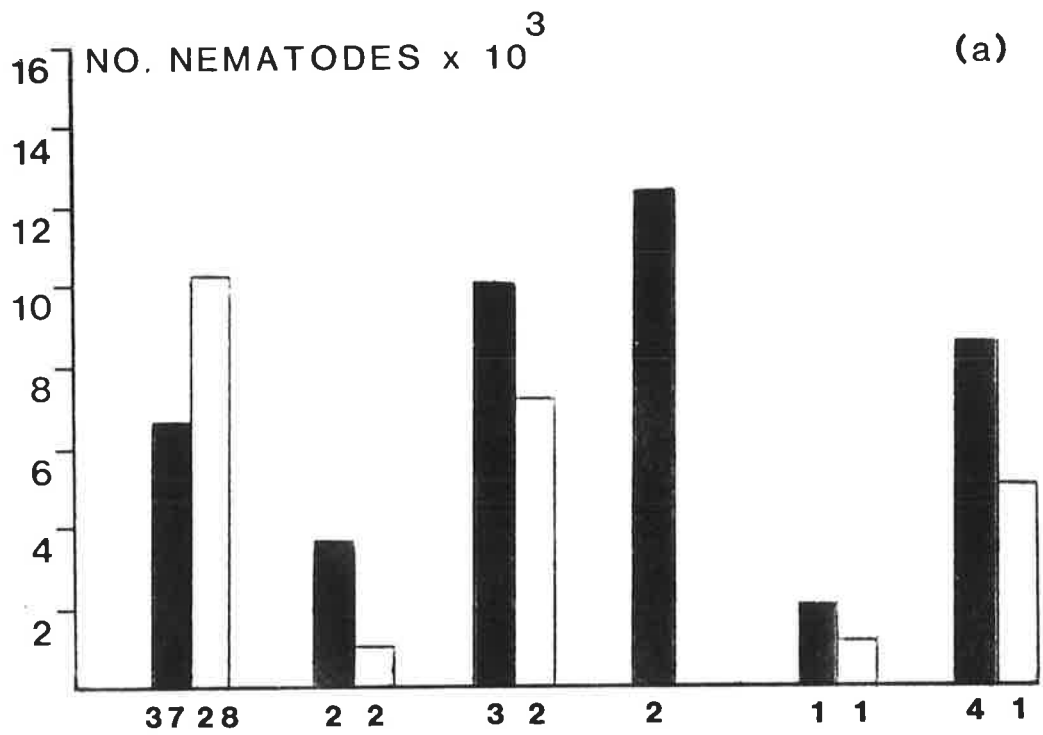
- (a) from pine-forest soil; *Botrytis*, *Mortierella*, *Paecilomyces*, *Pestalotia* and *Trichoderma*,
- (b) from wheat-field soil; *Acremonium*, *Broomella*, *Cochliobolus*, *Doratomyces*, *Graphium*, *Mortierella* and other Mucorales, *Paecilomyces*, *Periconia*, *Phoma*, *Torulomyces* and *Ulocladium* (Tables 28a and 29a). Concomitant, then, with the greater diversity of fungi found in wheat-field soil was a larger number of good food-hosts in that soil compared with the pine-forest soil. Mankau and Mankau (1963) and Townshend (1964) concluded that *A. avenae* exhibited a distinct preference for plant parasitic fungi but, as can be seen from the above, fungi which are presumed to be saprophytic (such as *Doratomyces*, *Graphium*, *Mortierella* and other Mucorales, *Paecilomyces*, *Periconia*, *Torulomyces* and *Trichoderma*) can serve adequately as hosts for

this nematode. Pine-forest *Trichoderma* isolates appeared to be more productive food-hosts for *A. avenae* than those from the wheat-field.

For many individual fungal genera and species groups, particularly those such as *Penicillium* in which it was possible to test a large number of isolates, variation in numbers of nematodes produced between both nematode isolates and fungal isolates was highly significant (Appendices 5 and 6). The interaction between the latter two terms was also often highly significant. It was concluded that within these fungal groups the nematode isolates differed in their rate of population growth, that some of the fungal isolates were better food-hosts for a given nematode than others and these also tended to be the same isolates that were better hosts for the other nematodes. Thus, fungal groups contained as much variability within themselves as between different groups in regard to suitability as food-hosts for these nematodes. This suggests that host suitability may depend on some property, such as some chemical factor, which varies below the generic and specific levels.

A similar pattern was seen with *A. avenae* treated with "Hibitane"; with the wheat-field soil isolate generally reaching higher populations than the pine-forest soil isolate and fungi such as *Penicillium* and *Aspergillus* being poor food-hosts in comparison with others such as *Fusarium* and sterile cultures (Figs. 19-21). Some apparent exceptions are seen in the case of certain fungi (other than *Penicillium*) from pine-forest soil (Fig. 21a), probably due to an insufficient number of isolates being tested. Populations of the *A. avenae* isolate from the *Suillus luteus* sporocarp were at least equal and, often, greater than those of the wheat-field soil isolate (Fig. 23a). The very low populations of the former isolate recorded on *Penicillium* and

FIG. 21: Mean numbers of pine-forest soil (shaded bars) and wheat-field soil (unshaded bars) isolates of *A. avenae* on common fungal genera and species groups from pine-forest soil. (Nematodes treated with chlorhexidine gluconate (a) and antibiotics (b)).



Mucorales reflect the fact that this isolate was tested upon only one isolate from each of these groups. With certain exceptions - *Aphelenchoides* isolates L and B on *Chaetomium* and *Sporotrichum* (?), and on *Broomella* respectively - the *Aphelenchoides* isolates produced lower numbers than *A. avenae* on fungi from both wheat-field and pine-forest soils (Figs. 22, 23b). The pattern of host suitability observed for the *Aphelenchoides* isolates was similar to that seen for *A. avenae*. Thus, *Penicillium* and *Aspergillus* were also poor food-hosts for the *Aphelenchoides* isolates while *Fusarium* and sterile cultures were more productive hosts (Fig. 23b). The most productive hosts for *A. avenae* were, firstly from pine-forest soil: *Botrytis*, *Fusarium*, *Pestalotia*, *Trichoderma* and *Verticillium* (Fig. 22a), and, secondly, from wheat-field soil: *Fusarium*, *Mortierella* and other Mucorales, *Phoma*, sterile cultures and *Ulocladium* (Fig. 22b). The mean population of the pine-forest soil isolate of *A. avenae* on five basidiomycetes from the pine-forest was comparatively low (Fig. 22a). Of these, *Suillus luteus* was by far the most productive host, yielding a mean population of 10,549 per Petri dish. Numbers of the *Aphelenchoides* isolate from the L-litter layer produced on these basidiomycetes, apart from *Suillus luteus* (mean population 13,075), were also very low.

Comparison of results with those of previous studies is frequently not possible as different conditions were used as well as different nematode and fungal isolates. Evans (1968) used an initial inoculum of five adult female *A. avenae* and media which were similarly "rich" in nutrients as the one used in this study. He obtained highest numbers of nematodes, 14,000 to 21,000 after two weeks at 25°C, with an isolate of *Rhizoctonia solani*. The isolate of *R. solani sensu lato* used in the present study was found to be a poor host for *A. avenae*, but marked differences in the suitability of

FIG. 22:

- (a) Mean numbers of pine-forest soil *A. avenae* (shaded bars) and *Aphelenchoides* from L-litter layer (unshaded bars) on fungal genera and species groups from pine-forest soil. Initial inoculum treated with chlorhexidine gluconate.

PEN = *Penicillium*; PAE = *Paecilomyces*; ASP = *Aspergillus*; FUS = *Fusarium*; TRI = *Trichoderma*; VER = *Verticillium*; BOT = *Botrytis*; MUC = *Mucor*; PES = *Pestalotia*; P16 = unknown Hyphomycete bearing conidia; BAS = Basidiomycetes; CHA = *Chaetomium*; SPO = *Sporotrichum* (?); PYT = *Pythium*; STE = sterile cultures; TOT = total.

- (b) Mean number of wheat-field soil *A. avenae* (shaded bars) and *Aphelenchoides* isolate from wheat-field soil, Quadrat B (unshaded bars) on fungal genera and species groups from wheat-field soil. Initial inoculum treated with chlorhexidine gluconate.

Fungal groups as above except for: MUC - Mucorales other than *Mortierella*; MOR = *Mortierella*; GLI = *Gliocladium*; CLA = *Cladosporium*; PHO = *Phoma*; ULO = *Ulocladium*; BRO = *Broomella*; TOR = *Torulomyces*; MYR = *Myrothecium*; MET = *Metarrhizium*; PHI = *Phialocephala*; ACR = *Acremonium*; RHI = *Rhizoctonia*; ALT = *Alternaria*.

Question marks indicate isolates not tested on those fungal groups.

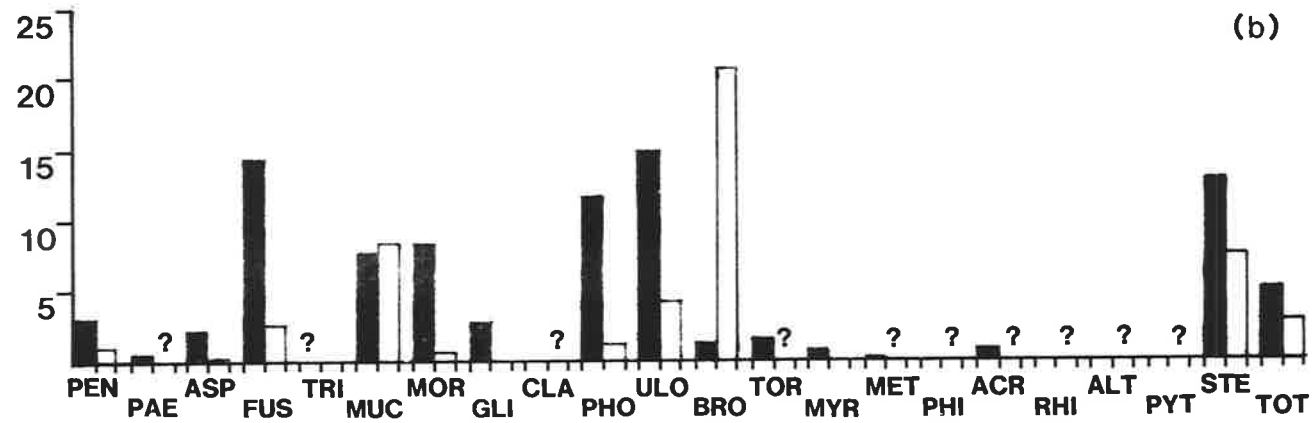
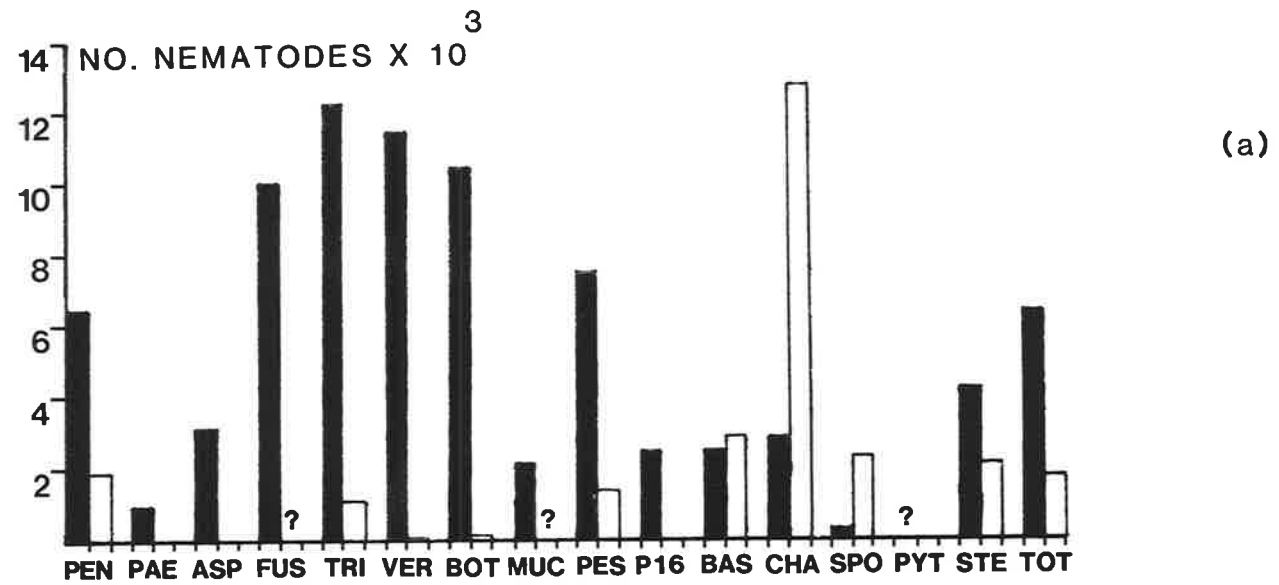


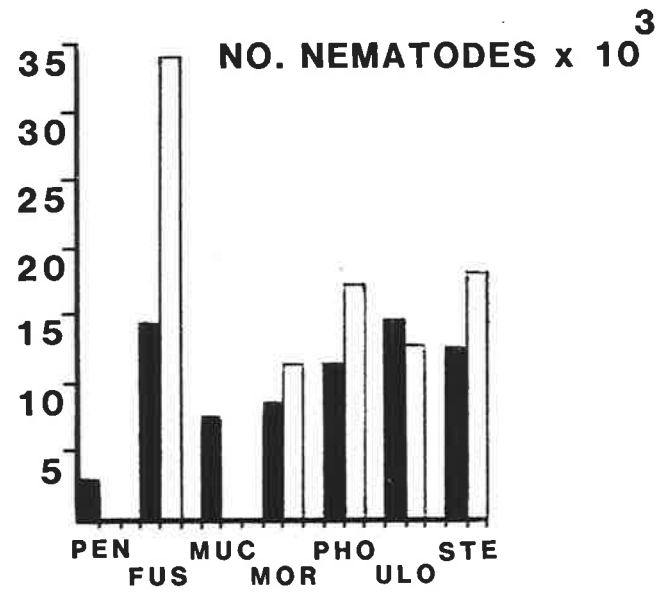
FIG. 23:

- (a) Mean numbers of wheat-field soil *A. avenae* (shaded bars) and *A. avenae* isolate from *Suillus luteus* sporocarp from pine-forest (unshaded bars) on wheat-field soil fungi. Initial inoculum treated with chlorhexidine gluconate.
- (b) Mean numbers of *Aphelenchoides* isolates on wheat-field soil fungi. Initial inoculum treated with chlorhexidine gluconate.

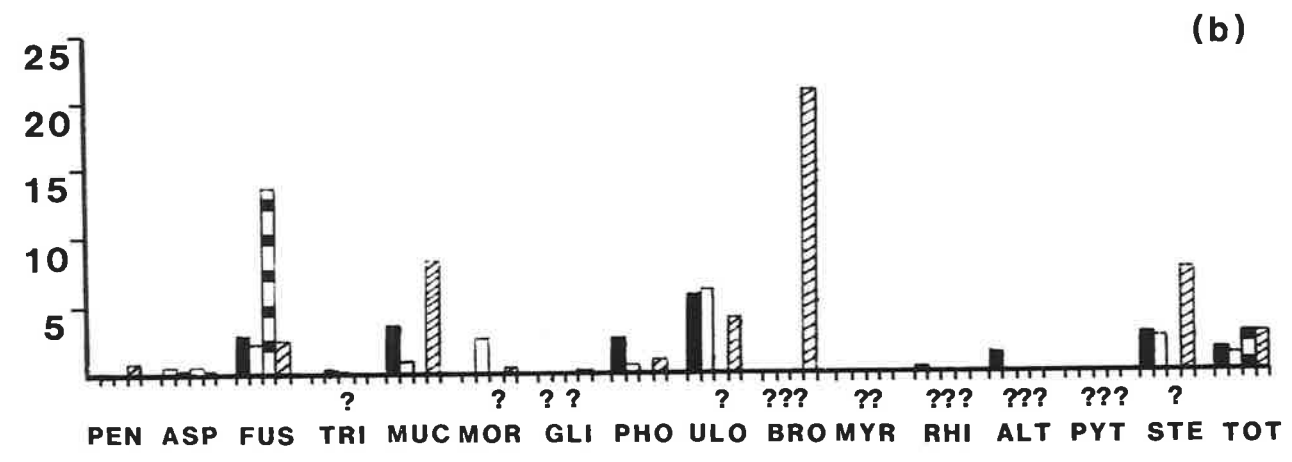
Shaded bars = L-litter layer isolate; unshaded bars = pine-forest soil, Quadrat C isolate; bars with shaded squares = isolate from *Rhizopogon luteolus* sporocarp from pine-forest; bars with cross-hatching = isolate from wheat-field soil, Quadrat B.

Fungal groups for both Figs. as in Fig. 22b.  
(Note: MUC = Mucorales).





(a)



(b)

*R. solani* isolates as hosts for *A. avenae* have been noted previously (Barker, 1964). Numbers of *A. avenae* on certain other fungi in this study matched and exceeded those observed by Evans (1968).

Isolates of the *Aspergillus niger* species group from both wheat-field and pine-forest did not support any population growth of either *A. avenae* or any of the *Aphelenchoides* isolates tested and few living nematodes were recovered from such cultures. Mankau (1969a,b) found that an isolate of this fungus was toxic to *A. avenae* and determined that this toxicity was due to oxalic acid produced by the fungus. Results reported here suggest that such toxicity may be widespread amongst isolates of these fungi.

Mankau and Mankau (1963) found that the pythiaceae fungi they tested were poor hosts for *A. avenae*. *Pythium* spp. (*P. acanthicum*, *P. afertile* species group, *P. irregulare*, *P. monospermum* and *P. paroecandrum*) tested here were similarly poor hosts for *A. avenae*. However, the large numbers of nematodes produced on many isolates of *Mortierella* demonstrated that not all pythiaceae fungi are such poor hosts.

Highest numbers of *A. avenae* were produced on a *Botrytis* sp. isolated from pine-forest soil. Of the fungi used to rear *A. avenae* by Townshend (1964), highest numbers were also produced on *Botrytis* spp..

Evans (1968) observed that the ratio of adults to larvae of *A. avenae* in culture underwent periodic fluctuations over time and interpreted this periodicity as reflecting the generation time of the nematode under the conditions given. He also noted significant variation in the adult/larvae ratio of *A. avenae* reared on several different host fungi and, in particular, observed the lowest ratio from the host which produced the highest numbers of nematodes. It was therefore of interest to determine

whether the adult/larvae ratio of the various nematodes here used varied significantly between host fungi and whether a relationship existed between the ratio and host suitability over the much larger number of fungi from the present study.

For this purpose, the data for antibiotic-treated nematodes reared on pine-forest fungi were chosen as a representative sample. The parameter analysed was the percentage of the population made up by adult nematodes at harvest (number of adult nematodes/total number of nematodes x 100%). An angular transformation was applied to this data and analyses of variance performed both for individual fungal groups (Appendix 7a-1) and for the data overall (Tables 30, 31).

Variation in % adults produced between both nematode isolates and fungal groups was highly significant, as was the interaction between the latter two variables ( $P = 0.001$ ; Table 31). It was concluded that there was significant variation in % adults produced both between nematode isolates and between fungal groups and that fungi which produced high (or low) % adults for one nematode tended also to produce high (or low) % adults for the other nematodes. Analyses of data for individual fungal groups showed that these sources of variation were equally significant for many groups between different fungal isolates (Appendix 7). *Penicillium* and *Verticillium* had the highest mean values of % adults over all nematode isolates. Mean % adults over all fungi was particularly high for the *Aphelenchoides* isolate from Quadrat C of the pine-forest (Table 30), a result which receives comment in the regression analysis below.

To determine whether a relationship existed between % adults and size of the population on different fungi, regression analysis was applied to (untransformed) data for several antibiotic-treated nematodes on pine-forest fungi. In all cases, there appeared to be a significant inverse

TABLE 30: Mean % adults of nematode isolates at harvest on fungal genera and species groups from pine-forest soil

Fungi	<i>A. avenae</i>			<i>Aphelenchoides</i> isolates				Total
	P	W	S	L	C	B	H	
<i>Penicillium</i>	25.57	19.78	22.28	32.64	76.03	100.00	-	31.45
<i>Paecilomyces</i>	18.62	13.95	-	32.17	13.39	28.88	-	18.76
<i>Trichoderma</i>	7.60	17.92	-	25.07	14.49	15.51	-	15.64
<i>Mortierella</i>	5.86	13.77	12.56	-	48.62	23.59	-	19.31
<i>Mucor</i>	7.78	14.17	-	-	-	-	-	9.70
<i>Fusarium</i>	16.18	15.50	38.33	19.85	28.80	17.96	-	18.95
<i>Verticillium</i>	22.14	25.84	-	18.92	100.00	41.13	-	38.45
<i>Pestalotia</i>	10.26	17.03	-	23.18	38.12	-	71.42	26.42
<i>Botrytis</i>	10.05	14.56	-	-	-	-	55.94	22.09
<i>Chaetomium</i>	8.64	7.34	-	-	-	-	-	8.08
Sterile fungi	15.69	19.53	25.14	31.54	60.91	10.38	-	25.31
P16*	13.03	7.51	9.95	-	100.00	-	-	24.95
Total	19.93	17.69	21.55	29.93	58.49	23.18	63.68	26.67

\* Unknown Hyphomycete bearing conidia.

TABLE 31: Analysis of variance of data as summarized in Table 30.  
Before analysis an angular transformation was applied to  
the data

Source of variation	df	Sum of squares	Mean square	F
Fungal genera	11	19830.7	1802.8	12.25 ***
Nematode isolates	6	85153.9	14192.3	96.41 ***
Interaction	36	37190.2	1033.1	7.02 ***
Residual	703	103484.4	147.2	
Total	756	245659.2		

\*\*\*F-value significant at  $P = 0.001$ .

relationship between the size of the nematode population achieved on different fungi in culture and the proportion of that population made up by adults (Table 32). The regression coefficient for the *Aphelenchoides* isolate from Quadrat C of the pine-forest was significantly different from those of all other nematodes examined (Table 32). The value of  $a$  (where the regression equation is in the form  $y = a + bx$ ) for this nematode was also much higher than for the others, reflecting the many fungal isolates upon which little or no reproduction of this nematode occurred such that the % adults approached the initial value (100%). On other fungi, reproduction was comparatively rapid and many larvae were produced; these factors combined to produce a significantly higher negative regression coefficient for this nematode. This result suggests that the *Aphelenchoides* sp. in question is comparatively more specialized in the range of fungal hosts it utilizes than either the other *Aphelenchoides* sp. from pine-litter or *A. avenae*. This apparent restriction in "niche breadth" could also be associated with utilization of food resources other than fungi by the particular *Aphelenchoides* sp.. That is, in comparison with the other nematodes, this species may be a facultative fungal feeder, utilizing other food resources such as plant roots while remaining only partially adapted to mycophagy upon a limited range of fungal hosts. This hypothesis will require further testing, in particular, the elucidation of alternative food sources.

Regression coefficients for the pine-forest and wheat-field soil isolates of *A. avenae* were not significantly different but the  $a$ -value was somewhat higher for the wheat-field isolate. This probably reflects the higher mean populations observed for this isolate as % adults for the two isolates were comparable (Table 30).

TABLE 32: Fitted regression lines for % adults on total population of various nematodes reared in agar cultures on (n) fungal isolates from pine-forest soil

Nematode isolate	n	Fitted Regression Line	Regression Coefficient $\pm$ S.E.
<u>A. avenae:</u>			
Pine-forest soil	54	$y = 27.06 - 0.0016 x$	$-0.00158^a \pm 0.00023$ ***
Wheat-field soil	38	$y = 35.19 - 0.0013 x$	$-0.00134^a \pm 0.00019$ ***
<u>Aphelenchoides:</u>			
L-litter layer	29	$y = 37.82 - 0.0020 x$	$-0.00196^a \pm 0.00506$ ***
Pine-forest soil (Quadrat C)	32	$y = 77.71 - 0.0412 x$	$-0.04124^b \pm 0.00538$ ***

a,b Regression coefficients with different letters are significantly different at  $P = 0.001$ .  
 Statistical methods as used by Bailey (1959); t-test with pooled sums of squares used where  $n < 30$   
 except when variance ratio significant at  $P = 0.05$ .

\*\*\* Regression coefficient significantly different from zero at  $P = 0.001$ .

The finding of periodic fluctuations in the ratio of adults to larvae of *A. avenae* reared on fungi by Evans (1968) suggests particular caution needs to be taken in comparing results for different fungi at a single harvest. However, it appears from the present study that results for different fungal groups are sufficiently consistent for valid comparisons to be made.



## VI. DISCUSSION AND CONCLUSIONS

Intraspecific variation between parthenogenetic populations of *A. avenae* from South Australian wheat-field soils was observed in karyotype and in the frequency with which males are produced at high temperatures. There appeared to be a distinct geographical distribution of populations with  $n = 8$  and  $n = 9$  chromosomes whereas the distribution of populations producing numerous males at high temperatures appeared to be haphazard; populations (and, perhaps, individuals within those populations) varied markedly in this capacity over the course of a few kilometres. Triantaphyllou and Fisher (1976) noted that the Californian population they examined with  $n = 9$  chromosomes produced numerous males at high temperatures whereas an Australian population with  $n = 8$  chromosomes did not. Results presented here suggest that these two traits are not causally related (that is, populations with  $n = 9$  chromosomes may or may not produce numerous males at high temperatures). The adaptive significance, if any, of these differences in karyotype and propensity to produce males, is not clear and such differences do not appear to be correlated with any obvious environmental variables. Gradual increases in chromosome numbers are thought to have taken place in other genera of plant parasitic nematodes through fragmentation of chromosomes or addition of whole chromosomes (Triantaphyllou and Hirschmann, 1980).

Intraspecific variation was also observed in rates of egg-laying and in rates of population growth on fungi in monoxenic cultures. Phenotypic differences observed between individuals of parthenogenetic animals are usually interpreted as indicating an underlying genetic diversity in the form of distinct clones which may or may not be adapted to different environments (for example, in earthworms - Jaenike *et al.*, 1980; spear-winged flies - Ochman *et al.*, 1980; and in the hybridogenetic fish *Poeciliopsis* -

Angus, 1980). Results presented here for *A. avenae* collected from a pine-forest indicate that major differences in phenotype (in rates of reproduction on fungi in monoxenic culture) may occur between individuals from within a three-hectare area. This suggests that such an area may encompass several clones or that several clones may co-exist at the one site, the clonal composition and frequency with which individual clones occur perhaps varying between sites as has been found for other parthenogenetic animals (Angus, 1980; Jaenike *et al.*, 1980; Ochman *et al.*, 1980). However, some phenotypic variation may possibly result from other mechanisms. In particular, the apparently haphazard distribution of populations and/or individuals with a propensity to produce males at high temperatures could conceivably arise through the action of transposable elements (that is, segments of deoxyribonucleic acid which can shift between *loci* within the genome and affect the expression of neighbouring structural genes). It has been suggested that unstable mutants observed in some eukaryotes may result from the action of such elements (Calos and Miller, 1980).

Evolutionary development, then, in *A. avenae* has not been brought to a standstill by the adoption of parthenogenesis as a mode of reproduction. Parthenogenesis in this nematode is automictic, that is, meiosis is retained (Triantaphyllou and Fisher, 1976). The genetic consequences of automixis depend upon the manner in which diploidy is restored and the pattern of chiasma localization (White, 1973; Maynard Smith, 1978). In *A. avenae* the second reduction division is suppressed such that a second polar body is not formed and it appears that diploidy may be restored by inclusion of both groups of chromosomes at telophase II in the same egg nucleus (Triantaphyllou and Fisher, 1976). Such a restitution nucleus would be formed by duplication of the chromatids which have separated in the egg cell,

a process genetically equivalent to fusion of sister pronuclei and expected to produce homozygosity for all *loci* between the centromere and the first chiasma of each chromosome (White, 1973; Maynard Smith, 1978). Triantaphyllou and Fisher (1976) found no evidence of centromeres on *A. avenae* chromosomes and indicated that a diffuse-kinetochore activity along the entire central euchromatic region of the chromosomes was possible. Such a modification could act to limit the tendency towards homozygosity, preserve existing heterozygosity over much of the length of the chromosomes and facilitate the genetic diversity apparent in this study generated by new mutations.

At 25°C, rates of egg-laying by parthenogenetic females of the Brownhill Creek isolate were equal to those of amphimictic females. Thus, a twofold advantage in reproductive rate (Maynard Smith, 1978) might be anticipated for the parthenogenetic isolate, although this would be partially offset by the higher initial rate of hatch of eggs observed for the amphimictic isolate at this temperature. However, the amphimictic isolate was not selectively displaced by the parthenogenetic isolate when inoculated together onto cultures. That is, a net advantage in reproductive rate was not observed for the parthenogenetic isolate in the presence of the amphimictic isolate. This may have been due to a reduction in fecundity and longevity of parthenogenetic females in the presence of amphimictic males (Fisher, 1972) or other factors (such as differences in ages of maturation of females between the two isolates) may have acted to obscure any potential differences in reproductive rate. In any case, these results suggest that the amphimictic form of *A. avenae* may be able to maintain itself in nature in the presence of parthenogenetic competitors.

The question then arises as to the reasons for the more widespread distribution of parthenogenetic compared with amphimictic forms of *A. avenae*. Parthenogenetic animals have often been observed to be more widespread in distribution than their sexual progenitors (Cuellar, 1977; Suomalainen *et al.*, 1979). Triantaphyllou and Hirschmann (1980) noted this in the case of certain plant parasitic nematodes, especially root-knot nematodes and believed that it reflected adaptation to environments of continuous monoculture of susceptible crop plants. Parthenogenetic animals are also widely known to be associated with newly created or disturbed habitats (Cuellar, 1977). In this study the parthenogenetic form of *A. avenae* was found to be abundant in soil under wheat in fields given over to rotation cereal cultivation. Significantly, the highest abundance of the nematode was recorded from a wheat-field which had been put under cultivation for the first time. *A. avenae* was far more abundant in these wheat-fields than has been found in less disturbed agricultural habitats such as permanent pasture (for example, the Waite permanent pasture in this study and pastures studied by Yeates (1981)) or the pine-forest of this study. Disturbed agricultural habitats are likely to be characterized by sudden declines in food resources and those organisms which utilize them. For example, in South Australia following harvest of wheat the soil remains fallow during the hot, dry summer and fungal activity - that is, abundance of viable hyphae and spores - declines markedly (Warcup, 1957). An organism such as *A. avenae* which depends upon fungal protoplasm from living hyphae will then face a food shortage if it is not limited before by the lack of soil water. A proportion of the population will survive over summer in a quiescent anhydrobiotic state (De Meure *et al.*, 1978) but we would expect the surviving population to be comparatively sparse on arrival of suitable conditions - rain and the planting of another crop - for population growth.

Under these conditions the ability of a parthenogenetic organism to establish a population from a single individual will be important. For *A. avenae* this is more likely since many soil fungi (particularly members of the Deutermycotina) appear to be active in soil over very short periods in restricted areas (Warcup, 1957). The preference of this nematode for plant parasitic over saprophytic fungi (Mankau and Mankau, 1963; Townshend, 1964) may reflect a partial specialization towards fungi which, through their parasitic mode of nutrition, have longer periods of activity in the mycelial state in the soil. However, soil fungi known to have long-lived mycelium in soil (Burgess, 1960) such as the basidiomycetes tested as food sources for *A. avenae* in this study did not appear to be particularly favoured hosts for the nematode. An ability to establish a population from a single individual would also be important for the spread of an organism which is dispersed passively by wind, particularly in an agricultural environment. Viglierchio and Schmitt (1981), in examining the nematodes recovered from dust raised by agricultural implements traversing dry fields and further carried by wind, found that *A. avenae* comprised over 29% on average of all living nematodes from such dust. The ability of *A. avenae* to undergo anhydrobiosis and adopt a tightly coiled form of decreased weight undoubtedly assists with this dispersal.

Gerritsen (1980) considered the problem of sexual reproduction in sparse populations entailed in finding a mate and developed a model to predict the critical density for successful sexual reproduction (that is, that required to sustain the population) in zooplankton. He was able to make a number of predictions from this model, certain of which can be tested against the data available for plant parasitic nematodes. Thus, encounter probability (between mates) is correlated with size of the organism and an

inverse relationship between size and critical density is predicted such that there may be a minimum size for obligate outbreeders. Using data published by the Commonwealth Institute of Helminthology (1972-77), plant parasitic nematodes can be classified according to maximum length of adult females and to breeding system (Table 33). Nematodes for which insufficient information were available were not included, as were those species whose adult females are sedentary since the model assumes both sexes are vagile. There is a significant association between these classifications and sexual reproduction is less common than expected due to chance amongst the smaller nematodes (Table 33). Of the species listed by the Commonwealth Institute of Helminthology with maximum length of adult females less than or equal to 800  $\mu\text{m}$ , obligate amphimicts comprised only 33% while parthenogenetic species made up 61%. Thus, plant parasitic nematodes appear to conform to the predictions of the above model, lending support to the hypothesis that parthenogenesis is advantageous to nematodes in disturbed habitats where the problems associated with sparse populations periodically occur. Unfortunately, not enough is known regarding the ecological correlates of different breeding systems in *A. avenae* to determine whether the amphimictic form tends to be more commonly found in less disturbed habitats than the parthenogenetic form. Certainly, the amphimictic population from Dangerous Reef occurred in a natural environment.

In wheat-field soil, abundance of *A. avenae* appeared to be correlated with a number of physico-chemical properties of the soil. For example, the nematode tended to be more abundant in more alkaline soils. Tests to examine the influence of these factors on population growth of *A. avenae* in culture revealed nothing of the nature of these correlations. Thus, higher populations of the nematode were produced on the more acid media which

TABLE 33: Association between size of plant parasitic nematodes and breeding system ( $\chi^2 = 6.96$ , significant at  $P = 0.05$ ). Data from Commonwealth Institute of Helminthology (1972-77). Expected frequencies in brackets.

Breeding System	200-600 $\mu$ m	600-1000 $\mu$ m	>1000 $\mu$ m	Total
Parthenogenetic (obligate and facultative)	11 (7.09)	16 (16.36)	9 (12.55)	36
Amphimictic	2 (5.91)	14 (13.64)	14 (10.46)	30
Total	13	30	23	66

supported a higher rate of growth of the host fungus. Although *A. avenae* tended to be relatively more abundant in more saline soils, in culture small increases in salinity of the culture medium tended to reduce the rate of growth of both the host fungus and the nematode population. Changes to composition of the culture medium appeared to affect population growth of the nematode through the host fungus. As expected, temperature appeared to exert a direct effect on the reproductive rate of the nematode in culture. These results, then, seem to point to marked differences between the soil environment and that provided in monoxenic cultures. In addition to the greater uniformity of the latter environment, in the soil physico-chemical factors could influence and be influenced by abundance of fungi and other biota such as predators and competitors of *A. avenae*.

In the pine-forest here studied, *A. avenae* was rarely found in soil. Other studies on the soil nematodes of coniferous forests have either not recorded *A. avenae* at all or only rarely (Bassus, 1962; Riffle, 1968; Boag, 1974; Sohlenius *et al.*, 1977; Magnusson, 1983a,b). In the Williamstown pine-forest a higher abundance of *A. avenae* was observed in certain more densely grassed areas, suggesting that the rarity of the nematode was more to do with a lack of suitable food sources - perhaps fungi associated with graminaceous plants - than with some other aspect of this environment such as the acid conditions which characterize coniferous forest soils. Yeates (1979) noted that these acid conditions were inimical to certain organisms such as lumbricid earthworms, millipedes and woodlice, but that fungal-feeding Tylenchida flourished in coniferous forest soils. Thus, nematodes which were, at least partially mycophagous and belonging to a closely related genus - *Aphelenchoides* - were comparatively abundant in the pine-forest, particularly in litter. Bassus (1962), Riffle (1968),



Boag (1974), Sohlenius *et al.* (1977) and Magnusson (1983a,b) also found *Aphelenchoides* spp. to be abundant in the coniferous forests they studied. These findings suggest the possibility of host specialization or competitive interactions between *A. avenae* and these nematodes.

Soils of the two study sites - the wheat-field and the pine-forest - differed markedly in texture, that of the wheat-field being classed as a clay-loam while that of the pine-forest being a sandy-loam. Correlations between soil type or texture and abundance of various nematodes have often been suggested by nematologists (see Wallace, 1973; Norton, 1978). Norton *et al.* (1971) found that *A. avenae* made up a higher proportion of the total nematode population and was more abundant in sandy-loam than in clay-loam soils, while Geraert (1967) concluded that it showed no preference for any soil type (sandy vs. heavy soils). It appears unlikely that the differences between the two study sites in soil texture were themselves responsible for the marked difference in abundance of *A. avenae*, particularly since the highest abundance was observed in the clay-loam soil. Of more potential importance to population densities of *A. avenae* between the two sites are differences in management practices and of rates of decay of organic material. Wallwork (1976) noted that absence of permanent plant cover or leaf litter (as in a field in fallow) and mechanical disturbance of the substratum caused by ploughing exposes the soil to marked fluctuations in temperature and moisture, producing high mortality among soil animals and leading to a reduction in species diversity. He observed that Collembola and Acari were particularly reduced in densities by such disturbance. Many collembolans and oribatid mites are known to feed on soil fungi (Griffin, 1972), and certain mites also feed on nematodes including *A. avenae* (Imbriani and Mankau, 1983). Thus, disturbed soil, such as that of the wheat-field, may harbour smaller and less diverse populations of

of important competitors and predators of *A. avenae* than pine-forest soil. Crop rotations also decrease species diversity of soil fauna (Wallwork, 1976). In comparing forest and grassland ecosystems, Wallwork (1976) observed that the latter was characterized by *in situ* decomposition of root systems and by higher rates of energy turn-over and breakdown of organic material. Mor humus, because of its acidity and high content of lignin, is particularly slow to break down. The higher rate of breakdown of organic material in a wheat-field compared with a pine-forest could be important for both saprophytic fungi and plant parasitic fungi in the saprophytic phase of their life cycles. Thus, many important wheat pathogens such as *Gaeumannomyces graminis* and *Rhizoctonia solani* are often found associated with organic fragments in the soil (Griffin, 1972). As against these factors is the observation that the acid conditions prevailing in coniferous forest soils promote the growth of soil fungi, leading to a proliferation of fungal-feeding Tylenchida (Yeates, 1979). From the present study, results with monoxenic cultures indicated that more acid conditions which favoured the growth of the host fungus could result in a higher rate of population growth of *A. avenae*. Hence it is by no means clear, on a *priori* grounds, why *A. avenae* should be comparatively rare in pine-forest soil.

Results presented for fungi isolated from wheat-field and pine-forest soils using a variety of methods indicated that these soils possessed characteristic and different mycofloras. Although dilution and soil plate methods of fungal isolation mainly record species present in the soil as spores (Warcup, 1957), a similar picture emerged with isolations by selective methods and from roots and organic debris. The pine-forest soil was dominated by *Penicillium* spp. and *Pinus* roots and organic debris were also dominated by these fungi and *Trichoderma* spp.. The wheat-field soil was

characterized by a more diverse mycoflora. The frequency with which *Penicillium* and *Trichoderma* spp. are isolated from coniferous forest litter and soil by various methods has often been remarked upon (Kendrick, 1958; Simpson, 1967; Widden and Parkinson, 1973; Widden, 1979; Nelson, 1982). These fungi are heavy sporulators and the fact of their high incidence - on dilution plates, for example, in the case of *Penicillium* spp. - is indicative of fungal activity mainly in that spores are a product of past active growth (Nelson, 1982). Nevertheless, the qualitative differences between the mycofloras of the two soils are clear. The positive correlation between abundance of *A. avenae* and diversity of the soil mycoflora in the two habitats suggests that composition of the mycoflora is an important influence on abundance of this nematode. Basidiomycete hyphae were abundant in pine litter as were mycophagous *Aphelenchoides*, but *A. avenae* was correspondingly rare, suggesting possible competitive exclusion or host specialization between these nematodes.

The soil bacteria and actinomycetes tested in this study were found not to be significant as food sources for *A. avenae* and it appears likely that the nematode is unable to utilize them as such. The stylet lumens of tylenchid nematodes are usually less than 1  $\mu\text{m}$  in diameter and are regarded as bacterial filters (Bird, 1971). Scanning electron micrographs of the head region of *A. avenae* show that the oral aperture, through which the stylet passes, is about 0.3 to 0.4  $\mu\text{m}$  in diameter (Hooper and Clark, 1980). The meaning of Tikyani and Khera's (1969) observation that *A. avenae* "cultivated on an unidentified bacterium when transferred in masses" is not clear. In the case of actinomycete hyphae, which are usually 1  $\mu\text{m}$  or less in diameter (Waksman, 1950) it is difficult to see how the nematode could maintain contact with a hypha to facilitate feeding. Feeding on fungal hyphae by *A. avenae* appears to require sustained contact by

body pressure as the lips of the nematode are pressed against the cell wall (Fisher and Evans, 1967).

The results of feeding trials with fungi from both soils showed that different fungi differed markedly in their suitability as hosts for *A. avenae* as indicated by the nematode's rate of population growth.

*Penicillium* spp. - those fungi which had been found to dominate pine-forest soil - were comparatively poor hosts. A greater range of more productive hosts were available for mycophagous nematodes among the more diverse mycoflora of the wheat-field soil than from the pine-forest soil. It is suggested that this is a major factor in determining the higher population density of *A. avenae* in wheat-field soil. Other possible factors, such as different and/or more numerous predators and competitors for fungi in pine-forest soil can not, of course, be entirely discounted. Since many soil fungi, particularly members of the Deuteromycotina, are active in soil for only short periods (Warcup, 1957), a greater diversity of suitable hosts would provide for a more constant supply of food across space and time.

Fungi that were good hosts for *A. avenae* also tended to be good hosts for the *Aphelenchoides* spp. from this study. Thus, it appeared that little host specialization had taken place between these nematodes to preclude the possibility of competition for food resources. The observed rarity of *A. avenae* in pine litter compared with mycophagous *Aphelenchoides* may have more to do with the nature of this habitat than the fungi present. For example, the thin water-films which surround needles in the litter may favour movement of the smaller-sized *Aphelenchoides* as against *A. avenae*. Different isolates of fungi belonging to the same genus varied markedly in their capacities to support population growth of mycophagous nematodes, supporting the suggestion (Mankau and Mankau, 1963; Townshend, 1964) that host suitability may be determined by some specific factor such as compo-

sition of protoplasm or production of antibiotics. Such factors would explain the similarity in response of the different nematode species to different fungi. However, *A. avenae* appeared to be more efficient at converting fungal protoplasm into biomass than any of the *Aphelenchoides* isolates.

Evans and Fisher (1970) ascribed differences observed in reproduction rates of isolates of *A. avenae* cultured on fungi to metabolic differences inherent in the nematodes. This conclusion is supported by results presented here which show that even within a comparatively small area there exist *A. avenae* with markedly different reproduction rates. Evidently, mutation rates and the automictic mode of reproduction of *A. avenae* allow for some degree of genetic flexibility such that considerable genetic diversity can exist in a parameter as important for survival as reproduction rate. The extent of genetic diversity displayed by *A. avenae* suggests that conclusions regarding ecological differences between populations of parthenogenetic nematodes which have been based on observations on experimental populations derived from single individuals be treated with caution. A number of plant parasitic nematodes have automictic modes of reproduction, including certain *Meloidogyne* spp. (Triantaphyllou and Hirschmann, 1980) - although parthenogenesis is not obligatory in this genus - and may also possess mechanisms to preserve heterozygosity. Further work on the clonal structure of parthenogenetic populations of *A. avenae* will be needed to establish the spatial and temporal dimensions of such clones.

VII. APPENDICES

## APPENDIX 1:

## Formulae for media

NDY (Warcup, 1955)

NaNO <sub>3</sub>	2.0 g
KH <sub>2</sub> PO <sub>4</sub>	1.0 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5 g
KCl	0.5 g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.01g
Yeast extract (Difco)	0.5 g
Sucrose	30.0 g
Agar	15.0 g
Distilled water	1 L

(NDY/6 consists of these ingredients in amounts one-sixth of those given above except for agar and water which remain unchanged)

Krainsky's Medium ("Plant Pathologist's Pocketbook", Commonwealth Mycological Institute, 1968)

Glucose	10.0 g
Asparagin	0.5 g
K <sub>2</sub> HPO <sub>4</sub>	0.5 g
Agar	15.0 g
Distilled water	1 L

Nutrient Agar (based on formula from "Plant Pathologist's Pocketbook", Commonwealth Mycological Institute, 1968)

Nutrient broth (Bacto)	8.0 g
Yeast extract (Difco)	2.0 g
Peptone (Bacto)	5.0 g
NaCl	5.0 g
Agar (Bacto)	15.0 g
Distilled water	1 L

continued/...

2% Malt Extract Agar

Malt extract (Difco)	20 g
Agar	15 g
Distilled water	1 L

Proprietary media (Potato dextrose agar, cornmeal agar, lima bean agar) were prepared according to instructions given by the manufacturer (Difco).

APPENDIX 2: Size classes of *A. avenae* separated on sieves of various mesh sizes. Mean lengths are given for ten randomly selected nematodes from amongst those passing through and retained by sieves after various periods of time

<i>A. avenae</i> isolate (age of culture)	Nemas/trial	Width of openings ( $\mu\text{m}$ )	Time (min)	Mean length of nemas ( $\mu\text{m}$ ) $\pm$ S.E.	
				Passed	Retained
Isolate 34 (6 wks)	52500	45	1	241 $\pm$ 7	371 $\pm$ 52
			2	287 $\pm$ 34	692 $\pm$ 84
			3	281 $\pm$ 30	613 $\pm$ 85
			5	281 $\pm$ 20	536 $\pm$ 87
Nemas retained on 45 $\mu\text{m}$ -mesh after 1 min.	18600	150	1	359 $\pm$ 45	702 $\pm$ 100
			2	323 $\pm$ 25	824 $\pm$ 51
			3	294 $\pm$ 25	1063 $\pm$ 126
			5	613 $\pm$ 83	744 $\pm$ 87
		250	3	369 $\pm$ 50	719 $\pm$ 100
Isolate 52 (8 wks)	73000	355	1	652 $\pm$ 56	979 $\pm$ 49
			3	967 $\pm$ 84	1055 $\pm$ 84
		450	1	814 $\pm$ 94	999 $\pm$ 35
Nemas retained on 45 $\mu\text{m}$ - mesh after 1 min.	73000	45	1	490 $\pm$ 56	-
		355	1	679 $\pm$ 58	888 $\pm$ 43



APPENDIX 3: Fungi isolated from wheat-field soil on dilution plates (dp) and soil plates (sp). Isolations recorded for Quadrats A-L; multiple isolations denoted by figures in brackets

Isolate No.	Fungus	dp	sp
<u>Fusarium isolates</u>			
W1	<i>F. roseum</i> sp. gr.	H,J(2)	A,F,G(2),I,J,K
W3	<i>F. roseum</i> sp. gr.		K
W33		A,B,E(3),F(2),G(3), J,L(3)	G,J,L(2),A(2), E(2),F
W55		A(2),B,H(2),L(2)	A,G,I,J(2)
W64		G	E(2)
W84	<i>F. solani sensu lato</i>	B(2),D,L(4)	C,D,F,K
W85		A,D,F,G,I,K(2)	A,E,H(2),K
W90		F,G,H	K
W93		A	H
W94			E
W112	<i>F. merismoides</i>	F	
W121		D	
W125			H
W126		C	
W127			C
W129	<i>F. oxysporum sensu lato</i>	F	
<u>Mucorales</u>			
W11	<i>Rhizopus (oryzae?)</i>	L(2)	
W21	<i>Mucor</i>	A,C	H,I(5)
W61	<i>Mortierella</i>		G(5),L
W62	<i>Mortierella</i>	K	G(2),K(4)
W65	<i>Mortierella</i>	I	A,D,J
W66	<i>Mortierella</i>	I	
W67	<i>Mortierella</i>	A,D,F(2),G,I(2), J,K(2)	B,C,H,L
W86	<i>Mucor (globosum?)</i>		I
W88	<i>Gongronella butleri</i>	L	I
W89	<i>Actinomucor elegans</i>	J	I
W105	<i>Absidia spinosa</i>	C	
W130	<i>Cunninghamella (elegans?)</i>	B	G
W182	* <i>Mortierella</i>		
<u>Trichoderma isolates</u>			
W51	<i>T. harzianum</i>	D(3),L	B
W83	<i>T. viride</i>	I	

continued/...

## APPENDIX 3/continued

Isolate No.	Fungus	dp	sp
<u>Aspergillus isolates</u>			
W3		C,F	
W6	<i>A. niger</i> sp. gr.	D	D(9)
W8			L(2)
W15	<i>A. terreus</i>	D(2)	
W35		B(3),E,F(2),H(2) J(6),K(3)	B(2),H,J(2),K
W36		I	
W37			A
W38		A,B(2),D(2),F,J(3), K	D,J,K(2)
W40			B
W71		C	
W91		C	
W101		B	
W157		G	
W158			F
W167		J	
W172		H	
W189	*		
<u>Penicillium isolates</u>			
W4	+Fd	B,F,K	C,E,F(2)
W5	Bs	A,D,E,F(2)	F(3)
W7	Pp	D(3),G(2)	E,I
W23	Ae	A,B,J(2)	B,D(4),J
W24	Ff	D,J	A,B,C,L(5)
W25	Ff	B(2),C(4),D,J	D,J
W26	Ff		B
W27	Ae	C	
W28	Ff		C(5)
W29	Ae		D
W30	Ae		A
W31	Ff		C
W32	Ff	A(2),C	
W41	Pp	A(2),D,F(12),H,J(3) K(4)	A(2),D(3),H(2), J,K(3)
W42	Pp		H
W43	Ff	I	
W44	Fd	A	
W45	Fd		F
W46	Fd	D	
W47	Fd		I
W48	Ff	B,C(2),F,J	A,C,J
W56	Ff		F
W69	Fd	D,F(3),H(2)	D,F(3),K(2)
W70	Bs	C,H,J	H,J
W72	Ff	G(2)	
W73	Ff	B,D	E
W74	Bs	A	
W75	Ae	L	
W76	Ff	L	

continued/...

## APPENDIX 3/continued

Isolate No.	Fungus	dp	sp
W77	Ff		C,I
W78	Aa	F,G	F,J
W79	Ae		L(4)
W102	Ff		I
W108	Bs		A
W109	Bs		L
W113	Ae	I	
W114	Ae		H
W115	Ff		I
W116	Bs		K
W117	Pp	A	
W118	Ff	B	
W119	Bs		J
W122	Bs	I	
W123	Bs	K	
W124	Pp		E
W128	Fd		G(2)
W137	Ae	B,F,J(2)	A,B
W138	Pp		C
W139	Fd	D	
W140	Ff		C
W141	Fd		C
W142	Aa	B	
W144	Ff		C
W145	Ff	I	
W146	Ff	I	
W147	Ff	I	
W148	Ff	J	
W149	Fd	C	
W150	Ae		I
W151	Ae	B	
W152	Ae		K
W153	Bs	J	
W154	Aa	D,J	
W163	Bs	F	
W166	Ae	G	
W168	Ff	D	
W169	Fd	D	
W170	Ae	J(2)	
W171	Ae	L	
W185	*Ae		
W188	*Ff		
W10,W82	<i>Cladosporium cladosporioides</i>	G,L	C,H(5),I,L
W13	<i>Metarrhizium anisopliae</i> var. <i>anisopliae</i>		C,E,J(2)

continued/...

## APPENDIX 3/continued

Isolate No.	Fungus	dp	sp
<u>Myrothecium isolates</u>			
W14,W92	<i>M. verrucaria</i>	J,L(3)	
W95	<i>M. cinctum</i>	B	
W133	<i>M. roridum</i>	G	
W16	<i>Paecilomyces lilacinus</i>	E(2),G,I(2),K	B
<u>Torulomyces isolates</u>			
W17		A,J	A,J(2)
W18		B,K	
W19		C	B
W20	<i>Phialocephala</i>	C,E	
<u>Acremonium isolates</u>			
W22		D(2),J	A(3)
W155			J
W159		B	
<u>Gliocladium isolates</u>			
W34	<i>G. catenulatum</i>	B(3)	L
W173	<i>G. roseum</i>		I
<u>Phoma isolates</u>			
W57		J	
W58			A
W104	<i>P. eupyrena</i>	A	
W181	*		
W187	*		
W60	<i>Ulocladium</i>		C,G
W64a	<i>Pythium</i>	G	E
W68	<i>Broomella</i>	A(6),B(8),C(4), F(7),G(2),H(2), I,J(2)	A(4),B(3),C(3), E(2),F(2),H,K, L
W100	<i>Broomella</i>	J	
W131	<i>Pestalotia sensu lato</i>		E

continued/...

## APPENDIX 3/continued

Isolate No.	Fungus	dp	sp
W107	<i>Graphium (bulbicola?)</i>	I	I
W110	<i>Chaetomium indicum</i>	B	
W111	<i>Stachybotrys</i>	F	
W162	<i>Conidiobolus</i>	I	
W164	<i>Coniothyrium (?)</i>	E	
W165	<i>Doratomyces</i>		G
W179	* <i>Cochliobolus sativus</i>		
W193	* <i>Periconia macrospinoso</i>		

Sterile cultures

W49		D(2),H,I	A,D,L(2)
W50		F,K	B,F,G(2),H(3)
W52		C,D	
W53			A(2)
W54			G
W59		A,E	D(2)
W80			F
W81		I	
W87			G
W97		B	
W98		A	
W99			C
W103		D,E(2),G,H(2),K(2)	
		L	
W106			A
W120		D	
W132		H	
W134			E
W135			A
W136		A	
W143			E
W156		B	
W161			C
W174	*		
W175	*		
W176	*		

continued/...

## APPENDIX 3:/continued

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W177	*
W178	*
W180	*
W183	*
W184	*
W186	*
W190	*
W191	*
W192	*
W194	*
W195	*
W196	*

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Unidentified hyphomycetes

W9	I(3),L
W160	I

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W12	Coelomycete (unidentified)	L(2)
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\*Isolated from wheat roots

+Sub-generic classifications of *Penicillium* isolates after Pitt (1979). Subgenus *Aspergilloides*: Section *Aspergilloides* (Aa), Section *Exilicaulis* (Ae); subgenus *Penicillium*, Section *Penicillium* (Pp); subgenus *Biverticillium*, Section *Simplicia* (Bs); subgenus *Furcatum*, Section *Furcatum* (Ff), Section *Divaricatum* (Fd).

APPENDIX 4: Fungi isolated from pine-forest soil on dilution plates (dp) and soil plates (sp). Isolations recorded for Quadrats A-L; multiple isolations denoted by figures in brackets

Isolate No.	Fungus	dp	sp
<u>Fusarium isolates</u>			
P16		E	
P20			C
P22	* <i>F. solani sensu lato</i>		
P23	<i>F. oxysporum sensu lato</i>	B(2),H	B,K(2)
P24			A
P25			A
<u>Mucorales</u>			
P13	<i>Mortierella</i>		E
P26	<i>Mucor plumbeus</i>	F	H
P122	<i>Mortierella</i>	A,H	B,E,I,K
P123	<i>Mortierella</i>	D,F,H,J	K,L
P124	<i>Mortierella</i>	J	
<u>Trichoderma isolates</u>			
P5	<i>T. hamatum</i>	A,L	C
P21	<i>T. koningii</i>	B,G	G,L
P115	<i>T. harzianum</i>	A,E,H,I	H(2)
<u>Aspergillus isolates</u>			
P3	<i>A. niger</i> sp. gr.	E	
P32		A	
P117			A
<u>Penicillium isolates</u>			
P2	+Fd	K	
P6	Ae	H	
P9	Ae		I
P29	Bs	B	
P30	Ae	D	
P31	Aa	J	
P33	Ae		A(2)
P34	Fd	B	
P35	Ae		J
P36	Ff		B
P37	Ae	D,L	
P38	Fd	B,L	L
P39	Bs	J	
P40	Ae	B	

continued/...

## APPENDIX 4/...

Isolate No.	Fungus	dp	sp
P41	Ff		B,C
P42	Ae		G,I
P43	Bs		A
P45	Pp	B	
P46	Aa	F	
P47	Bs	J	
P48	Bs	A(3),B(2),G,H,K	
P49	Bs	A,B	
P50	Bs		A(2)
P51	Ae	D	
P52	Ae	F	
P53	Ff	H	
P54	Ff	I	
P55	Aa	H(2)	
P56	Fd		A
P57	Fd	D	
P58	Ae	A(2),C	
P59	Ae	L	
P60	Fd		G
P61	Fd		E
P62	Ff	B(4),E	
P63	Fd	H	
P64	Fd	D,G(2),H(2),I(2), J(3),K,L(2)	H(2),L
P65	Ae	H	
P66	Ae	D	
P67	Ae		C
P68	Ae	D	
P69	Ae	D	
P70	Ae		C
P71	Aa	K	
P72	Ae		A,B
P75	Aa	E,F,G(5),I,J(2),L	E,F(2),H,B,C, D(4)
P77	Ff	A,D	
P78	Ae		E
P79	Fd		L
P80	Ff	D	C
P81	Fd		D
P82	Ae	D	
P83	Ae	D	
P84	Ae	D	
P85	Aa	A(3),B,C(2),D(26), E(5),F,G(3),I(6), J(4),K(7),L(5)	A,C(2),D(12),E, F(6),G,I,J(6), K,L(3)
P86	Fd	A,B(2),D,E,F,H(2), I(2),J(2),L(2)	G(2),H(2),I, J(2),K,L(2)
P87	Ff	B	
P88	Ae	D	D
P89	Ae	D,F,G(5),H,I(11), J(3),K(9),L(4)	G,I(2),L(2)
P90	Ae	D	

continued/...



## APPENDIX 4/...

Isolate No.	Fungus	dp	sp
P91	Fd	A,B,E	B(2)
P92	Ae	A(6),B(3),C(4), D(7),E(2),F(2), G(4),H(3),I(6), J(2),K(5),L(4)	F(5),G(1),H(6), I(6),K(5)
P93	Ae	A(4),B(3),C(2), D,E(2),F(6),G(3), H(7), I(6),J, K(6),L(2)	G(4),H(2),I(2), J(5),K,L(3)
P94	Fd	A(3),B,D(4),E(2), H,J(3)	C,D,F
P95	Ae	A(2),B(2),C,H(2), J(3),L	B,G,H,I(5),J
P96	Ff	I	
P98	Fd	A(2),C,H,I,J,K,L	H,L
P99	Ae	I	
P100	Aa	J,K	E,F,G,K
P101	Fd	D	
P103	Fd	A(7),B(4),C(9),D(8), F(2),G(9),H(5),I, J(2),K(4),L(6)	B,C,E,F(2),G(5) H(2),I,J(3),K, L(3)
P104	Ff	A,B,D	
P105	Ff	L	A(2),B(4),C(5), E(3)
P106	Ff	B(3),C,D(2),E,G(2), K	A(5),B(4),C,D, E(7)
P107	Ff	E	
P108	Fd		E
P109	Ff		B
P110	Fd	B	B
P111	Ff	D	
P112	Fd	D	
P113	Ff		A
P114	Ae	F(2),G(3)	
P116	Ae	E	
P118	Fd		F
P119	Fd	A	
P120	Fd	I	
P121	Bs	A(5),B(2),C(2), D,E(2)	
P125	Bs	L	
<u>Paecilmyces isolates</u>			
P1	<i>P. lilacinus</i>	B,H,J(3),L	E,F
P44	<i>P. carneus</i>	G	
P4	<i>Sporotrichum (?)</i>	J	G

continued/...

## Appendix 4/...

Isolate No.	Fungus	dp	sp
P7 P11 P12	<i>Verticillium</i> isolates	I J H	
P8	<i>Pestalotia sensu lato</i>	D	
P14	<i>Botrytis</i>	L	
P19	<i>Chaetomium</i>	A,B,H,J	G
<u>Sterile cultures</u>			
P10 P15 P17 P18 P27 P28 P73	*  * *  *  *	    K,L  C	     J(2)  D
P16	Unidentified hyphomycete	E	

\*Isolated from roots of unidentified grass sp. + subgeneric classifications of *Penicillium* isolates as for Appendix 2.

APPENDIX 5: Analyses of variance for data of Table 28a pertaining to individual fungal genera and species groups from wheat-field soil.

Appendix 5a: Wheat-field soil *Penicillium* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Penicillium</i> isolates	9	536.7	59.64	83.42 ***
Nematode isolates	4	364.4	91.09	127.42 ***
Interaction	16	87.4	5.46	7.64 ***
Residual	108	77.2	0.71	
Total	137	1065.7		

Appendix 5b: Wheat-field soil *Fusarium* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Fusarium</i> isolates	12	172.5	14.38	31.47 ***
Nematode isolates	5	543.5	108.70	237.91 ***
Interaction	37	240.3	6.50	14.22 ***
Residual	216	98.7	0.46	
Total	270	1055.0		

Significance levels for variance ratios: P = 0.001 (\*\*\*);  
P = 0.01 (\*\*); P = 0.05 (\*); N.S. - non-significant.

continued/...

Appendix 5c: Wheat-field soil *Mortierella* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Mortierella</i> isolates	4	454.78	113.69	175.42 ***
Nematode isolates	3	29.04	9.68	14.93 ***
Interaction	5	4.08	0.82	1.26 N.S.
Residual	54	34.99	0.65	
Total	66	522.89		

Appendix 5d: Wheat-field soil mucorales<sup>z</sup> isolates

Source of variation	df	Sum of squares	Mean square	F
Mucorales isolates <sup>z</sup>	2	6.75	3.38	8.54 ***
Nematode isolates	5	276.33	55.27	139.79 ***
Interaction	6	66.64	11.11	28.09 ***
Residual	69	27.28	0.39	
Total	82	377.00		

<sup>z</sup>Other than *Mortierella*.

continued/..

Appendix 5e: Wheat-field soil *Trichoderma* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Trichoderma</i> isolates	1	2.45	2.45	9.00 **
Nematode isolates	4	6.61	1.65	6.09 **
Residual	22	5.97	0.27	
<b>Total</b>	<b>27</b>	<b>15.03</b>		

Appendix 5f: Wheat-field soil *Aspergillus* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	5	142.56	28.51	64.8 ***
Residual	22	9.68	0.44	
<b>Total</b>	<b>27</b>	<b>152.24</b>		

Appendix 5g: Wheat-field soil *Metarrhizium* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	1	2.77	2.77	6.39 *
Residual	6	2.60	0.43	
<b>Total</b>	<b>7</b>	<b>5.37</b>		

continued/...

Appendix 5h: Wheat-field soil *Cladosporium* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	1	7.50	7.50	11.23 *
Residual	6	4.01	0.67	
Total	7	11.51		

Appendix 5i: Wheat-field soil *Paecilomyces* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	1	4.55	4.55	11.49 *
Residual	5	1.98	0.40	
Total	6	6.53		

Appendix 5j: Wheat-field soil *Acremonium* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Acremonium</i> isolates	1	208.23	208.23	460.23 ***
Nematode isolates	4	107.21	26.80	59.24 ***
Interaction	1	6.57	6.57	14.52 **
Residual	20	9.05	0.45	
Total	2	331.06		

continued/..

Appendix 5k: Wheat-field soil *Gliocladium* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	4	99.89	24.97	116.13 ***
Residual	15	3.23	0.22	
Total	19	103.11		

Appendix 5l: Wheat-field soil *Graphium* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	3	60.08	20.03	26.59 ***
Residual	10	7.53	0.75	
Total	13	67.61		

Appendix 5m: Wheat-field soil *Broomella* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	5	159.79	31.96	46.02 ***
Residual	27	18.75	0.69	
Total	32	170.54		

continued/...

Appendix 5n: Wheat-field soil *Phoma* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Phoma</i> isolates	2	4.26	2.13	4.73 *
Nematode isolates	5	106.19	21.24	47.14 ***
Interaction	5	18.47	3.69	8.20 ***
Residual	41	18.47	0.45	
<b>Total</b>	<b>53</b>	<b>147.39</b>		

Appendix 5o: Wheat-field soil *Cochliobolus* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	1	1.53	1.53	9.58 *
Residual	4	0.64	0.16	
<b>Total</b>	<b>5</b>	<b>2.17</b>		

Appendix 5p: Wheat-field soil *Myrothecium* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Myrothecium</i> isolates	3	212.86	70.95	156.41 ***
Nematodes isolates	5	365.10	73.02	160.96 ***
Interaction	9	77.23	8.58	18.92 ***
Residual	52	23.59	0.45	
<b>Total</b>	<b>69</b>	<b>678.78</b>		



Appendix 5q: Wheat-field soil *Torulomyces* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Torulomyces</i> isolates	1	5.99	5.99	20.35 ***
Nematode isolates	1	5.45	5.45	18.50 **
Interaction	1	0.05	0.05	0.16 N.S.
Residual	12	3.53	0.29	
<b>Total</b>	<b>15</b>	<b>15.02</b>		

Appendix 5r: Wheat-field soil *Doratomyces* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	3	74.99	24.99	44.78 ***
Residual	16	8.93	0.56	
<b>Total</b>	<b>19</b>	<b>83.92</b>		

Appendix 5s: Wheat-field soil *Coniothyrium* (?) isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	2	35.22	17.61	41.33 ***
Residual	13	5.54	0.43	
<b>Total</b>	<b>15</b>	<b>40.76</b>		

continued/...

Appendix 5t: Wheat-field soil *Periconia* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	1	0.18	0.18	0.26 N.S.
Residual	8	5.53	0.69	
Total	9	5.71		

Appendix 5u: Wheat-field soil *Ulocladium* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	3	178.68	59.56	269.99 ***
Residual	15	3.31	0.22	
Total	18	181.99		

## Appendix 5v: Wheat-field soil sterile fungi isolates

Source of variation	df	Sum of squares	Mean square	F
Sterile fungi	14	571.7	40.83	64.26 ***
Nematode isolates	5	944.8	189.00	297.36 ***
Interaction	44	534.1	12.14	19.10 ***
Residual	197	125.2	0.64	
Total	260	2175.8		

APPENDIX 6: Analysis of variance for data of Table 29a pertaining to individual fungal genera and species groups from pine-forest soil.

Appendix 6a: Pine-forest soil *Penicillium* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Penicillium</i> isolates	32	1219	38.09	80.87 ***
Nematode isolates	4	1307	326.80	693.78 ***
Interaction	56	375	6.70	14.23 ***
Residual	266	125	0.47	
<b>Total</b>	<b>358</b>	<b>3027</b>		

Appendix 6b: Pine-forest soil *Trichoderma* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Trichoderma</i> isolates	1	3.69	3.69	17.29 ***
Nematode isolates	4	81.47	20.37	95.39 ***
Interaction	2	1.31	0.66	3.07 N.S.
Residual	32	6.83	0.21	
<b>Total</b>	<b>39</b>	<b>93.30</b>		

continued/...

Appendix 6c: Pine-forest soil *Mortierella* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Mortierella</i> isolates	1	0.64	0.64	2.86 N.S.
Nematode isolates	4	163.01	40.75	183.09 ***
Interaction	4	106.69	26.67	119.83 ***
Residual	32	7.12	0.22	
Total	41	277.46		

Appendix 6d: Pine-forest soil *Mucor* isolates

Source of variation	df	Sum of Squares	Mean square	F
Nematode isolates	1	0.46	0.46	4.48 N.S.
Residual	8	0.83	0.10	
Total	9	1.29		

continued/...

Appendix 6e: Pine-forest soil *Fusarium* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Fusarium</i> isolates	3	45.44	15.15	39.83 ***
Nematode isolates	5	380.04	76.01	199.90 ***
Interaction	9	101.23	11.24	29.58 ***
Residual	88	33.46	0.38	
Total	105	560.17		

Appendix 6f: Pine-forest soil *Paecilomyces* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Paecilomyces</i> isolates	1	10.92	10.92	34.04 ***
Nematode isolates	4	49.48	12.37	38.55 ***
Interaction	1	0.38	0.38	1.17 N.S.
Residual	28	8.98	0.32	
Total	34	69.76		

continued/...

Appendix 6g: Pine-forest soil *Verticillium* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Verticillium</i> isolates	1	134.97	134.97	274.88 ***
Nematode isolates	4	214.85	53.71	109.39 ***
Interaction	2	33.70	16.85	34.32 ***
Residual	29	14.24	0.49	
Total	36	397.76		

Appendix 6h: Pine-forest soil *Pestalotia* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	4	149.33	37.33	119.57 ***
Residual	24	7.49	0.31	
Total	28	156.82		

continued/...

Appendix 6i: Pine-forest soil *Botrytis* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolate	2	186.14	93.07	226.49 ***
Residual	16	6.57	0.41	
Total	18	192.71		

Appendix 6j: Pine-forest soil *Chaetomium* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	1	0.13	0.13	0.73 N.S.
Residual	5	0.89	0.18	
Total	6	1.02		

continued/...

## Appendix 6k: Pine-forest soil sterile fungi isolates

Source of variation	df	Sum of squares	Mean square	F
Sterile fungi isolates	3	53.10	17.70	50.75 ***
Nematode isolates	5	304.24	60.85	174.47 ***
Interaction	9	133.11	14.79	42.41 ***
Residual	60	20.92	0.35	
Total	77	511.37		

## Appendix 6l: Pine-forest soil unknown Hyphomycete (P16)

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	3	83.67	27.89	22.94 ***
Residual	15	18.23	1.22	
Total	18	101.90		



APPENDIX 7: Analyses of variance for % adults at harvest of nematode isolates on fungal groups from pine-forest soil. Data subjected to an angular transformation.

Appendix 7a: Pine-forest soil *Penicillium* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Penicillium</i> isolates	32	19290	602.8	6.24 ***
Nematode isolates	4	51660	12920.0	133.61 ***
Interaction	56	21610	385.8	3.99 ***
Residual	248	23970	96.7	
<b>Total</b>	<b>340</b>	<b>116500</b>		

Appendix 7b: Pine-forest soil *Trichoderma* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Trichoderma</i> isolates	1	111.25	111.25	1.79 N.S.
Nematode isolates	4	880.62	220.16	3.54 *
Interaction	2	157.63	78.81	1.27 N.S.
Residual	32	1990.79	62.21	
<b>Total</b>	<b>39</b>	<b>3140.29</b>		

continued/...

Appendix 7c: Pine-forest soil *Mortierella* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Mortierella</i> isolates	1	904.53	904.53	13.70 ***
Nematode isolates	4	9618.59	2404.65	36.41 ***
Interaction	4	12922.81	3230.70	48.91 ***
Residual	31	2047.47	66.05	
Total	40	25493.40		

Appendix 7d: Pine-forest soil *Fusarium* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Fusarium</i> isolates	3	2240.53	746.84	9.02 ***
Nematode isolates	5	3980.41	796.08	9.61 ***
Interaction	9	4837.57	537.51	6.49 ***
Residual	85	7039.70		
Total	102	18098.21		

continued/...

Appendix 7e: Pine-forest soil *Paecilomyces* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Paecilomyces</i> isolates	1	30.91	30.91	0.35 N.S.
Nematode isolates	4	1411.97	352.99	3.92 *
Interaction	1	39.63	39.63	0.44 N.S.
Residual	28	2521.13	90.04	
Total	34	4003.64		

Appendix 7f: Pine-forest soil *Verticillium* isolates

Source of variation	df	Sum of squares	Mean square	F
<i>Verticillium</i> isolates	1	1821.26	1821.26	26.29 ***
Nematode isolates	4	28013.57	7003.39	101.11 ***
Interaction	2	345.48	172.74	2.49 N.S.
Residual	27	1870.24		
Total	34	32050.55		

continued/...

Appendix 7g: Pine-forest soil *Pestalotia* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	4	11572.52	2893.13	30.93 ***
Residual	24	2245.02	93.54	
Total	28	13817.54		

Appendix 7h: Pine-forest soil *Botrytis* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	2	5875.6	2937.8	9.49 **
Residual	16	4953.6	309.6	
Total	18	10829.2		

Appendix 7i: Pine-forest soil *Chaetomium* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	1	2.88	2.88	0.08 N.S.
Residual	5	188.41	37.68	
Total	6	191.28		

continued/...

Appendix 7j: Pine-forest *Mucor* isolates

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	1	81.10	81.10	7.50 *
Residual	8	86.49	10.81	
Total	9	167.59		

## Appendix 7k: Pine-forest sterile fungi

Source of variation	df	Sum of squares	Mean square	F
Fungal isolates	3	5826.65	1942.22	20.11 ***
Nematode isolates	5	19432.77	3886.55	40.24 ***
Interaction	9	16852.50	1872.50	19.39 ***
Residual	59	5698.33	96.58	
Total	76	47810.25		

## Appendix 7l: Pine-forest soil unknown Hyphomycete (P16)

Source of variation	df	Sum of squares	Mean square	F
Nematode isolates	3	13007.38	4335.79	104.16 ***
Residual	15	624.42	41.63	
Total	18	13631.80		



continued (i)

3. W43			70	180	2	2	1	4		
			110	260	2	2	3	4		
			300	630	1	1	1	1		
			320	540	0	1	0	0		
4. W48J			195	705			4	15		
			345	1245			2	14		
			510	1410			6	16		
			345	1305			5	6		
5. W56			105	345			1	3		
			360	1335			4	9		
			285	1380			2	5		
			195	660			1	2		
6. W70H	5	18	91	196	1	1	11	52	17	65
	13	77	165	399	1	6	39	127	7	40
	11	24	168	501	1	1	28	81	36	71
	12	46	99	372	0	0	40	142	13	58
			100	410						
			50	210						
			50	180						
			20	150						
7. W73B	400	4300	800	9600			300	6120	450	630
	100	4700	4000	27250			600	3720	420	1070
	900	7800	1500	16750			630	2730	330	1090
	300	4100	3000	23750			25	120	270	680
			3500	13000						
			1000	7000						
			2500	18000						
			2000	17500						
8. W75			60	480			330	1140		
			320	2760			390	2145		
			50	370			225	2325		
			250	1310			255	3135		

continued (ii)

9. W79	260	870	300	9200		126	1494	240	360
	200	810	900	10300		5	17	190	330
	160	420	800	8100		20	91	300	400
	90	150	600	10700		17	74	440	680

10. W108			450	4800		84	222	46	51
			750	5100		132	246	92	128
			900	9450		168	276	77	107
			600	6150		90	156	65	73

Fusarium isolates

1. W55L	250	3750	2000	16000		750	2400	90	260
	1000	10500	400	3800		600	1740	240	440
	500	6750	350	2750		480	1140	170	240
	250	4500	1500	8000		780	3060	240	650
	1250	9000	2000	9500					
	2000	19750	1750	8500					
			2750	12250					
			1250	4750					

2. W64	400	9000	1400	14600	768	4224	1240	4000	21	25
	1000	6400	600	18400	1440	5220	1680	7569	19	25
	400	13600	2400	28600	1020	2160	1080	3180	9	13
	600	15400	2400	27600	600	2160	2280	8040	3	6
	4000	18000	13000	33000						
	4500	27500	13000	34000						
	1000	12000	14000	41000						
	2000	12500	7000	33000						
			7500	41500						
			9500	44000						
			11500	37500						
			7500	37000						



continued (iii)

3. W84K	150	2070	1500	12150	60	405	180	990	360	1890	30	50	
	150	480	600	6750	80	1216	120	1650	150	630	100	140	
	30	960	2850	20250	150	645	180	2790	300	1440	20	20	
	90	720	750	10950	75	675	150	1350	300	1260	10	20	
	800	6000			150	2730			180	1150			
	150	1620			120	1080			110	940			
	500	3200			390	1920			80	660			
	600	4700			300	2430			210	1220			
4. W85I	375	7750	5500	20500	800	6300					7	9	
	1950	17400	2500	22500	200	4400					28	29	
	450	4950	7500	26500	800	6300					7	9	
	1200	10650	6000	21500	200	4400					28	29	
	1400	14400	2000	22500	400	5400					11	16	
	2600	11000	1500	16250	700	3500					29	29	
	1600	9200	3750	35250									
	4400	17400	3500	25750									
			2000	11500									
			2500	15500									
		3000	18000										
		1000	17500										
5. W90G	7000	46500	750	7500			30	270	11	55			
	3000	34000	750	5250			50	230	8	32			
	4000	41000	1000	4750			40	100	2	24			
	1000	26000	750	6500									
			1650	5100									
		750	4950										
6. W93A	800	7000	3750	16500									
	900	18600	1250	5250									
	1500	12000	2750	12750									
	800	12400	1750	11250									

continued (iv)

7. W96	150	6000	1750	14500	240	2370	540	4800	210	1650	120	720	
	330	2420	2100	19500	150	1830	60	600	840	6060	270	1200	
	600	3450	1500	22500	180	1521	180	780	540	3360	300	1230	
			1800	15600	330	2250	240	3840	360	3840	150	1050	
								300	2400				
								180	420				
								240	2460				
								1140	5400				
8. W112G	400	3800	200	4000			10	20	180	450	150	480	
	200	2000	1100	5400			10	10	420	1080	120	270	
	800	4600	400	2600			20	40	10	60	140	230	
			900	2500					60	180	90	120	
9. W121D	500	4500	2500	19000							90	435	
	2000	13500	5000	18000							135	540	
	1250	9500	5000	15500							165	510	
	1500	17500	7500	26500							45	450	
			1000	13250									
			1000	9250									
			750	14500									
			3750	23250									
10. W125	240	1500	540	5100									
	360	2040	1200	10320									
	300	2520	1140	9360									
	240	3240	1260	8760									
11. W126	100	2100	2400	12450			580	3240	80	235	240	1740	
	400	3500	1650	3150			96	888	5	10	120	1050	
	300	2600	2550	13050			10	12	390	1350	270	2704	
	400	3700	2250	11850			26	76	300	810	300	1590	
							15	52					
12. W127	750	7750	4500	19000			400	3000	690	3420	300	710	
	1750	11000	1750	12500			400	4600	960	3930	220	500	
	1750	13250	3250	14750			800	7000	2800	10400	160	520	
	1750	11750	2250	25750					1600	9300	340	1010	

continued (v)

13. W129

1500	13500	2500	23500
1000	9000	7000	27000
1000	11500	3500	15500
1000	9000	4000	18000

90	390
90	360
150	150
10	10

900	5400
1200	7800
1500	6900
1500	6150
270	1830
510	1890
330	1650
1080	2880

Mortierella isolates

1. W62G

300	4400	60	1740
500	2900	240	4440
400	6300	300	3360
200	2100	4000	23000
4500	24750	4500	21500
2750	29500	9500	30000
1750	21750	10000	31500
2000	29000	9000	22000

60	1800
150	1590
120	1290
150	1440

2. W66

600	4600	1200	20100
600	11400	300	8700
1400	19100	1500	17400
200	6900	1050	6450

3. W67F

2	4
2	2
4	5
2	3

1	1
2	2
0	0
0	0

4. W67L

1000	26800	200	22300
400	17800	1000	32400
800	17400	200	26800
1200	20600	1000	20400
500	3700	1625	8125
700	3800	600	6300
1700	8100	750	3300
400	4500	750	4050

continued (vi)

5. W182	240	4800	1750	22000				7250	30000	350	1260	
	720	7040	500	13250				5000	17500	380	1160	
	320	4960	250	4000				3750	16500	470	1550	
	640	9040	1250	6500						120	350	
<u>Mucor isolates</u>												
1. W86	300	4900	1000	10500	330	1560	180	1620	540	1800	22	29
	200	4500	1500	20000	150	1320	150	1650	420	1560	34	41
	600	5300	3000	21000	360	2250	180	780	240	1500	40	48
	400	4200	5000	38500	300	1110	30	90	180	1500	32	42
			2000	10000	55	260			480	1980	330	420
			1000	17500	90	465			660	2700	150	260
			3000	18000	195	855			360	1080	220	330
			2500	13500	70	325			360	2640	260	340
<u>Gongronella isolates</u>												
1. W88I	800	14400	2750	32500	150	1920					7	11
	1250	8000	2750	19250	150	2430					7	10
	250	8750	5000	41000	150	1170					5	7
	750	11000	5000	26500	60	1170					2	2
	1350	12900	3000	31000								
	450	6000	2500	14500								
	450	6450	2500	17000								
	1050	11700										
<u>Cunninghamella isolates</u>												
1. W21G	240	5220	1020	7620					60	180	120	520
	240	6060	450	3450					50	140	210	490
	120	1260	600	6000					20	80	200	640
	100	1740	450	8550					10	100	270	930
			600	5200								
			400	5600								
			700	7700								
			500	6400								

continued (vii)

Trichoderma isolates

1. W51D

56 792  
135 1320  
150 1320  
180 1995

2. W83

240	840	300	4600	1650	10950	240	1200	270	3270
120	1740	100	4800	300	1650	180	2910	900	6270
60	1560	100	2100	300	4500	240	2430	960	9420
300	1080	200	3500	1050	6000	420	3870	750	9750
		100	1100						
		200	2000						
		700	3800						
		600	2100						

Aspergillus isolates

1. W71

4	76	140	290	0	0	0	0	90	174	230	430
4	33	110	320	0	0	0	0	16	22	130	380
5	179	60	240	0	0	0	0	2	5	220	290
4	54	130	430	0	0	0	0	2	5	130	230
6	76										
12	69										
0	53										
3	49										

Metarrhizium isolates

1. W13E

300	2300	1000	10250
1050	6300	1000	6750
90	750	750	8750
450	3900	500	7750

Cladosporium isolates

1. W10H

180	720	1100	8600
480	4080	1000	6300
180	840	100	3700
60	300	1100	8600

continued (viii)

Paecilomyces isolates

1. W16I

600	4950	1500	12750
1050	5700	3900	20400
240	1230	2100	27300
		1800	10800

Acremonium isolates

1. W22A

255	915	9500	60500
105	525	6500	49500
360	2160	13000	77000
195	690	20500	68000

2. W155

20	30	90	170
40	60	50	260
20	30	80	170
		60	150

Gliocladium isolates

1. W173

80	160	130	650
150	430	200	770
90	300	170	700
30	170	50	530

Graphium isolates

1. W107

400	5200	3000	21500
1100	10300	1500	8000
400	8400	2000	20500
400	11800	3000	19500

Broomella isolates

1. W68C

1250	6750	1950	9600
4250	19750	750	8700
1000	10750	2100	17400
200	2250	1350	12300
		20000	72000
		24000	75000

126	408
180	492
228	594
246	864
110	1250
580	3940
320	1990
180	600

1	1	1	1	25	33
1	1	0	0	29	41
0	0	3	4	47	71
0	0	1	1	198	660
7	14	0	0	210	270
13	22	0	1	230	420
11	18	0	0	100	160
5	8	1	1	75	80
144	480	70	500		
400	1872	30	250		
		10	30		
		10	40		
204	888	162	882	13	19
21	64	410	1810	6	9
7	53	140	940	6	12
		310	1330	8	10
		70	440		
		130	440		
		120	520		
		40	560		

continued (ix)

Phoma isolates

1. W57	2100	14100	500	6500	630	3690	315	975	300	3360	130	240
	1050	7050	2000	8500	570	3810	100	1140	60	2100	470	1850
	2100	16950	3000	14000	360	1860	165	1425			270	1570
	1350	5400			120	660	255	1920			160	680
2. W58	400	3700	900	7000	203	742						
	1100	8300	1700	11200	182	560						
	600	8200	800	5500	77	392						
	1000	8000	800	4300	91	343						
	750	13500										
3. W104	700	7300	1800	10500			21	31	120	390		
	1100	14400	400	5200			39	143	110	520		
	2400	26800	18000	67000					220	710		
	1200	21600	13500	67000					110	360		
	1500	6750	12500	56500								
	2250	16500	10500	60000								
	1000	4750										
5000	19500											

Cochliobolus isolates

1. W179	3200	15000	2500	22000								
	600	5000	14000	28000								
			5000	27000								
			1500	24500								

Myrothecium isolates

1. W14	780	4140	2700	13200	0	0	0	0	1	1	68	95
	360	660	2250	12750	0	0	0	0	9	32	112	171
	240	840	750	4950	0	0	0	0	1	2	196	332
	30	200	900	8550	0	0	0	0	2	3	248	372
	10	100										
2. W92	20	30	170	1870			3	4	2	2	110	210
	30	100	270	1470			0	0	3	3	60	90
	30	100	90	780			0	0	1	1	20	50
	80	180	240	810			3	3	0	0	30	70

continued (x)

3. W95	100	3000	240	2460	21	42	300	2520	35	145
	400	4600	540	4140			180	2220	50	170
	600	3100	300	6060			180	2400	130	350
	200	4100	560	4830			60	1140	75	225
4. W133	250	1350	550	2500						
	125	727	700	3150						
	300	1900	850	4500						
	325	1900	400	3050						
<u>Torulomyces isolates</u>										
1. W18B	2000	11250	3500	64000						
	2500	23500	3000	44000						
	2250	12750	7000	30500						
	250	8000	4000	52000						
2. W19B	500	5000	250	19000						
	200	1400	250	17500						
	120	6480	750	11750						
	300	6900	500	5500						
<u>Doratomyces isolates</u>										
1. W165	40	260	1500	25250			40	80	780	2400
	220	1190	100	1750			60	70	300	1560
	10	120	200	5500			40	80	480	2280
	20	370	250	9750			30	70	660	2160
			500	15500						
			5500	25000						
			3000	23000						
			500	7000						
<u>Coniothyrium isolates (?)</u>										
1. W164	480	1640	210	2445			30	57		
	420	2550	390	4035			17	40		
	300	1170	600	6360			53	117		
	440	2400	360	4140			27	65		
			420	2220						
			360	840						
			240	1080						
			180	720						



continued (xi)

Periconia isolates

1. W193	3500	21000	4500	21500
	1250	15750	6000	28750
	750	3500	5500	27750
	500	7500	750	4250
	5500	31000		
	1500	14000		

Ulocladium isolates

1. W60G	1400	11900	3000	11000	12	29	23	28
	100	9200	5000	25000	14	25	6	8
	600	6700	2500	10000	25	54	16	17
			4000	15000	5	10	22	23
			300	7500				

Sterile cultures

1. W50H	1600	11800	450	10800	0	0	150	810	111	123
	600	4000	2100	12450	0	0	50	150	108	129
	3400	15600	300	8700	5	67	100	350	177	288
	1000	3800	2250	15000	2	73			159	210
2. W53C	1500	9750	10500	25000	420	2040			29	35
	3250	13750	7500	22500	1500	5100			22	29
	2400	12600	12000	39000	1500	5520				
	1250	11750	20000	49000	2940	7200				
3. W54	1250	13500	750	9500						
	250	10750	250	11750						
	750	11750	375	8750						
	1500	10000								
4. W59A	800	6800	100	6800	10	180	310	1250	390	1140
	300	5500	900	15600	70	520	320	1020	270	2370
	1000	6800	200	2700	120	1540	160	450	360	2250
	300	4700	2200	20400	170	2030	400	1280	390	1920
	1200	11400								
	900	8250								
	600	6150								

continued (xii)

5. W80	750	8850	2000	13250	0	0	105	465	15	22	70	160	
	900	9450	1000	5500	4	12	150	720	7	22	140	190	
	1800	11850	2250	11500	0	1	135	1125	16	41	130	190	
			500	13750	6	13	60	375	7	13	150	200	
			1050	6300					60	570			
			750	5700					345	1335			
			450	2550					75	510			
			1050	8400					270	825			
	6. W81	390	1410	180	1560			1	1	7	13	34	35
		145	315	780	4500			8	8	7	10	55	59
52		106	660	3480					21	66	71	102	
			400	1240					5	7	84	98	
7. W97	700	3300	1800	12300	480	4020	0	0	720	3480	190	310	
	200	4900	2200	15200	720	5100	0	0	300	1560	350	490	
	3900	15300	1800	10050	720	4140	0	0	480	1560	270	350	
	1500	9600	900	7650	540	3600	0	0	480	2280			
8. W103D	600	6300	7500	31000	720	2220					5	6	
	100	2400	10000	31000	240	1020					2	3	
	700	6400	11500	41000	240	1260					7	10	
	400	3500	10000	34000							7	8	
			1600	9900									
			700	5800									
			3000	19100									
			2250	11500									
			1500	7750									
			750	8750									
9. W136			1500	12250									
			2250	19250	7500	32000	12	12	9500	29500	435	1020	
			1250	23500	11250	62500	0	12	7500	39500	345	870	
			1000	23250	7500	40000	24	384	10500	30000	405	660	
			2500	24000	7750	36750			13000	32500	105	195	

continued (xiii)

10. W176I			6000	39000				3150	13200	450	960	
			3500	16000				5250	19500	150	660	
			1000	17500				5250	15300	180	930	
			3500	41000				5400	18900	150	570	
11. W177K			13	35			0	0		210	360	
			3	11			2	9		130	190	
			14	38			0	1		150	250	
			26	58			0	0		300	490	
12. W178I	100	460	2000	5500	105	480	1	1	280	910	170	260
	30	150	3150	12300	60	525	1	1	360	2040	170	170
	60	240	2400	13500	70	410	5	10	120	540	90	200
	160	2000	3000	10800	15	30	18	23	80	200	260	360
13. W194			2200	10600	270	520	0	0	540	2520	90	330
			2000	12100	190	580	0	0	120	2280	210	590
			1100	6600	70	320	0	1	120	960	110	220
			3600	14700	190	390	0	0	360	1800	40	80
14. W195C	300	3000	2000	33750						190	370	
	510	3510	5000	52000						330	840	
			10000	73000						540	990	
			6000	25000						250	480	
15. W196			3300	13600						390	2580	
			1800	29700						270	1620	
			3800	20900						330	1590	
			2700	12100						210	210	

(B) Populations of nematodes (pre-treated with antibiotics) on fungi from pine-forest soil

Fungi	<i>Aphelenchus avenae</i>						<i>Aphelenchoides</i> spp.					
	a P b		a W b		a S b		Pine-forest			Wheat-field		
	a	b	a	b	a	b	a L b	a C b	a B b	a H b		
<i>Penicillium</i> isolates	720	3180	345	1815	6000	21500	22	36				
	360	2700	2240	10560	13250	40000	15	17				
	1. P40	450	2850	1620	8250	6250	26750	7	15			
		120	1590	1600	4440	10750	43750	5	12			
2. P41C	55	99	11	30								
	20	50	11	45								
	20	40	21	72								
	20	30	21	63								
3. P42	50	70	2750	13750								
	140	230	2250	15500								
	190	740	3750	21000								
	260	590	7500	19750								
4. P43	180	900										
	180	990										
	120	450										
	120	390										
5. P46	300	2800	1000	6900								
	200	1400	500	3000								
	300	1300	900	4700								
	100	1000	1200	4900								
6. P48B	180	810	480	6240			65	151				
	90	720	800	5400			26	125				
	180	1170	900	5100			20	81				
	150	600	700	6800								
7. P49	90	645										
	165	600										
	75	360										
	105	510										

8.P50	225	615							
	330	1560							
	105	525							
	180	580							
9.P52	60	190	4000	12250					
	130	950	1250	10250					
	360	2160	750	8000					
10.P53	30	90			2000	13000	10	120	
	210	1410			2500	13250	150	660	
	300	3420			4250	17250	100	520	
	330	5580			1250	15000	140	830	
11.P54	360	2340	6000	25050	1000	14500	4	10	1
	800	4800	6600	33000	4500	13500	48	88	8
	320	2360	1800	14400	9500	26000	2	7	1
	150	4650							1
	660	3180							
	480	3480							
	720	3660							
	540	8340							
12.P55	150	525							
	105	465							
	105	765							
	105	345							
13.P56	130	460			1800	10200	13	35	
	50	240			600	10950	16	47	
					900	10500	17	112	
				1350	12450	160	436		
14.P57	1500	27500	3500	25500					
	2500	14000	4500	32000					
	2500	12000	2500	16000					
	3000	26000	1000	19500					
15.P58	240	330			4500	17500	150	720	2
	450	1640			2500	16000	210	330	0
	200	330			7000	11500	210	480	0
	270	570			3000	11000	330	870	0

	360	1500								
	240	1560								
	540	1680								
16.P59	120	3560								
	440	1240								
	480	3040								
	360	1400								
17.P60	1000	3500	5500	29000	16500	39500	176	640	2	2
	1200	4600	9000	29000	4000	21500	150	225	13	30
	200	500	7000	29000	500	2700	195	1050	2	2
	400	2800	7500	39500			420	1140	2	2
18.P61	120	250	2000	14000	1350	7350	540	2490		
	250	1420	3000	16500	900	6150	240	1440		
	150	550	4500	23500	300	1350	120	1320		
	330	1400	1500	5000			2040	8100		
19.P62	80	170	130	520						
	80	210	290	970						
	50	200	230	940						
	50	110	600	4380						
20.P63	4	14	40	110	7	37	3	3	0	0
	2	12	10	120	9	72	0	0	2	2
	1	14	30	150	12	36	0	0	1	2
	3	14	10	90			0	0	1	1
21.P64I	180	360	1200	8400			132	560	3	3
	240	480	800	5900			204	384	0	0
	450	1470	500	1700			324	1500	2	2
			1300	4900			288	1128		
22.P65	160	560	600	5220						
	240	930	480	5520						
	120	480	800	8600						
	390	1920	1200	10800						
23.P75B	190	410	1200	6000			270	1230	1	1
	340	1150	750	6450			450	2310	0	0
	130	680	2850	13200			150	600	0	0
	330	740					270	1770	1	1

24.P85C	130	450			552	1452	0	0
	160	290			948	2244	0	0
	190	390			456	1356	2	9
	300	670			60	156	0	0
25.P86B	90	170			3	7	0	0
	30	200			0	0	0	0
	100	220			1	1	1	1
	10	70			0	0	2	2
26.P89I	600	4500			10750	61750		
	800	4000			7500	35000		
	800	4000			2500	17000		
	1300	11400			1000	5000		
27.P92C	250	9250			42	784	1	1
	750	3750			120	1260	0	0
	350	1000						
	400	1000						
28.P93I	400	7000	1000	8000	540	2100	2	2
	400	3800	3500	17500	3430	13860	2	2
	320	2720	2750	14250	360	2400	2	2
	320	1760	5500	17500	1380	5100		
			1350	12450				
			750	7950				
			3600	25650				
		1800	18900					
29.P94B	500	2300			110	620	10	14
	240	1440			130	600	16	47
	120	450			330	1590	27	70
				380	1590			
30.P95C	240	960			780	4680		
	210	420			420	1140		
	150	690			300	1620		
	90	210			60	180		
						1	1	
31.P98C	10	200	72	296	66	336	4	4
	130	540	76	280	84	372	3	3
	72	232	88	300	42	180	5	5
	30	110	80	192	84	396	3	26

32.P103I	17	116			15	20	1	1		
	12	106			10	20	3	8		
	7	83			5	35				
	9	65			35	145				
34.P105B	10	50			6	11	3	3		
	10	60			11	21	3	3		
	10	70			14	32	4	4		
	10	120			14	36				
<u>Trichoderma isolates</u>										
1.P21	1950	19200	2000	16500	300	1950	60	615	320	1270
	1650	16200	1000	10500	200	750	90	405	195	1575
	2100	21300	6000	21500	96	624	45	165	105	1065
	1650	15300	4500	15000	144	336	375	2940	195	1335
			2500	12750						
			2500	12500						
			1000	17500						
			1250	15750						
2.P115	400	8700	4000	15000			60	600		
	300	700	4000	18500			60	840		
	600	8400	5500	16000			90	630		
	400	10600	4500	20000			90	720		
			3000	27000						
			2250	17250						
			2500	16250						
			2000	21750						
<u>Mortierella isolates</u>										
1.P122B	250	10000	150	5700	450	8850	240	1470	420	1560
	500	8000	450	900	600	7200	210	2670	660	2460
	250	5250	500	3125	150	8550	150	1830	240	1020
	500	10500	625	6125			270	3390	540	1320
2.P123	800	21800	1500	25000	7000	24500	1	1	320	2752
	1600	16200	2500	22500	2000	18500	2	2	300	1200
	400	10600	2000	30500	2000	26500	0	0	1080	6420
	1000	13200	1500	19500	4000	15500	2	2	1140	6660
	1250	16750								
	750	9000								
	750	13750								





4.P25	1200	4200	1200	7500			270	1460	110	1100	150	1065
	800	5700	520	4940			360	3870	140	1570	210	1725
	1000	7300	1625	11250			330	1500	90	540	285	3060
	800	3900	1250	5750			100	780	90	540	180	1980
	800	9400	3500	15750								
	1400	8800	3750	21000								
	600	4400	2500	32250								
	600	4800	3250	29750								
<u>Sterile cultures</u>												
1.P10	1200	9000	750	4350					0	0	8500	49000
	1200	11600	300	1050					4	4	700	43500
	600	4400	1350	2250					4	4	5500	51000
	600	8600							4	4	5500	37000
2.P17	300	1110	600	7800	1050	6600	2	4	2	2		
	180	990	200	900	450	1950	2	3	1	1		
	150	1620	300	2400	1950	8700			2	2		
	240	720	200	600								
			960	3840								
			900	8100								
			600	4620								
			280	3850								
3.P18	800	3900	700	4900	3250	9000	416	1920	150	948		
	300	4200	1600	6300	2750	11500	420	2340	96	368		
	400	3600	400	7600	2500	8500	360	1650				
	500	4600	1000	8700			30	270				
	420	720	900	8200								
	660	3660	1400	9000								
	300	5100	1000	5200								
	1000	7750	900	7800								
	50	5750										
	700	3300										
4.P27	700	9500	4250	20250					72	368	20	740
	600	8000	8250	41000					104	560	60	680
	700	7200	6750	17500					80	240	80	720
	1800	8300	1750	9750					104	592	60	380

Paecilomyces isolates

1.P1	150	2850	3250	14250	1260	3540	300	2070	20	210
	600	2400	250	8000	270	660	300	1770	120	750
	450	4050	1500	10500	120	600	240	1830	40	80
	900	6300	2000	17750			660	7320	40	100
2.P44	375	5500	1400	7900						
	375	3000	4100	20000						
	400	1900	2100	14900						
	1000	2833	1400	14100						
	510	2370	1250	14250						
	600	2610	3000	13000						
	420	2100	1250	14500						
390	1410	2500	19000							

Verticillium isolates

1.P11	3	15	220	860			3	3			
	5	15	130	290			0	0			
	2	15	60	220			4	4			
	10	50	40	80			2	2			
	70	140									
	30	80									
2.P12	1000	11750	1500	9250	420	2070	2	2	4000	7750	
	1500	12250	2500	14250	810	3510	2	2	550	1700	
	500	6500	500	4750	300	1950	4	4	1500	3700	
	750	4000	2000	9000	330	1950	0	0	1600	4000	
			4750	17250							
			3750	18500							
		4500	20250								

Pestalotia

1.P8	600	4650	625	10500	3750	18750	420	1050	34	41
	750	6900	2750	8500	4950	22950	60	450	7	13
	300	2400	1875	12875	2250	10800	510	1230	17	20
	900	12900	4000	23750	3000	9900	450	780	23	36
	960	11820	5000	33750						
	900	10800	5000	30500						
	600	6300	5000	46500						
	1000	7800	10500	46500						
			7500	39500						

Botrytis

1.P14

1000	9750	13500	63000
750	6250	12000	63000
125	9250	9000	37500
4500	44000	7000	45000
5500	33500	500	13750
		2250	21500
		3250	23250
		2000	18750
		13000	63500
		3000	47000

1	11
9	13
6	6
10	22

Chaetomium

1.P19B

400	5000	900	5000
200	3600	200	11100
1000	8900	200	900
800	8200		

Unidentified hyphomycete

1.P16

300	3100	720	6000	1200	12450
200	600	1000	20200	2550	24300
300	5800	500	11750	1200	13050
300	1200	750	8500	1350	12900
400	4800				
180	3840				
150	5925				
1350	8700				

8	8
4	4
408	408

(C) Populations of nematodes (pre-treated with chlorhexidine gluconate) on fungi from pine-forest soil

	P		W		S		L		C		R		B	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
<i>Penicillium</i> isolates														
1. P6	1000	14000	220	760			0	0						
	1000	6000	60	380			0	0						
	3200	15200	270	1600			0	0						
	2600	15600	420	4320			0	0						
2. P9	261	1674	420	2870			177	369						
	189	2376	980	4900			3100	10000						
	558	3231	175	1435			4100	9500						
	873	6975	350	6400			2700	8900						
3. P29	78	288	55	210										
	30	159	49	120										
	108	498	51	155										
			26	69										
		50	95											
4. P30	1100	15200					1	5						
	700	15300					1	12						
	1300	17300					22	49						
	1500	12800					8	89						
5. P31			34	79										
			175	545										
			73	116										
			46	140										
			342	903										
			630	2355										
6. P33			150	590										
	1500	9200	420	1980										
	2500	8800	180	1500										
	2600	19500	120	3120										
	2500	27800	150	750										
			196	672										
		214	1572											

7. P34	700	14300	850	4250	12	70		
	300	6100	800	4800	6	43		
	400	9000	100	630	5	20		
	1000	15000	140	790	6	39		
8. P35	1800	14600	70	770				
	400	5200	100	400				
	1000	9800	660	5340				
	600	3400	315	2660				
9. P36	1300	14400	250	1780				
	600	6000	120	900				
	800	6800	290	1460				
	2600	22800	250	2460				
10. P37	300	1836	37	100				
	168	804	68	151				
	120	1440	84	285				
	312	1272	7	47				
11. P38	3400	15400	120	720	13	51		
	1400	14400	420	3660	30	127		
	4200	27800	120	3240	57	249		
	3000	14600	240	3120	78	387		
12. P39	800	4900	2400	13800	2760	14160		
	4400	21000	1200	6000	2640	8160		
	5600	27600	100	4800	800	9200		
	3200	24600	1900	7300	3840	19680		
13. P40	480	2520	1600	3650			20	88
	135	765	4800	18600			360	1950
	270	1320	5400	24000			105	1095
			2100	7200			180	1410
14. P41C			5	18				
			8	26				
			22	59				
			30	56				
							60	270
							420	2580

15. P42

7000 28000  
3250 11750  
3250 20000  
3000 15500

16. P43

60 60 210 630  
360 2160 600 6950  
240 1200 500 2250  
480 2880 330 3780

192 1344  
84 1116  
168 1428

270 1450  
40 790  
90 1030  
120 1060

156 804  
288 684

17. P45

210 2640 690 4740  
270 4215 600 5820  
600 3870  
780 5670

18. P48B

350 3200  
250 3150  
440 4120  
440 6560  
8 28  
70 430  
385 3273  
210 2888

17 98  
19 104  
6 30  
130 960

19. P49

285 1200 135 1155  
105 930 330 2115  
285 1095 90 660  
345 2925 300 3540

200 2220 120 440  
204 1596 400 3960  
336 1752 160 1360  
300 1812 200 1880

20. P50

390 2760 450 6500 225 3525  
570 5730 420 3240 600 5520  
420 5340 300 2430 560 6960  
420 2040 870 6090 1200 6300

116 1000 30 390  
150 1128 60 450  
78 1056 750 4890  
156 1410 240 2850

21. P51

870 3900  
625 10000  
1000 8500  
1250 9500

0 13  
260 624  
26 91  
65 221

22. P52	3000	18750	2600	28600						
	750	5250	3800	27400						
	500	2500	3200	32800						
	2000	11500	2200	35200						
23. P53	750	5850	4000	45000	125	500	287	1694	100	240
	900	8700	5500	27000	2000	9250	270	615	306	1278
			13500	52000	1000	7250	90	920	612	2184
			4500	34500	1750	13625	75	915	480	3420
24. P54	4000	17250	7000	29500						
	4000	12250	3000	22750						
	6250	19750	11000	65000						
	5250	18500	5750	25750						
25. P55	270	2700	120	1380	352	1952	117	465	70	212
	330	1560	360	5040	288	1952	80	1020	114	435
	360	1260	240	1980	102	714	34	205	57	198
	450	1230	240	1800	576	3744	30	171	54	141
26. P56	1400	14800	2700	15600	900	5800	10	26	2	7
	400	7800	5500	36250	600	5900	63	137	3	9
			750	13750			72	252	4	13
			2500	19125			26	71	2	2
27. P58	3750	15750	10250	41250						
	2800	10300	9500	40750						
	6000	17000	10750	50000						
	2333	9000	9000	38000						
28. P59			1000	2000						
			8250	29250						
			6000	18500						
			9250	27250						
29. P61	130	590	200	2300			255	3060	82	150
	140	615	500	6300			400	2000	26	44
	330	1800	350	2000			60	270	36	84
			100	650			120	720	84	182

3360 16720



30. P64I 108 909  
 90 225  
 45 135  
 9 153

31. P65 2000 17800  
 600 8400  
 1400 8200  
 800 9600

32. P75B 7 14  
 90 210  
 52 114  
 1400 12900  
 1500 13250

33. P85C 33 88  
 253 1309  
 44 286  
 5000 17000  
 3000 26000

34. P86B 32 64 0 0  
 7 14 0 0  
 23 94 0 0  
 18 45 0 0  
 0 0

35. P89I 240 1120  
 196 1120  
 8000 26000  
 750 6000  
 36 64

36. P92C 187 1738  
 2200 21600  
 1000 17000  
 21 32  
 6 9

37. P93I  
 99 242  
 209 1375  
 660 2736  
 77 1111  
 800 7800  
 1600 6400

38. P94B  
 900 6900  
 3200 18240  
 2800 16800  
 1920 11040  
 20 120  
 150 650  
 500 7100  
 500 5250

39. P95C  
 0 2  
 0 5  
 70 1106  
 1050 3885  
 2905 11795

40. P98C  
 90 420  
 240 2080  
 154 836  
 5500 27500  
 2250 9750

41. P103I  
 0 0 0 1  
 1 1 1 1  
 1 2 0 0  
 0 0 1 2

42. P105B  
 68 380 0 0  
 80 292 0 0  
 41 236 0 0  
 34 100 0 0  
 42 68 0 0  
 9 15 0 0  
 216 684  
 243 666

7 14  
 0 0  
 2 5  
 8 37  
 20 42  
 0 0

43. P121B	1	4		
	1	3		
	24	59		
	22	59		
	279	2925		
	81	1341		
<u>Basidiomycetes</u>				
1. H	43	172		
	33	100		
	51	361		
	72	399		
2. L	19	40	0	0
	94	655	1	1
	32	139	0	0
	264	1464	0	0
3. C	144	924	0	0
	108	828	0	0
	114	744	0	0
	54	612	0	0
4. A	103	441	0	0
	50	302	0	0
	52	316	0	0
	52	316	0	0
			0	0
			0	3
5. <i>Suillus luteus</i>	22	8932	1600	11500
	330	9130	1700	16800
	88	8404	1500	12000
	440	15730	1300	12000

Pythium isolates

1. *P.irregulare*

1 1  
0 0  
0 0  
0 0  
0 0  
0 0  
0 0

Trichoderma isolates

1. P5C 400 12500  
1200 10600  
6400 23000  
4400 22200

1 1  
0 0  
1 1  
0 0

2. P21 1480 7120  
880 9160  
880 7760  
1080 6040

420 1078  
840 4170  
91 1197  
252 2282

Aspergillus isolates

1. F3 1 2 0 0  
0 0 0 0  
0 0 0 1  
1 1 0 0

0 0  
0 0  
0 1  
1 1

2. P32 280 8610 5 50  
630 7280 160 2570  
140 5950 110 1500  
110 4170  
72 1188

Fusarium isolates

1. P22 420 1050  
2400 6600  
1190 5110  
665 1855

2. P23  
 800 2650  
 1650 8700  
 200 3500  
 600 3200

3. P24  
 4800 16800  
 1000 4200  
 4400 12200  
 3800 9900

4. P25  
 4200 15800 500 2675  
 3800 19000 1300 9300  
 5800 28400 375 1350  
 8000 24600 75 825

Sterile cultures

1. P10  
 3 15 64 216  
 72 456 64 72  
 231 1488 12 52  
 136 896

2. P17  
 300 2375  
 75 1750  
 75 475  
 25 475

3. P27  
 540 5790 800 8400  
 1740 11190 1200 14200  
 1680 13600 1000 7800  
 1440 8480 1600 8500

Verticillium

1. P7  
 40 120 3 19  
 24 184 4 11  
 8 24 3 7  
 56 64 2 3

2. P11  
 270 714  
 168 392  
 210 805 3 3  
 875 2590 0 1  
 2400 6400 5 5  
 1 1

1215 3945  
 330 1230  
 2010 7530  
 1290 4260

0 2  
 1 1  
 3 3  
 7 21

0 0  
 0 0  
 1 1  
 0 0

12 36  
 24 72  
 4 20  
 4 16

3. P12	2250	16250	108	620	0	0
	13500	48500	1700	9500	0	0
	6000	28000	600	4900	4	4
	6500	32500			0	0
	850	7600			54	102
	2000	25200			40	104
	2600	13000			82	223
	1600	12400			35	75
<u>Paecilomyces isolates</u>						
1. P1	91	1036	240	1275	3	3
	99	948	204	1751	2	2
	119	837	195	840	0	0
			150	675	1	1
<u>Sporotrichum(?) isolates</u>						
1. P4	5	30	33	53	1080	2190
	35	120	120	320	540	2220
	210	660	90	370	1680	3540
	35	155			450	960
<u>Pestalotia isolates</u>						
1. P8	5	10	300	700	320	1824
	4	8	20	200	528	1680
	36	121	1100	7000	102	646
	64	76	50	5150		
	160	2080				
	480	4640				
	3750	36000				
	3000	17500				
<u>Botrytis isolates</u>						
1. P14	600	5800			0	1
	600	11200			84	168
	1800	18900			54	113
	500	6200			29	161
<u>Chaetomium isolates</u>						
1. P19B	150	300			750	13350
	300	2650			600	12900
	400	5550			900	11700
	450	3150			1050	13200

Mucor isolates

1. P26	143	2365	49	308
	187	2783	238	896
	165	1441	270	1365
	77	1761	315	2025

Unknown hyphomycete

1. P16	100	630	2	6
	690	3610	9	22
	630	1490	4	5
	990	4590	4	12

(D) Populations of nematodes (pre-treated with chlorhexidine gluconate) on fungi from wheat-field soil

Fungi	P		W		S		L		C		R		B	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
<u>Rhizoctonia solani</u> <u>sensu lato</u>			20	85			91	144						
			3	15			152	604						
			2	2										
			5	32										
<u>Alternaria</u> <u>alternata</u>			15	28			265	655						
			7	22			480	1224						
			11	35			510	2010						
			2	8										
<u>Pythium isolates</u>														
1. <u>P.acanthicum</u>			5	19			2	2						
			3	5			3	3						
			2	4			4	4						
			4	10			4	4						
2. <u>P.afertile</u> sp. gr.			7	28										
			2	3										
			2	2										
			5	8										
3. <u>P.irregulare</u>			2	2										
			1	6										
			3	7										
			3	5										
4. <u>P.afertile</u> sp. gr.			26	198										
			23	52										
			39	153										
5. <u>P.monospermum</u>			1	1										
			0	24										
			1	11										
			0	1										



6. *P. paroecandrum*

	4	7	37
	0	26	
	3	3	
		5	

Aspergillus isolates

1. W3C	140	976	65	175
	24	92	20	107
	120	688	63	307
	128	768	36	187

2. W6	0	0	0	0
<i>A. niger</i> sp. gr.	0	0	0	0
	0	0	1	1
	0	0	0	0

3. W8	220	1740	195	1620
	440	3140	165	2160
	80	1320	30	1080
	140	640	170	1020

4. W15	245	1155	174	576
<i>A. terreus</i>	280	4340	60	990
	280	5110	90	498
	210	2170	78	198

5. W36			165	435
			90	529
			180	608
			120	293

6. W37			300	2280
			180	510
			450	1170
			210	930
			1375	11875
			1000	15625

	13	58	210	450	20	72	33	63
	35	80	64	204	28	73	57	183
			60	192	13	71	108	312
			72	546	15	33	39	87
					124	440		
					44	284		
					116	424		
	110	495	60	568	585	3495	120	332
	160	415	56	260	156	668	120	732
	160	505	96	532	90	600	44	260
			68	380	195	285	36	212

7. W38F			264	636			260	1340	24	73	136	944	14	52
			192	2136			65	315	45	93	180	830	208	684
			440	2160			270	635	35	82	60	250	46	121
			264	3420			50	540	49	182	60	660		
			1125	7875										
8. W39			240	1344			15	196			5	33	108	276
			216	2016							12	76	128	520
			336	3984							3	12	88	328
			240	1632							4	17	8	16
9. W40			72	96			12	30	58	212	7	28	104	180
			48	90			126	306	84	510	63	140	148	236
			312	1368			204	612	42	176	189	763	64	192
			300	3350					18	134	49	623	144	432
			750	8950										
			700	7100										
10. W71			48	84									0	0
			135	460									0	0
			102	414									0	0
			324	828									1	1
<u>Sterile cultures</u>														
1. W53C	1000	10250	3000	26500	10500	28500	2200	10000	660	4740			1530	3420
	500	5000	5500	22500	9500	42000	700	3000	175	4340			840	1560
	1250	4500	7500	31000	9000	24500	2100	8300	280	1960			600	1560
	1500	7500	6500	20000	5500	12000	100	2200	270	3180			576	3540
2. W54	20	1000	420	7320			735	2790	704	5536			7000	39500
	625	6500	704	4416			882	3096	416	2464			8500	47500
	250	3250	704	7360			510	3960	512	4192			7000	41500
	250	2000	360	9540					210	840				
3. W59A	60	120	3700	20100	3375	14500	350	520	450	3210			210	430
	420	2100	3000	14250	875	8875	340	1120	420	3600			470	900
	960	5880	1500	6500	3750	15875	240	370	120	1320			450	640
	180	660	4500	15500	2125	9250	1080	3900	480	3600			240	290
4. W80			1875	5875									2	8
			1450	5200									6	25

4. W80			550	1850									26	125
			550	1950									1	3
5. W103D	3400	30400	2300	16000	500	1000	93	300	84	448			114	192
	1200	12600	800	4700	16500	41000	84	462	32	420			162	258
	4400	31800	4000	27100	3000	11500	111	459	76	520			114	192
	2600	14800	1600	9200	3500	8000			80	664			96	162
<i>Fusarium isolates</i>														
1. W1I	1040	12720	560	4720										
<i>F. roseum</i> sp. gr.	960	8400	780	5700										
	2560	18560	480	2640										
	160	9120												
2. W2K	1320	12000	210	2135										
<i>F. roseum</i> sp. gr.	2760	17880	735	6020										
	1364	8680	735	3290										
	2040	20160	490	3150										
3. W33L			18	48			1650	5250	1040	8100	4250	23250	630	4680
			45	175			3750	11850	1800	9240	3500	17250	390	3060
			51	312			1200	4650	1080	6720	5000	26000	480	3600
			12	22			1800	4050	832	6656	1750	8250	600	3180
			5000	25000										
			6500	24000										
4. W55L	500	2950	320	1440			2	3	40	192			68	308
	3100	11600	1520	6400			0	1	36	158			76	236
	200	1300	180	960			2	4	16	50			68	244
			720	1880									36	80
5. W64	1000	3000	9000	31500	6250	17250	450	2190	135	1485	1300	10500	1200	5300
	900	4100	6000	26500	9750	22250	960	4440	368	2064	625	4875	900	4400
	600	3800	6500	28500	15000	33000	450	3060	270	2970	2500	12250	1100	7100
	100	1300	9500	30000	7500	18750	148	1360	135	2820	1250	7000	500	3600
6. W84K			2400	12600									960	7360
			4400	17600									640	1920
			200	4000									320	2400
			1600	9800									320	1760
			2800	9800										
7. W85I	120	300	6400	21600			330	2760	189	567			60	138
	70	220	4200	14400			105	1845	161	294			294	768
	150	830	7200	39000			255	2145	203	721			228	492
			5400	16400			120	2925	140	377			318	996

8. W96			2100	12100								210	1650		
			2600	16800								1320	5640		
			3100	12700								1860	5160		
			700	2600								840	4200		
9. W121D	90	510	16000	51000	3400	10200	180	720	42	273		104	224		
	1140	2760	11000	35000	10800	29000			35	168		104	196		
	1440	4380	9000	36000	14000	43500			6	102		160	328		
	2040	6960	8000	27000	36000	98000			12	96		128	476		
<u><i>Gliocladium</i> isolates</u>															
1. W34L			48	1740			0	0	0	0	0	0	0	2	3
<i>G. catenulatum</i>			192	3240			0	0	5	17	0	0	0	7	11
			168	3768			0	0	3	6	0	0	0	81	179
			132	864			0	0	35	61	0	0	0	1	3
			720	3840											
<u><i>Cladosporium</i> isolates</u>															
1. W10H	136	544	5	29											
<i>Cladosporioides</i>	230	1140	23	67											
	100	920	26	38											
	110	650	35	73											
<u><i>Mucorales</i> isolates</u>															
1. W11	240	12880	200	3440											
<i>Rhizopus</i> sp.	240	11840	240	7920											
	160	10080	120	2920											
	160	14240	120	2160											
2. W86												1000	7000		
<i>Mucor</i> sp.												10500	54000		
												11500	45000		
												5500	44000		
3. W63	15	34	15	145	18	36	56	462	9	96		40	220		
<i>Actinomucor</i> sp.	2	4	36	168	14	24	42	336	12	138		10	160		
	12	23	28	300	8	31	84	413	6	111		10	160		
	13	28	75	370	9	14	42	238	18	369		60	200		
4. W88I	304	1776	4500	34500			700	7300	225	1995		90	180		
<i>Gongronella</i> sp.	135	2325	1500	47500			900	11900	300	1290		420	1085		
	60	510	3500	33500			300	2000	90	1320		210	1295		
	240	2250	5000	36000			800	6000	90	2280		120	570		

5. W21G			204	592				1	1
<i>Cunninghamella</i> sp.			204	472				0	0
			192	676				1	1
			54	186				0	0
			50	290					
			84	644					
			40	160					
			20	124					
6. W130			120	4320				540	4320
<i>Cunninghamella</i> sp.			90	2010				120	2220
			210	2445				540	5490
			150	2790				90	1440
<u>Metarrhizium isolates</u>									
1. W13E	350	2765	57	156					
	560	4095	55	171					
	105	1295							
	35	2240							
<u>Myrothecium isolates</u>									
1. W14			14	24	0	0		0	0
<i>M.verrucaria</i>			126	495	0	0		0	0
			58	117	0	0		1	1
			42	261	0	2			
			153	882					
			162	1134					
			378	1854					
<u>Paecilomyces isolates</u>									
1. W16I	735	5670	140	510					
<i>P. lilacinus</i>	210	3815	56	252					
	385	2940	49	196					
	385	3465	119	483					
<u>Torulomyces isolates</u>									
1. W17A	900	2950	110	480					
	500	3600	240	3880					
	400	5300	400	3320					
	650	10100	200	2300					
2. W18B	50	1300	69	213					
	700	12900	345	3570					
	300	7200	330	1200					
			240	1305					

3. W19B	120	1890	4	8										
	400	4725	33	73										
	160	1760	80	240										
	340	2860												
<u>Phialocephala isolates</u>														
1. W20C			30	72										
			7	12										
			18	81										
			6	14										
<u>Acremonium isolates</u>														
1. W22A			108	372										
			207	279										
			345	1725										
			285	1260										
<u>Phoma isolates</u>														
1. W57	2750	25250	6000	24750	12000	41500	1860	6780	270	1190	300	1360	80	140
	2000	18500	5500	20250	9500	30000	1200	4560	330	1790	70	320	420	1701
	4250	28500	6000	23750	6000	18000	480	1740	200	1420	150	340	231	455
	4000	32500	2250	10500	8500	21500	660	4140	170	600	100	440	340	867
2. W58	1400	16600	1040	5200	690	2310	420	1022	8	45	6	11	60	435
	3000	12400	1150	4150	480	1680	728	1988	21	55	36	280	546	2268
	2400	16600	550	1700	1980	6480	98	882	16	61	4	500	42	196
			650	2550			98	625	25	73	210	1290	756	2492
							615	1665						
<u>Broomella isolates</u>														
1. W68C			180	830									4800	22100
			190	1210									1900	12000
			180	1020									3500	22800
			300	1600									4300	25700
<u>Ulocladium isolates</u>														
1. W60G	20	200	2750	8500	3800	8800	1575	7125	390	5340			256	2768
	2000	12600	2400	24400	3000	17200	450	4350	120	7980			320	2528
	600	6100	3700	18300	4200	13400	375	5925	300	5340			1344	9984
	700	6800	2600	8200	3200	12400			420	5700			256	992

Penicillium isolates

1. W5F	840	5180	195	603
	70	2800	115	800
	770	4830	105	600
	1680	8330	115	670
2. W7E	35	75	3	3
	312	1134	5	12
	168	486	2	2
	258	864	1	7
3. W23B	50	1400	1250	5100
	3200	14000	80	410
	1600	8200	20	990
	3000	20600	50	350
4. W24L			34	82
			204	2724
			140	1036
5. W25J	6000	14500	30	240
	3400	7000	280	3255
	2600	7400	120	2790
	5800	14000	210	1140
6. W26	300	1680	111	483
	150	930	168	1288
	90	660	156	864
	150	630	144	471
7. W27	1000	5100	140	790
	900	7300	160	4560
	600	3300	240	1335
		120	2970	
8. W28	3600	19600	864	2976
	4000	21200	192	1984
	2000	20400	270	2970
	1000	9400	240	2790
	1300	11600		
	3100	22700		
	2300	7500		
5400	15400			

648  
 450  
 138  
 573  
  
 5  
 221  
 13  
 40  
  
 3570  
 2310  
 1470  
 1110  
 140  
 1104  
 2736  
 903  
  
 22  
 20  
 13  
 67  
  
 5340  
 8000  
 7980  
 11580  
  
 294  
 210  
 635  
 1905  
  
 49  
 76  
 64  
 38  
  
 408  
 504  
 576  
 336

8  
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 48  
 246  
  
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 76  
 104  
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18. W45	32	46	144	492				1	7	1	2	
	27	39	132	732				4	5	1	1	
	55	74	144	636				2	4	4	6	
	35	52	180	636				2	11	1	1	
19. W48J	100	232	85	174				1	4	1	2	
			109	237				2	2	1	4	
			116	230				1	1	0	2	
			56	138				5	33	1	3	
20. W56	6	11	129	264	0	0		1	1	0	0	
	6	17	245	546	0	0		1	1	0	0	
	7	11	150	240	0	0				0	0	
	3	11	162	300						0	0	
21. W70H			80	240						4	12	
			148	892						4	5	
			48	212						3	7	
			88	400						22	46	
22. W73B			2200	10400						420	6840	
			3000	8600						120	1020	
			4200	13400						960	9180	
			600	4200						300	4740	
23. W73D			2800	10050						660	3570	
			5000	26000						420	1920	
			3000	7200						96	448	
			3800	17000						270	1800	
24. W75			800	9700						144	600	
			300	5600						152	536	
			200	2600						368	1456	
			1300	7900						224	1248	
25. W79			1500	30000						300	4080	
			4000	24000						120	2400	
			1000	17000						60	1140	
			600	9400								
26. W102	12	54	1	26	22	46	2	3	54	354	5	17
	17	35	1	10	15	33	5	23	54	372	1	6
	20	82	7	23	33	63	3	10	48	270	2	6
			0	8	23	31	5	19	36	216	9	18
							3	12				

27. W108			700	3250						250	715	
			250	750								
			600	1650								
			750	3550								
<u>Mortierella isolates</u>												
1. W62G	480	4800	1250	10500		25	58	6	60	480	1920	
	1080	4500	1000	2250		0	0	24	128	60	270	
	300	2100	1750	9750		1	2	24	216	510	2070	
			500	12250				144	1120	224	704	
2. W66	400	10600	1000	10100		6	11	600	6300	105	765	
	400	6300	200	16900		14	34	320	3040	75	450	
	500	11300	200	8200						165	540	
	300	8300	800	16800						368	1472	
3. W67F			2	4						1	1	
			2	2						2	2	
			4	5						0	0	
			2	3						0	0	
4. W67L	900	10100	120	8700	100	13500	4	5	150	4650	4	6
	1100	17100	240	10500	250	12750	5	21	100	3600	8	25
	200	6200	60	13260	50	9625	8	45	50	2900	4	10
	200	8700	180	16320	50	10125	9	19	100	3850	2	12
							27	35				
<u>Trichoderma isolates</u>												
1. W51D	24	132					1	1	23	66	2	28
	12	68							35	97	4	10
	28	116							38	96	4	17
	16	156							18	46	4	18

## APPENDIX 9: Publications

Papers accepted for publication (approximate date  
of publication March, 1984).

WALKER, G.E. (1984). Ecology of the mycophagous nematode *Aphelenchus avenae* in wheat-field and pine-forest soils. *Plant and Soil* (14 text pages, 8 tables, 1 figure).

WALKER, G.E. (1984). Feeding trials of *Aphelenchus avenae* on soil bacteria and actinomycetes. *Plant and Soil* (5 text pages, 1 table).

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