

**INTERACTIONS BETWEEN MYCOPHAGOUS NEMATODES,
MYCORRHIZAL AND OTHER SOIL FUNGI**

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To my mother Haroula

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ABSTRACT

The hypothesis that mycophagous nematodes feed on the mycelia of VAM fungi and consequently reduce the spread of vesicular-arbuscular mycorrhizal (VAM) infection in plant roots and the growth response of plants to this infection was investigated in experiments carried out under controlled environmental conditions.

Preliminary experiments indicated that nematodes might be able to reduce VAM infection by *Glomus clarum* if they were added to soil at the same time as the fungal inoculum. However, nematodes did not affect the growth response of red clover to VAM infection even though there was an indication that nematodes may directly affect plant growth possibly by causing damage to root hairs which could facilitate the entry of pathogens. There seemed to be little effect of mycorrhizal status on the numbers of nematodes which could be recovered from the growth medium. The two standard methods used to extract nematodes in these preliminary experiments were found to give low and variable recoveries. A method which gave greater and less variable recoveries was developed and the conditions for its successful use were defined.

The four species of nematodes used in this study differed in their ability to reduce the growth of a range of fungi and in their ability to increase in number as a result of feeding on these fungi. Both experimental data and a mathematical model, which was developed subsequently, showed that the suitability of a fungus as a source of nutrition could be measured by the increase in numbers of nematodes and the reduction in weight of mycelium as a result of grazing. The model therefore provided a rationale for ranking different fungi in terms of their suitability as hosts for a given species of nematode.

Of the four species of nematodes, *Aphelenchus avenae* was the most damaging to the growth of a range of fungi. However, when added at the same time as, or later than, the VAM inoculum, *A. avenae* had no effect on the spread of VAM infection or on the response of plants to VAM infection. The numbers of *A. avenae* recovered were similar in non-mycorrhizal and mycorrhizal treatments. A mathematical model was developed which simulated the spread of VAM infection and the change in numbers of nematodes with time. Close agreement between predictions of the model and experimental results was only achieved if it was assumed that the nematodes were not capable of feeding on the VAM fungus.

Aphelenchoides composticola multiplied most when cultured in agar plates but grew poorly when cultured on its own in soil. When organic material (bran) colonized either by *Agaricus bisporus* (the nematodes' preferred host) or by volunteer fungi was introduced into soil, the numbers of *A. composticola* increased by up to one hundred times. Neither VAM infection by *G. clarum* nor the mycorrhizal responses of the plant were affected by these high numbers of *A. composticola*, by the introduction of bran alone or by bran colonized by *A. bisporus*. In soil without added bran or saprotrophic fungi numbers of *A. composticola* were not affected by the presence of *G. clarum*, indicating that the available food sources in unamended soil were sparse. Mycorrhizal plants appeared to benefit more than non-mycorrhizal plants from the mineralization of phosphorus by the saprotrophic fungi.

Factors which may determine the suitability of some fungi as food sources for mycophagous nematodes are discussed. The possibility that VAM fungi are immune to grazing by these nematodes is considered. Ways in which mycophagous nematodes may influence the growth of plants are also discussed.

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CHAPTER 1. GENERAL INTRODUCTION

The practice of intensive agriculture has made it necessary to use great amounts of fertilizers and control tightly the activities of plant pathogens with chemical pesticides. This has tended to generate research activity which concentrates on particular narrow aspects such as plant nutrition or disease while ignoring biological interactions. For example the direct and economically disastrous effects of plant parasitic nematodes on crop yield are extensively researched and documented (see Southey 1978; Nickle 1984; Brown & Kerry 1987) but the important role of free-living nematodes in soil processes, which is less obvious, has only recently begun to be appreciated (Freckman 1982a).

Interest in biological interactions in the soil and the rhizosphere is increasing as shown by recent publications (e.g. Fitter 1985; Mitchell & Nakas 1986; Curl & Truelove 1986; Jensen, Kjoller & Sorensen 1986). The appreciation that fertilizers and pesticides may be toxic to people and the environment in general, was one important reason for stimulated interest in the exploitation of such interactions in agriculture and forestry – for example, the biological control of crop diseases (Mangenot & Diem 1979; Lynch 1983). In his introductory speech at the opening of the 1st European Symposium on Mycorrhizas in 1985, Harley noted that stimulation of plant growth by mycorrhizal infection is often so remarkable that artificial inoculation of plant crops with mycorrhizal fungi may be valuable in agriculture as well as in forestry. However, results of research on these topics, which are often contradictory, are there to remind us of our continuing ignorance.

It has often been argued that, by using partially sterilized soil in experiments on the various aspects of enhancement of plant growth by mycorrhizal infection, potentially important contributions of other organisms are ignored. On the other hand, infection of roots by mycorrhizal fungi in natural or agricultural soils is the rule rather than the exception and it is within this context that the present study of the role of mycophagous nematodes in the soil becomes relevant.

1.1. SOIL FUNGI

Fungi may account for about 70% of microbial biomass in soil (Lynch 1983) and can usually be classified as either saprotrophic or plant-infecting. However, this division is blurred in the case of some unspecialized plant-infecting fungi which may be able to grow and reproduce saprotrophically in the absence of their hosts. Examples of this group include *Rhizoctonia solani*, *Pythium* spp. and *Phytophthora* spp. (Curl 1982). Specialized root infecting fungi can only grow significantly in the presence of a suitable host, in the absence of which they survive as resting spores, sclerotia or rhizomorphs. Examples of such fungi are the species of *Fusarium* and *Verticillium* causing vascular wilt.

The symbiotic mycorrhizal fungi also belong to the plant-infecting group. Some ectomycorrhizal species may have a limited saprotrophic capability whereas all known vesicular-arbuscular mycorrhizal species appear to be totally host dependent and will not grow in the absence of live roots (Harley & Smith 1983). The latter are discussed in greater detail below, since this thesis is primarily concerned with their interactions with mycophagous nematodes.

1.1.1. VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) FUNGI

These fungi, in symbiosis with roots, form a vesicular-arbuscular mycorrhiza consisting of three main components, the root of the host plant itself, the hyphae which grow inside and between the cells of the root and the external mycelium. Two types of characteristic structures are produced, arbuscules which are formed inside the cortical cells and vesicles which may form inside or between the cortical cells or on the external mycelium.

After mycorrhiza formation, the plant often benefits from improved nutrition, particularly of phosphorus, as a result of which its growth is stimulated. At the same time the plant supplies the fungus with the carbohydrates which are essential for its growth.

VA mycorrhizas are ubiquitous geographically and may occur in most plants and cultivated crops (see Harley & Smith 1983; Moore 1987).

Infection of roots by VAM fungi

A typical VAM infection originates from soil-borne propagules which may be single spores and sporocarps, hyphae and intact or fragmented mycorrhizal roots. When a hypha encounters a susceptible root it may penetrate between or through the epidermal cells. After reaching the cortex, the hypha branches and spreads out longitudinally, forming side branches with arbuscules inside cortical cells, thus giving rise to an infection unit. As infection progresses, infection units may overlap and interpenetrate each other. If the hypha that enters the root originates from a source other than intact mycorrhizal roots, infection is termed primary, otherwise it is termed secondary. The dynamics of mycorrhizal infection have been described in detail with the aid of several mathematical models (Sanders & Sheikh 1983; Sheikh 1984; Al-Nahidh 1985; Sanders 1986a,b).

The role of the external mycelium in the VAM symbiosis

Secondary infection results in the formation of a profuse external mycelium which has the ability to absorb and translocate phosphate from considerable distances away from the root (Harley & Smith 1983; Tinker 1984). Phosphate ions diffuse very slowly in soil whereas their translocation through hyphae is usually much faster. Sanders & Tinker (1973) found that this may lead to an increase of the inflow of phosphorus to the roots which was subsequently found to be proportional to the amount of external mycelium (Sanders *et al.* 1977). Taking the matter further, Graham, Linderman & Menge (1982) suggested that lack of growth enhancement could reflect limited production of external hyphae rather than limited root colonization.

Quantification of the external mycelium is often difficult because this involves its extraction from soil and separation from debris and other non-mycorrhizal mycelia (see Sanders *et al.* 1977; Graham, Linderman & Menge 1982; Sylvia 1988). To avoid this difficulty, there have been several attempts to find a universal relationship between the

quantities of mycelium inside and outside the root. The percentage or fraction of root length which is mycorrhizal, termed percentage or fractional infection respectively, can be measured quite efficiently by combining standard staining and scoring techniques (Phillips & Hayman 1970; Giovannetti & Mosse 1980). Although frequently used as a measure of the extent of VAM infection, percentage infection should be treated with caution since it is not directly related to fungal biomass (Kucey & Paul 1982). Hepper (1981) found that the ratio of external to internal mycelium on mycorrhizal plants grown *in vitro*, was 0.98:1. This was in agreement with the results of Sanders *et al.* (1977) who showed that the dry weight of external mycelium of three VAM species, was proportional to the total infected root length. Sylvia (1988) used a technique which allowed active and dead external hyphae to be distinguished. The correlation between the length of active external hyphae and root colonization was significant whereas that between the length of total external hyphae and root colonization was not. Graham, Linderman & Menge (1982) found that internal root colonization could exceed 95% without a proportional development of external mycelium and attributed this to restrictive soil factors.

Species of VAM fungi may differ in the quantity of external mycelium produced (Bevege & Bowen 1975). It was found by Abbott & Robson (1985) that the length of external hyphae of *Glomus fasciculatum* per unit of infected root length was smaller than that of *Gigaspora calospora*. This was in contrast with the extent of mycorrhizal infection which was greater in roots colonized by *G. fasciculatum*.

Effect of soil phosphorus availability on mycorrhizal infection

Plant growth in agricultural soils is often limited by phosphorus deficiency which may need to be corrected by the application of phosphate fertilizers. Because mycorrhizal infection appears to be dependent on the phosphorus status of the soil, fertilizer applications are expected to influence its development.

In a given soil, an increase of the concentration of phosphorus usually results in a decrease of percentage infection of mycorrhizal root length (Sanders & Tinker 1973; Mosse 1973; Harley & Smith 1983; Amijee, Tinker & Stribley 1989). This may result from increased root growth associated with increased phosphate concentrations in the soil

(Abbott & Robson 1977) or may be due to a decreased growth and spread rate of the fungus itself (see Amijee, Tinker & Stribley 1989).

By applying phosphorus to onion leaves, Sanders (1975) was able to demonstrate that it is the concentration of phosphorus inside the plant that influences percent infection. More recently, Amijee, Tinker & Stribley (1989) showed that at high concentrations of phosphorus there was an increased resistance to penetration of the root by a VAM fungus which in turn increased the delay in the establishment of infection.

It has repeatedly been shown that the growth responses of plants to mycorrhizal infection may be reduced by increasing the level of phosphorus available in the soil (Mosse 1973; Abbott & Robson 1984; Amijee, Tinker & Stribley 1989).

1.2. SOIL NEMATODES

1.2.1. ABUNDANCE AND TROPHIC GROUPS

Soil nematodes are largely free-living and together with those associated with plant roots constitute one of the most abundant groups of soil fauna. Potentially, they are major contributors in soil processes because they are of a size (0.5-1.5 mm long) which enables them to move freely along root surfaces and into crevices and microhabitats occupied by fungi and bacteria. In lightly managed agricultural systems population densities may range from 10^5 to 10^6 per m^2 (Stinner & Crossley 1982). The numbers of nematodes extracted by Ingham *et al.* (1986a) from the top layers of a semi-arid grassland soil ranged from 30 to 165 nematodes per g of soil, depending on the season.

In the classification of nematodes into trophic groups it is common practice to characterize the groups according to morphology of the stoma (Southey 1978; Freckman 1982b). Thus nematodes with a relatively simple, open and unarmed stoma are usually bacteria feeders (microbivores) and can be easily distinguished from another group of free-living nematodes, the mycophagous nematodes or fungivores, which have a hollow stylet resembling a hypodermic needle.

Mycophagous nematodes are very abundant in field soils (Magnuson 1983a; Ingham *et al.* 1985a; Sohlenius & Bostrom 1986) but have not so far been shown to cause any major direct losses to crop yield. Instead, their economically important effect has been the destruction of mushroom crops (Goodey 1960; Arrol & Blake 1968; Hesling 1974; Hooper 1974). *Ditylenchus*, *Aphelenchus* and *Aphelenchoides* are the genera most commonly associated with these effects. Surveys in mushroom farms in Australia showed that after a normal establishment of the spawn in the compost and of a sufficient yield in the first harvest, the mycelium very often degenerated. Later harvests were reduced or cropping stopped completely and numbers of the above genera of nematodes were found to exceed 10000 per 20 g of moist compost (Arrol & Blake 1966).

Mycophagous nematodes can be cultured on agar plates with mycelia of a great variety of fungal species (Townshend 1964; Nickle & McIntosh 1968; Riffle 1971; Shafer, Rhodes & Riedel 1981) and have been shown to reduce the weights of fungal mycelia (Wasilewska, Jakubczyk & Paplinska 1975; Giannakis & Sanders 1990). The mechanism of the feeding of nematodes on fungal hyphae has been described in detail (Doncaster 1966; Fisher & Evans 1967). The nematode thrusts its stylet until it manages to penetrate the hyphal wall and then it sucks out the contents of the cell. Stylets are the common feature of both mycophagous and most plant feeding nematodes. In fact, the latter feed on plant cells in very much the same way that mycophagous nematodes feed on hyphae (Wyss 1981).

The trophic groups of stylet-bearing nematodes are not always definitive (see Yeates 1971; Klekowski, Wasilewska & Paplinska 1972; Freckman 1982b; Magnuson 1983b). For example, several plant parasitic species such as *Ditylenchus destructor*, *Aphelenchoides blastophthorus* and *Aphelenchoides arachidis* have been cultured on fungi (Faulkner & Darling 1961; Hooper 1973, 1975; Bridge & Hunt 1985).

On the other hand the primarily mycophagous nematode *Aphelenchus avenae*, one of the most frequently recorded species in soil around diseased roots (Minz 1957), fed and reproduced readily on plant callus tissue in pure culture (Barker & Darling 1965).

Even though this nematode species was not found to reproduce on or affect the growth of a variety of crops (Barnes, Russell & Foster 1981b), it has often been associated with root damage. Steiner (1936) found *A. avenae* inside roots and commented upon its potential to damage them mechanically. The results of Hussey & Roncadori (1981) indicated that *A. avenae* reduced cotton root weights whereas two other mycophagous species, *Aphelenchoides composticola* and *Aphelenchoides cibolensis*, had a similar effect on the roots of pine seedlings (Riffle 1975).

Chin & Estey (1966) observed *A. avenae* using its stylet to puncture root hairs of several species of seedlings, on some of which it managed to feed ectoparasitically. Many ectoparasitic nematodes are now known but until recently they have received little attention. This was because they cannot be found inside roots and because the symptoms of damage are not always characteristic of nematode injury (Christie 1953). It is therefore possible that plant parasitic tendencies of the free-living mycophagous nematodes have largely escaped our attention.

1.2.2. METHODS FOR THE EXTRACTION OF NEMATODES FROM SOIL

Various techniques for extracting nematodes are described in detail by Hooper (1986). The methods are of two types, direct or mechanical, which physically separate the nematodes from the soil, and indirect or dynamic, by which nematodes are driven out of a soil, usually by their own behavioural reaction to some stimulus such as water (Freckman *et al.* 1986). Combinations of these two types of method have also been used (Seinhorst 1956).

Direct or mechanical techniques

Decanting followed by sieving is the simplest representative of the mechanical type and was first introduced by Cobb (1918). Other methods are based on elutriation, the principle of which is that particles such as nematodes may remain suspended in an upward flowing current of water (see Seinhorst 1956, 1962; Byrd *et al.* 1976). The technique of Seinhorst (see Hooper 1986) in which one or two specially shaped flasks

are used to separate nematodes from soil particles, relies on the different settling rates of nematodes compared with soil particles.

Centrifugation has also been employed in the effort to separate nematodes from soil debris. In the case of centrifugal flotation, small samples of soil are suspended in water and centrifuged. The supernatant is then discarded and pellets resuspended in a dense solution (e.g. sugar) and centrifuged again. The second centrifugation serves to separate the soil from the nematodes which are then collected on a sieve. An alternative is to suspend and centrifuge soil directly in sugar solution (Willard & Petrovich 1972).

Indirect or dynamic techniques

These are generally much simpler in design. They make use of the motility of the nematodes and their ability to swim from an adequately wetted sample of soil through a screen, on which the soil is supported, to water below. The pioneer of this method was Baermann (1917). Many workers have modified this technique and replaced the funnel by a tray or a bowl (Townshend 1963; Whitehead & Hemming 1965).

Efficiencies of extraction and factors which may influence them

Ideally, all methods of extraction should be accurate and precise. In other words they should have an efficiency of recovery of nearly 100% and a very small variation between replicate samples (McSorley 1987). In addition, the extraction should produce clear suspensions of nematodes with very little other material (Seinhorst 1962), be equally efficient when used under a different experimental conditions and free of operator errors.

Unfortunately, there are no methods which fulfil all these requirements. Goodell (1982), considered that most methods have extraction efficiencies as low as 10-25%, being extremely variable and depending on a variety of factors. Furthermore, there is disagreement on the relative efficiency of different methods, which makes it difficult to select the most appropriate. Therefore, it becomes necessary to test carefully and quantify the efficiency of a given technique, which is difficult because it is not possible to know the exact nematode population of a soil.

The most common means of obtaining an efficiency of recovery has been by adding a known number of nematodes to a sterile sample of soil followed by immediate extraction (Goodell 1982). However, it may be difficult to extract nematodes from soils in which they have lived for long periods of time (see Flegg 1967; McSorley 1987).

An alternative to this method is to compare the numbers extracted by a particular technique with those counted directly in a suspension of soil. Such direct counts appear to be the most accurate way of determining populations of nematodes in soils but are rarely used because they are immensely laborious and time consuming (Oostenbrink 1971). Minagawa (1979) counted directly fixed and stained nematodes in soil samples and was able to calculate the efficiencies of recovery of various extraction methods, by comparing the numbers counted directly to those following extraction. The use of direct examination of soil samples in quantifying the recoveries of extraction techniques has also been suggested by Anderson *et al.* (1979) who described a more efficient way of staining nematodes by treating them first with the enzyme collagenase.

The effect of temperature

The temperature of storage of soil or the temperature at which extraction from soil occurs can significantly influence efficiencies of extraction, either by affecting the rates of settling of nematodes in sedimentation procedures, or by affecting the activity of nematodes in motility-dependent methods.

Barker, Nusbaum & Nelson (1969b) found that storage of soil at -15°C increased the recovery of nematodes by centrifugal flotation in sugar whereas storage of soil at 2°C reduced the recovery by a Baermann funnel. In another study with a modified Baermann funnel method, storage temperature had variable effects on the recovery of different species of nematodes and the difference in numbers recovered in two locations were attributed to the temperature difference between the locations (Kerr & Vythilingam 1966). This is in agreement with the results of Flegg (1967) who concluded that the optimum temperature for extraction yields of two species of *Xiphinema* was directly related to their geographical distribution. A north European species had an optimum of $14-20^{\circ}\text{C}$ whereas the yield of a mediterranean species increased up to 25°C .

Barker, Nusbaum & Nelson (1969a) studied the seasonal differences in numbers of nematodes using three extraction procedures. Baermann funnels gave the highest yield of *Meloidogyne* spp. and *Pratylenchus zae* in the autumn, when nematodes were most active, and the lowest in winter when their motility decreased. The optimum temperature for extraction of a variety of species using a dynamic technique, was 18 °C and only a quarter as many nematodes were extracted at 10 °C or 25 °C (Whitehead & Hemming 1965).

The effect of soil type

The efficiency with which nematodes are extracted has been shown to vary substantially with soil type (Viglierchio & Schmitt 1983b). The stability of soil may be lost when it is in contact with water and many nematodes may become immobilized during extraction by a dynamic method. Peat and loam are stable soils but sand is unstable and this was probably the reason why Harrison & Green (1976) extracted significantly fewer nematodes from sand with a dynamic method than with three mechanical techniques. The same workers found that soils with fine particles such as peat, did not favour procedures which relied primarily on sieving because the resulting suspensions were too dirty for accurate counting.

Kimpinski & Welch (1971b) compared a sugar flotation extraction and a Baermann funnel extraction and concluded that the former was more suitable for soils such as clay, which tend to compact. For such heavy soils, Willard & Petrovich (1972) found that it was better to omit the first step of centrifugation in water and centrifuge the residues from soil suspensions directly in sugar solution.

Despite these differences, Whitehead & Hemming (1965) claimed that their modified Baermann technique extracted similar numbers of nematodes from clay or sandy soils.

The effect of nematode size

The genus of nematode may determine the efficiency with which it may be extracted from soil (Viglierchio & Schmitt 1983b). Large dorylaims, such as *Longidorus*,

were extracted less efficiently by centrifugal flotation which was a better method for the extraction of other smaller genera (Harrison & Green 1976). Lack of sufficient activity by other species such as the ectoparasites *Criconemoides ornatum* and *Xiphinema americanum* resulted in poor results with methods dependent on nematode motility (Barker, Nusbaum & Nelson 1969a; Townshend 1963). Mechanical methods appear to be generally unsuitable for estimating populations which are primarily in the roots such as for example *Meloidogyne* spp. (McSorley 1987).

The effect of sieves

The sieves used to concentrate suspensions of nematodes and separate them from soil debris usually have a 45 or 53 μm aperture and are a major source of error and variation in the extraction of nematodes from soil. If sieves are too coarse they allow many nematodes to go through them, especially if the nematodes do not fall horizontally across the screen. If sieves are too fine they become clogged by fine soil particles (Hooper 1986). Viglierchio & Schmitt (1983b) found that, despite careful operator handling, losses by sieving occurred from suspensions of nematodes in water (i.e. without soil) and predicted that the efficiency of sieving would decrease if soil was included in the procedure.

The semi-automatic elutriator described by Byrd *et al.* (1976) is the method most widely used at present in the USA (Freckman *et al.* 1986) but still suffers from the disadvantages outlined above. For example, only 20-40% of second stage larvae of *Meloidogyne* were recovered by Byrd *et al.* (1976) even with 26 μm sieves. Seinhorst (1956) suggested that the ratio of the number of worms that remain on a sieve to the number which pass through, depends on the ratio of length of worms to the aperture of the sieve, the volume of suspension, the diameter of the sieve and the number of repeated sievings. He found that nematodes could be separated quantitatively from a 4000 cm^3 suspension, by relatively few sievings through a sieve with a diameter of 20 cm and an aperture not wider than 1/10 of the length of the nematodes.

The effect of period of extraction

The efficiency of techniques in which sedimentation of nematodes occurs is dependent on the duration of sedimentation since different species of nematodes may show different rates of sedimentation (Viglierchio & Schmitt 1983a). Dynamic techniques in which a soil sample is left on a screen in contact with water will unavoidably be influenced by the time allowed for extraction (Minagawa 1979).

According to Seinhorst (1956) practically all *Rotylenchus uniformis* passed through a screen within 20 hours and only a fifth of the total population which were in the process of moulting were still on the screen after 44 hours. In another study with several species of *Xiphinema*, an average of 72% of a 48 hour yield was obtained after 6 hours and 94% after 20 hours (Flegg 1967). Whitehead & Hemming (1965) allowed 24 hours for extraction of a variety of genera of active nematodes by a modified Baermann funnel technique and observed extraction of only a few extra nematodes after a further 24 hours. By contrast, significant numbers of *Meloidogyne incognita* were recovered after 48 or even 72 hours of extraction (Viglierchio & Schmitt 1983a).

Eggs may hatch in a soil sample during an indirect extraction, and give a skewed distribution of juveniles in the extract (Freckman *et al* 1986). Using an extraction period of 1-10 days, Minagawa (1979) showed that the number of species extracted by a Baermann funnel increased with time and this was partly attributed to the hatching of eggs. Townshend (1963) extracted three ectoparasitic and one endoparasitic species for a period of 8 days by modifying a technique described by Oostenbrink (1960) and found that only the yields of the endoparasite increased with time. This was probably due to the longer extraction periods which allowed more nematodes to come out of the roots (see Whitehead & Hemming 1965).

Quantity of soil processed

Quantity of soil may influence the efficiency of extraction, particularly with centrifugal flotation and the dynamic methods. Fewer nematodes were extracted from a sample of 20 g than samples of 5 or 12.5 g, following centrifugation in tubes with a

volume of 50 cm³, probably because the lost specimens were trapped by the mass of precipitating particles (Harrison & Green 1976). Extraction efficiency was inversely proportional to the amount of soil spread on the screen resting in a funnel (Yeates 1972). Sohlenius (1980) supported the use of small amounts of soil with Baermann funnels and Townshend (1963) suggested that a soil sample should not exceed 0.4 g per cm² of screen area. In contrast, Whitehead & Hemming (1965) claimed that their dynamic method, extracted similar numbers from (100, 300 and 500 cm³) 0.2, 0.6 or 1 g of sandy or clay soil per cm² of screen.

Comparison of mechanical to dynamic techniques

Vigliarcho & Schmitt (1983b) tested 5 methods with 4 species of nematodes and concluded that the methods were exceedingly poor quantitative tools. Out of the 60 combinations tested, three barely achieved a 50% recovery of the original numbers of nematodes introduced to the soil (Table 1.1).

Sohlenius (1980) proposed that the Baermann funnel was the best technique if small samples were processed. However, lack of oxygen at the bottom of the funnel neck of the original apparatus as described by Baermann resulted in loss of activity of the nematodes which collected there (Flegg 1967) and in some cases, no nematodes were collected from the bottom of the funnel because they lodged on its inclined surfaces (Minagawa 1979). The modifications of the Baermann funnel proposed by Whitehead & Hemming (1965) and Townshend (1963) may have corrected these faults by replacing the funnels with shallow trays or pans which may allow better oxygenation of the water and avoid losses on the inclined walls of the funnel.

Although Whitehead & Hemming (1965) concluded that their dynamic method was more efficient than three other mechanical techniques, required less labour, was cheaper and occupied less space, Harrison & Green (1976) found that the particular technique was less efficient compared to centrifugal flotation with sugar. McSorley (1987) characterized all dynamic techniques as the least efficient but his conclusions were drawn from work with plant-parasitic species (McSorley & Parrado 1981) which are generally not active.

TABLE 1.1. Some reported efficiencies of recovery of nematodes from soil.

Method	Recovery efficiency (% of initial number)	Reference
Decanting + BF	86-92	Flegg 1967
	25-26	Kimpinski & Welch 1971a, b
	94	Yeates 1972
	60	Anderson <i>et al.</i> 1979
	40	Sohlenius 1980
Sieving of soil suspension + BF	3-45	Viglierchio & Schmitt 1983b
Sieving + BF in mist chamber	7-36	Viglierchio & Schmitt 1983b
BF in mist chamber	3-40	Viglierchio & Schmitt 1983b
Sieving + centrifugal flotation	2.5-48	Viglierchio & Schmitt 1983b
	38-42	Kimpinski & Welch 1971a, b
Centrifugal flotation	54-65	Byrd <i>et al.</i> 1976
	36.5	Willard 1972
Semi-automatic elutriator + centrifugal flotation	35-79	Byrd <i>et al.</i> 1976
Tray method (modified BF)	34-42	Procter 1977

BF: Baermann funnel.

One important disadvantage of the dynamic techniques appears to be that, by depending on the motility of the nematodes, they tend to recover some species of nematodes better than others (Oostenbrink 1971). Tissue paper, which is often used as part of the screening device, was found to have dramatic effects on the recovery of nematodes and some tissues retained a high proportion of the nematodes even though soil was absent (Viglierchio & Schmitt 1983a).

Centrifugal flotation with sugar solutions, a method which is preferred by some workers (Harrison & Green 1976; Elmiligy & De Grisse 1970), was found by Minagawa (1979) to be unsuitable for large nematodes such as *Longidorus* because they tended to

sink together with soil particles when centrifuged in sugar solution. In addition, centrifugal flotation methods appear to be appropriate only for small soil samples unless sieving precedes centrifugation.

Elutriation may be utilized to process large soil samples and Seinhorst (1956) described an elutriator which could handle 500 g of soil. More recently, Byrd *et al.* (1976), designed two semi-automatic elutriators capable of processing 500 to 1000 cm³ of soil, without premixing, which reduced the variability due to sub-sampling and operator handling and gave low coefficients of variation (20-30%). Speed and ease of operation are other advantages of the elutriators but low efficiency, cost and the extraction of both live and dead animals are their serious disadvantages.

The techniques of Seinhorst (see Hooper 1986) in which one or two specially designed flasks are used and that of Cobb (1918) which involves decanting of soil suspensions with nematodes through a sieve are laborious, time consuming and prone to operator errors because they rely a lot on sieving. They may not be reliable for quantitative recoveries especially when small species of nematodes are involved (Oostenbrink 1971).

In conclusion, the dependency of methods of extracting nematodes on so many factors can make the choice of a suitable method quite a difficult task. Since losses incurred by extraction are bound to be progressive with an increasing number of steps (Viglierchio & Schmitt 1983a), dynamic techniques of extraction may be advantageous because they usually include only two steps. However, measurements and comparisons of efficiencies of the various methods by different authors have lead to conclusions which are often contradictory. It would not be possible, therefore, to select one method over another purely on the basis of this literature review. The factors on which the efficiency of recovery of a particular method depends need to be defined and kept constant each time the method is used.

1.3 SOME BIOLOGICAL INTERACTIONS IN THE RHIZOSPHERE

1.3.1. INTERACTIONS BETWEEN VAM FUNGI AND PLANT PARASITIC NEMATODES

Plant parasitic nematodes could, by attacking the root system, influence the extent of its colonization by hyphae of mycorrhizal fungi whereas mycorrhizal fungi could counteract the damaging effects of the nematodes by infecting the roots. The latter possibility has received great attention in the last 15 years or so because of its potential for biological control of diseases caused by plant parasitic nematodes (see Hussey & McGuire 1987). However, it should be noted that, as discussed above, plant parasitic nematodes are by no means the only ones which could adversely affect plant growth. Those which are primarily mycophagous may occasionally feed or attempt to feed on roots and may be capable of damaging them and according to Curl (1988) this is more likely to occur under conditions of limited fungal food supply. Therefore the observations described below could also be found to apply to interactions between mycophagous nematodes and mycorrhizas.

The results of a field experiment by Rich & Bird (1974), indicated that the damage caused to the roots by plant parasitic nematodes may be magnified through the detrimental effects on the associated VAM fungi. Since that time several experiments have shown that nematodes may reduce sporulation or VAM infection of the roots. In the majority of cases these effects were accompanied by reduced yields of mycorrhizal plants (Atilano, Menge & Van Gundy 1981; Thomas *et al.* 1989; Heald, Bruton & Davis 1989). Significantly fewer vesicles or even none at all may be formed in the cortex of roots infected with nematodes (O' Bannon & Nemeč 1979; O' Bannon *et al.* 1979). Suppression of mycelial growth and vesicle formation was attributed to the physical disruption of the cortical tissue by the nematodes, leaving little space for colonization by the fungus (O' Bannon & Nemeč 1979).

Alternatively the effect of the nematodes on mycorrhizal development may be physiological. Atilano, Menge & Van Gundy (1981) suggested that endoparasitic

nematodes may interfere with the movement of metabolites required by mycorrhizal fungi by disrupting the transport of water and metabolites through the roots. Whichever the mechanism it seems that the effects of root feeding nematodes on mycorrhizas will occur via their effect on the root, contrary to those of mycophagous nematodes which are more likely to affect the fungus directly.

Plants are either resistant to or tolerant of nematode attack. These responses may be modified by the presence of VAM fungi in the roots. Resistance is denoted by low nematode reproduction (Fox & Spasoff 1972; Saleh & Sikora 1984), reduced penetration of the roots by nematodes (Sikora 1978; Sitaramaiah & Sikora 1981) or a delayed development of larvae to adults (Sikora & Schonbeck 1975; Smith, Hussey & Roncadori 1986). In contrast tolerance of the plant to nematode infection is shown when the reproduction of nematodes remains unaffected while at the same time there is little or no suppression of plant yield (Roncadori & Hussey 1977; Germani, Ollivier & Diem 1981; Thomson Cason, Hussey & Roncadori 1983; Kotcon *et al.* 1985).

The mechanisms of resistance or tolerance in plants infected with VAM fungi are not clearly understood. It has been suggested that VAM fungi may increase resistance by competing with the nematodes for space inside the root (Hussey & Roncadori 1978). However, Smith, Hussey & Roncadori (1986) found that nematode numbers were suppressed even when only half of the root system was colonized by a VAM fungus. They suggested that competition for host photosynthates or possibly the production of a nematostatic compound from the host-symbiont system could better explain the development of resistance. Altered attraction of the nematodes to the roots induced by VAM infection could also explain increased resistance of mycorrhizal plants to nematode attack (Sikora 1979).

Smith (1988) proposed that the improved nutritional status of the host resulting from VAM infection may cause qualitative or quantitative changes in root exudates which could reduce penetration of the root by nematodes. Improved phosphorus nutrition of the plant, due to mycorrhizal infection, may increase resistance as was suggested by Smith

& Kaplan(1988) but other factors may be more important (Cooper & Grandison 1986, 1987).

Increased uptake of phosphorus from soil by VAM plants seems more often to increase their tolerance to infection by nematodes. Experiments in the greenhouse (Kotcon *et al.* 1985) and in the field (Smith, Roncadori & Hussey 1986), have shown that both the number of nematodes penetrating the roots and the growth of nematode infested plants may be increased by mycorrhizal infection.

Clearly the interactions between plant parasitic nematodes and VAM fungi may have variable effects on the growth of the host plant. Results of experiments may vary from one combination of fungus-nematode-host to another (Schenck, Kinloch & Dickson 1975; Strobel, Hussey & Roncadori 1982; Thomas *et al.* 1989). Their results clearly indicated that not all species of VAM fungi are equally able to influence the damage caused by nematodes. The ability of a VAM fungus to counteract plant-parasitic nematodes should also be considered during selection of species of VAM fungi for inoculation of crops, in addition to other attributes such as those outlined by Sanders (1986).

With a given combination of fungus, nematode and plant species, the outcome of interaction experiments may depend on the amount of root colonized by the mycorrhizal fungus. Saleh & Sikora (1984) showed that increasing densities of spores in the VAM inoculum increased subsequent levels of mycorrhizal infection and reduced nematode reproduction. Plant growth, however, was only increased at the highest spore density.

Another factor which may determine how successful a fungus may be in counteracting the adverse effects of nematodes on its host, is the number of nematodes initially present in the soil. Seed yield of a nematode-susceptible variety of mycorrhizal soybean and the sporulation of its symbionts, decreased with increasing nematode inoculum (Schenck, Kinloch & Dickson 1975). In another study, the presence of mycorrhizas enhanced plant growth at low initial levels of nematodes but not when initial numbers were high (Atilano, Menge & Van Gundy 1981).

A key factor in these interactions is the sequence in which plants become mycorrhizal and are infected by nematodes. By allowing the VAM fungus to establish itself first inside the roots fewer nematodes were able to enter them (Sitaramaiah & Sikora 1981). It is probable that when VAM fungi are inoculated first, they will be more effective in reducing nematode penetration, development and reproduction of the nematodes as well as in counteracting their adverse effects (Smith, Hussey & Roncadori 1986; Cooper & Grandison 1986, 1987).

To conclude, VAM fungi are certainly capable of interfering with the growth and reproduction of nematodes. The mechanisms of these interactions are not very well understood but some progress has been made. Unfortunately there *is* little information on interactions between plants, VAM fungi and ectoparasitic nematodes whose free-living habits resemble those of the mycophagous group. Nevertheless, it is entirely possible that mycorrhizas may also influence the reproduction of mycophagous nematodes which are abundant in the rhizosphere of plants, and that conversely, mycophagous nematodes by feeding on mycorrhizal fungi may influence host nutrition and growth.

1.3.2. DISEASE COMPLEXES INVOLVING FUNGAL PATHOGENS OF THE ROOTS AND NEMATODES

The extensive wounds of the epidermis and other tissues, caused by the activities of the nematodes, may make roots more prone to infection by pathogenic fungi (Powell 1971; Pitcher 1978). However, other mechanisms may be more important in the occurrence of such disease complexes. In a detailed review, Powell (1971) reported the role of nematodes in wilt, root-rot and seedling diseases caused by fungi and pointed out that nematodes may also predispose plants to severe infection by otherwise harmless fungi. Furthermore, he suggested that in agricultural soils disease complexes involving fungi and nematodes probably constitute the rule rather than the exception.

Even though prior root infection of the roots by nematodes appears necessary in disease complexes, the influence of the nematodes is greater if they have preceded the fungus for 3 to 4 weeks (Golden & Van Gundy 1975; Griffin & Thyr 1988). Therefore

factors other than nematode wounding, for example the altered physiology of roots attacked by nematodes, may be important (Powell 1971). Golden & Van Gundy (1975) showed that *Rhizoctonia solani* preferred root galls formed by *Meloidogyne incognita* which were also the only sites of sclerotia formation. They hypothesized that this was due to leakage of nutrients from the root galls.

Sedentary and migratory endoparasitic as well as ectoparasitic nematodes have been involved in disease complexes (Powell 1971; Ruehle 1973). Nematodes which did not parasitize roots were also implicated in increased damage by soil fungi (see Powell 1971) but the role of nematodes which appear to be primarily mycophagous has generally been ignored. Steiner's (1936) early observations indicated that *Aphelenchus avenae* may enter and migrate through the root tissues inflicting mechanical injury. Later on, Chin & Estey (1966) published what seems to be the only report on interactions between plants, mycophagous nematodes and pathogenic fungi. They observed that *A. avenae* fed ectoparasitically on the root hairs of six plant species but found nematodes only inside the roots of tomato plants. In further tests with tomato plants inoculated with both *Verticillium albo-atrum* and *A. avenae*, the percentage of wilting plants was considerably higher and the plant weights lower than in the case of plants inoculated with either organism alone. The authors postulated that nematodes followed the infecting fungus inside the root and fed on the fungus rather than the root tissue. Their results indicated that mycophagous nematodes may possibly stimulate fungal diseases either by mechanical damage resulting from probing of cells of the root epidermis or their movement within the cortical tissues.

VAM fungi may be able to suppress disease complexes involving nematodes and fungi since they are able to influence these organisms individually (see Bagyaraj 1984; Smith 1988; Schenck 1981). In fact, control of the disease complex can often be accomplished through the control or removal of one component (see Golden & Van Gundy 1975; Powell 1971).

1.3.3. CONTROL OF FUNGAL ROOT DISEASE BY MYCOPHAGOUS NEMATODES

Mankau & Mankau (1963) discussed the potential use of mycophagous nematodes in the biological control of fungal diseases of the roots after finding that *Aphelenchus avenae* showed a distinct dietary preference for fungi commonly associated with diseased plant roots. After sampling a wheat field soil and a forest soil, Walker (1984a) found that *A. avenae* was more abundant in the former soil while there was a positive correlation between abundance of *A. avenae* and the diversity of soil mycoflora, especially that of plant parasitic fungi.

In factorial pot experiments, several species of mycophagous nematodes have been shown to damage severely the mycelia of pathogenic fungi and limit diseases of the roots caused by them (Rhoades & Linford 1959; Riffle 1973; Barnes, Russell & Foster 1981a; Roessner & Umland 1983; Gupta 1986). It appears that the nematodes can lower the inoculum potential of root-infecting fungi by destroying the mycelium growing out of propagules in the soil. This was demonstrated by Klink & Barker (1968) who fed *A. avenae* on germinating sclerotia of the root-rotting fungi *Rhizoctonia solani* and *Sclerotium* sp.

Riffle's results (Riffle 1971, 1973) indicated that nematodes may be more effective in preventing initial development of the fungus than suppressing fungal growth and subsequent root colonization after the fungus becomes well established in the root tissue. This could mean that the addition of the nematodes to the soil at or before sowing may be more effective in reducing root infection than when the nematodes are introduced after sowing.

It has often been necessary to apply artificially high numbers of nematodes to pots in order to inhibit fungal invasion and disease of the roots (Rhoades & Linford 1959; Barnes, Russell & Foster 1981a; Hong & Estey 1985). In pot experiments, indices of plant disease caused by *R. solani* were negatively correlated with initial numbers of nematodes (Barker 1964). It has been frequently claimed that nematodes may not be as

effective in suppressing disease in the field where their numbers are often substantially lower (Rhoades & Linford 1959; Barker 1964).

There is little information on population dynamics of the nematodes associated with fungi in the rhizosphere, which would be useful to aid understanding of the relationship between nematode numbers and root infection. Even numbers of nematodes recovered from the soil at harvest are rarely reported, although they would be more closely related to the responses of plants to root-infecting fungi which are also measured at harvest. This is probably because the techniques for the extraction of nematodes from soil are often very unsatisfactory (see section 2 in this chapter). In addition, factorial experiments may become unmanageable if they include the sequential harvesting necessary for monitoring nematode population dynamics.

Where final numbers in the soil have been measured, they are often far smaller than the numbers initially added (Riffle 1973; Roy 1973). This is not unexpected since numbers added are often too great to be sustained by the limited fungal biomass. In contrast, it appears that in soils with a high level of organic substrate, which is readily colonizable by fungi acceptable to the nematodes, small initial numbers can multiply to give a population able successfully to control pathogenic fungal infection of the roots. The use of organic additives in order to enhance the role of nematodes in suppressing diseases by fungi was suggested by Klink & Barker (1968) and Barnes *et al.* (1981) defined two approaches to this possibility for biological control:

1. Incorporating into the soil certain organic materials that promote an increase in the existing populations of organisms antagonistic to pathogens.
2. Adding cultures of highly active antagonists to soil in order to increase populations of existing antagonists.

Mankau (1962) showed that certain groups of nematodes indigenous to a field soil, were differently influenced by a range of organic materials. Numbers of bacteria-feeding nematodes increased in the untreated control and all other treatments whereas mycophagous species increased most in soils treated with particular organic materials,

such as chopped alfalfa. A species of plant parasitic nematode was only able to reproduce in soil in which tomato seedlings were growing.

In field experiments, plant materials in bags buried in soil were colonized almost exclusively by bacteriophagous and mycophagous nematodes which multiplied significantly (Wasilewska, Paplinska & Zielinski 1981; Wasilewska & Bienkowski 1985). These two groups of nematodes apparently fed on the bacterial and fungal saprotrophs which colonized the plant material and had different population levels depending on the part and species of the plant material as well as on the period of decomposition.

Roy (1973) measured a substantial increase in *A. avenae* added to pot soil also amended with organic material. However, neither he nor Gupta (1986) found that organic amendments enhanced the effect that this nematode had on reducing both the growth of fungal pathogens and their constraint on plant growth. Nevertheless, treatment of the soil with organic matter, rather than introducing nematodes at high rates, is probably a more effective means of increasing the numbers of mycophagous nematodes in previously sterilized pot soils.

An equivalent situation may often occur in the field. In recent years, the use of minimal tillage systems in agriculture has resulted in a greater concentration of plant residues in the surface layer of the soil (Lynch 1983; Lynch 1985). The residues which become rapidly colonized by saprotrophic fungi and bacteria, may promote a build-up in the population of mycophagous nematodes, with unknown consequences for the seedlings and the mycorrhizas of the subsequent crop.

1.3.4. THE INFLUENCE OF ORGANIC MATTER AND ITS SAPROTROPHIC COLONIZERS ON VAM INFECTION

In an early study on mycorrhizas of grasses in the field, Nicolson (1959) observed that external mycelium of VAM fungi grew profusely over decaying roots and fragments of organic debris indicating the possibility of a partial saprotrophic habit. St. John & Coleman (1983) reported that VAM extramatrical hyphae switched from an

undirected to a branching mode of growth after accidental encounter with fragments of decomposing organic material possibly because these were nutrient rich microsites.

There have been several investigations on the saprotrophic ability of VAM fungi, as part of the attempts to grow them in pure culture. Mosse (1959), while trying to identify the possible growth requirements of mycelium from VAM spores germinated on agar, found that glucose and sucrose were inhibitory whereas certain organic acids were beneficial. It has been claimed that VAM fungi may be able to survive in the soil in the absence of a host plant and that organic matter may play an important role in this (Warner & Mosse 1980; Hepper & Warner 1983; Warner 1984; Hetrick 1989) but this has not so far been proven conclusively.

The increase of soil microflora following the addition of organic substrates to soil, may lead to immobilization of nutrients and increased production of CO_2 which may be limiting for the VAM fungi. Avio & Giovannetti (1988), found that the response of lucerne plants to mycorrhizal infection was reduced when cellulose was added to a sterile sandy soil. Spread of VAM infection in roots was less rapid in a sterile sandy soil amended with cellulose than in unamended soil. The effect was reduced by the addition of extra nitrogen of NH_4NO_3 but was independent of the cellulose source and species of VAM fungus. The authors suggested that increased competition for nitrogen between soil microflora which had colonized cellulose and the VAM fungus was one reason for the negative effects of cellulose on mycorrhizal activity. However, competition for nitrogen between the plant itself and the microflora may have also influenced the development of mycorrhizas.

Little work has been done on the interactions between VAM fungi and soil saprotrophs even though they may share the same niche and mutual interference through antibiosis or competition for nutrients may occur (Avio & Giovannetti 1988; Wilson, Hetrick & Kitt 1989). Hetrick, Wilson & Kitt (1988) found that although the presence of saprotrophic fungi lead to increased growth of non-mycorrhizal plants in sterile soil, mycorrhizal plants were occasionally smaller.

Apart from competitive interactions, saprotrophic organisms interact synergistically with VAM fungi. Microorganisms in the rhizosphere may produce compounds such as plant hormones (Azcon, Azcon-G de Aguilar & Barea 1978), amino acids and vitamins which can increase root exudation and possibly stimulate growth of VAM hyphae or facilitate their penetration of roots (Azcon-Aguilar & Barea 1985). Germination and hyphal growth of *Glomus mosseae* on water agar was stimulated by some unidentified saprotrophic fungi (Azcon -Aguilar, Diaz-Rodriguez & Barea 1986a).

The influence of soil microflora on the availability of phosphorus in the soil by mineralization and solubilization may also have important effects on the uptake of phosphorus by mycorrhizal roots (see Hetrick 1989). For example, synergistic interactions between phosphate solubilizing bacteria and VAM fungi may improve phosphorus uptake and plant growth (Barea, Azcon & Hayman 1975; Azcon, Barea & Hayman 1976; Raj, Bagyaraj & Manjunath 1981). Several soil fungi are also known to solubilize phosphate in pure culture, possibly by decreasing the pH of the medium and/or producing large quantities of organic acids (Agnihotri 1970). However, *Aspergillus niger*, which was the most effective solubilizer of phosphate in Agnihotri's experiments, failed to stimulate the uptake of phosphorus or yield of mycorrhizal onion (Manjunath, Mohan & Bagyaraj 1981). In fact it is possible that mineralization of organic phosphorus may be the more significant contribution of saprotrophs to the increased availability of nutrients (Alexander 1977; Curl & Truelove 1986 ; Hetrick 1989).

Such evidence has been presented by Dighton, Thomas & Latter (1987) who found, in a microcosm experiment, that a saprotrophic fungus interacted synergistically with ectomycorrhizal fungi in increasing shoot and total plant weight of pine seedlings. They postulated that the saprotroph was able to derive nutrients from a fraction of the growth medium that was unavailable to the ectomycorrhizal fungi and the roots.

1.3.5. THE EFFECTS OF NEMATODES ON THE DECOMPOSITION OF ORGANIC MATTER BY SAPROTROPHS

Grazing of bacteria by protozoa or nematodes often stimulates mineralization of nitrogen and phosphorus in the soil (Coleman *et al.* 1977; Cole *et al.* 1978; Baath *et al.*

1981; Trofymow *et al.* 1983). As suggested by Trofymow & Coleman (1982) this could occur by an enhancing effect of grazing on bacterial activity and multiplication, by the release of nutrients that would otherwise remain immobilized in the bacteria or simply by increased dispersal of the bacteria.

Relatively little is known about the effects of fungal feeding nematodes on nutrient cycling. Trofymow & Coleman (1982), in soil amended with cellulose, found that less carbon and nitrogen were mineralized by a saprotrophic fungus grazed by *A. avenae* than by the fungus on its own. They suggested that the nematodes which were added in high numbers may have overgrazed the fungus thus reducing its capacity for mineralization. However, the results of a later experiment with the same organisms showed that nitrogen mineralization was increased by the grazing of *A. avenae* on the fungus (Trofymow *et al.* 1983).

Ingham *et al.* (1985b) conducted a similar but more detailed study which also included plants. They predicted that in soils where nutrients were limiting, mineralization of phosphorus and nitrogen by saprotrophic fungi and bacteria should provide additional inorganic nutrients for uptake by plants and thus increase plant growth. Mycophagous and bacteria-feeding nematodes may increase rates of mineralization leading to faster plant growth. Blue grama grass was grown in microcosms containing soil low in inorganic nitrogen, with or without chitin amendments as a source of organic nitrogen. The soil was inoculated with *Pseudomonas* spp. or *Fusarium oxysporum*. Half of the treatments with bacteria were also given bacteria-feeding nematodes and half of the treatments with fungi received the mycophagous *Aphelenchus avenae*.

However, the predicted results were found only for the bacteria, the fungi and the grazing of bacteria-feeding nematodes. The mycophagous nematodes increased mineralization only slightly and had little effect on nutrient uptake and plant growth even though their numbers increased when fungi were present. It was suggested that the quantities of nutrients that were made available to the plant via mineralization by the fungus alone were probably sufficient for plant growth and that microbial grazing may be

important only when mineralization by microflora alone is not sufficient for the plant's needs.

1.3.6. INTERACTIONS BETWEEN MYCORRHIZAL FUNGI (OTHER THAN VA) AND MYCOPHAGOUS NEMATODES

Clark (1964) examined some *Rhododendron* cuttings from a commercial nursery which had failed to grow. No plant-parasitic nematodes were found but he counted very high numbers of a mycophagous *Deleadanus* species. He suggested that *Deleadanus* was destroying the mycorrhizas by feeding on the mycelia and that such effects may well be occurring in tree nurseries where mycorrhizal seedlings frequently grow poorly.

Since then, several species of mycophagous nematode have been shown to feed, reproduce and reduce the growth of a number of ericoid and ectomycorrhizal mycelia growing in pure culture (Sutherland & Fortin 1968; Riffle 1971; Shafer, Rhodes & Riedel 1981; Giannakis & Sanders 1990). Mycophagous nematodes have been reported to suppress the formation of ectomycorrhizas on pine seedlings by *Suillus granulatus* in agar culture (Sutherland & Fortin 1968; Riffle 1975). *Aphelchoides composticola* appears to reduce the growth of mycorrhizal pine seedlings in tube culture with soil (Hickson pers. comm.) and more experiments are currently under way in order to quantify this effect using pot-grown seedlings.

1.3.7. INTERACTIONS BETWEEN VAM FUNGI AND MYCOPHAGOUS NEMATODES

Mycorrhizal infection and stimulation of plant growth in field soils

Much evidence about the potentially beneficial effects of VAM fungi on nutrient uptake and growth of crops has been obtained from laboratory experiments, using partially sterilized soil. However, in non-sterile and field soils mycorrhizal responses are often weak or absent (Gerdemann 1964; Ross 1980; Hetrick, Kitt & Wilson 1986; Wilson, Hetrick & Kitt 1988, Hetrick *et al.* 1988). Mosse (1975) reported that considerable amounts of external mycelium were attached to roots infected with an

introduced endophyte in irradiated soil. In non-sterile soil however, very little mycelium was attached to roots infected by indigenous endophytes. Intermediate amounts were found ⁱⁿ non-sterile soil with roots infected by the introduced endophyte.

Amendments of sterile soil with either organic matter, non-sterile soil filtrates or sievings or different amounts of non-sterile soil reduced sporulation of the mycorrhizal fungi and levels of mycorrhizal infection or plant growth (Ross 1980; Hetrick, Kitt & Wilson 1986; Hetrick *et al.* 1988; Wilson, Hetrick & Kitt 1988, 1989). It has been suggested that these effects may be due to the activity of soil microorganisms which in nonsterile soils may compete for phosphorus. However, bacteria, actinomycetes and fungi are usually able to re-colonize heat-sterilized soils quite rapidly (Warcup 1957). In contrast, mycophagous soil fauna such as nematodes, springtails and mites which are frequently present in field soils (Curl 1988) are eliminated by those treatments designed to produce partial sterilization. Therefore, mycophagous fauna may be a consistently missing biological factor in experiments with partially sterile or sterile soils.

Even though stimulation of mycorrhizas in field soils by applying nematicides or insecticides has not always been achieved (Menge, Johnson & Minassian 1979), the results of such experiments have inspired research on interactions between soil fauna and VAM fungi. Ocampo & Hayman (1980) found that aldicarb, a nematicide/insecticide in general use, greatly increased the number of spores produced by VAM fungi as well as percentage infection of the roots in a field of barley. In a study where the target organisms of nematicide applications were more specifically identified, reduction in numbers of plant-parasitic nematodes was associated with an increase in VAM infection. It was suggested that the nematicide eliminated nematodes competing for the same infection sites on the roots as the VAM fungi (Bird, Rich & Glover 1974).

Bird, Rich & Glover (1974) did not measure the population changes of the abundant mycophagous nematodes, though these may have been equally affected by the nematicide. It is possible that mycophagous nematodes may have reduced mycorrhizal infection by feeding on the mycorrhizal hyphae. For example, Barker (1964) suggested that feeding of mycophagous nematodes on the mycelia of root-pathogenic fungi may be

responsible for variable appearance of root rots between seasons or fields. Ingham *et al.* (1986b) found that a reduction in numbers of nematodes when a nematicide was applied to a short grass prairie resulted in 6-10 fold increases in active arbuscular colonization and 1.5 fold increases in total VAM colonization. One possible explanation was that the nematicide killed nematodes which were feeding directly on the mycorrhizal mycelia. In another study, increases in mycorrhizal infection, rate of phosphorus accumulation and shoot growth were attributed to a reduction of numbers of Collembola caused by insecticide application (Finlay 1985).

Effects of mycophagous nematodes on VAM fungi

Studies on the grazing of VAM fungi by mycophagous nematodes are comparatively rare (see Ingham 1988). This is probably because the mycelia of these fungi have not yet been cultured successfully on agar, though considerable progress has been made towards understanding the factors involved in their independent growth (Mosse 1959; Warner & Mosse 1980; Hepper 1983; Azcon-Aguilar, Diaz-Rodriguez & Barea 1986a).

Salawu & Estey (1979), were the first to show that the simultaneous addition of a *Glomus sp.* and a high number of *Aphelenchus avenae* reduced the number of mycorrhizal spores and the yield of soybean infected with the fungus. Because nodulation by *Rhizobium*, which is often stimulated in mycorrhizal plants by the improved uptake of phosphorus, was also reduced, they concluded that *A. avenae* destroyed the mycelium of *Glomus* and consequently reduced uptake of phosphorus from the soil. The final numbers of nematodes were only 13% of those added.

The effects of *A. avenae* on the growth of mycorrhizal cotton were not so clear-cut (Hussey & Roncadori 1981). When nematodes were applied at a rate of 75 or 150 per 100 cm³ of soil and at the same time or 3 weeks after inoculation with *Gigaspora margarita*, there was no subsequent influence on nematode numbers and mycorrhizal infection and growth were unaffected. In a second experiment, greater numbers of *A. avenae* were applied (250, 1000 or 2000 per 100 cm³ of soil) simultaneously with inocula of *G. margarita* or *Glomus etunicatus*. On this occasion, nematodes at the two

higher rates reduced the shoot and root growth of plants which were mycorrhizal with *G. margarita*. The growth response of shoots to infection by *G. etunicatus* was reduced by the nematodes only when they were applied at the lowest rate. Mycorrhizal infection by either endophyte was not affected and the nematode numbers recovered at the end of the experiment were much lower than those in the first experiment. It could be inferred that the outcome of any interactions between VAM symbioses and mycophagous nematodes may be influenced by the sequence of inoculation with VAM fungi and nematodes, the species of plant or symbiont and the size of the initial nematode population.

Experiments using mycophagous Collembola rather than nematodes have given more consistent results. Although levels of mycorrhizal infection may not be reduced by Collembola, they may reduce the uptake of phosphorus by mycorrhizal plants and hence their growth response (Warnock, Fitter & Usher 1982; Finlay 1985). Finlay's results indicated that there was a non linear relationship between the initial density of Collembola and plant growth and nutrient uptake. Warnock, Fitter & Usher (1982) and Finlay (1985) found hyphal fragments and spores in the guts of Collembola and concluded that the animals had fed on the external mycelium of the fungi. This was shown directly by Moore, St. John & Coleman (1985).

1.4. AIMS AND OBJECTIVES OF THIS WORK

Two of my predecessors studied the effects of temperature and planting or propagule density on the spread of mycorrhizal infection and its stimulation of phosphorus uptake and plant growth (Sheikh 1984; Al-Nahidh 1985). Experimental data was compared with the predictions of theoretical models, an approach which appears to be useful in the understanding of the mechanism of infection spread (Sanders & Sheikh 1983; Sanders 1986 α,b).

The ultimate aim of the work described in this thesis, was to extend the methods developed by Sheikh (1984) and Al-Nahidh (1985) to investigate the hypothesis that mycophagous nematodes may reduce rates of colonization of root systems by VAM fungi and hence influence plant response to mycorrhizal infection.

By considering the factors determining rates of primary and secondary infection spread, it is possible to construct a simple model to describe how the nematodes may influence infection and plant growth. Spores of VAM fungi germinate in soil under adequately warm and moist conditions and produce a limited amount of mycelium. When a hypha encounters a root it may penetrate the epidermis and spread in the cortex forming an infection unit. Secondary infection occurs rapidly by hyphae which infect other roots or the same root further along its axis and a profuse external mycelium is formed. When fractional infection is plotted against time from planting, three distinct phases of VAM infection become apparent in a typical sigmoid curve (Fig. 1.1a). During the lag phase spores germinate, germ-tubes form and the host roots become penetrated. The logarithmic phase begins with the establishment of primary infection and continues with the rapid growth of external mycelium along the root surfaces initiating secondary infection units. This is followed by the phase of constancy during which the rate of increase in the length of mycorrhizal root is equal to that of total root length.

Mycophagous nematodes probably congregate in the rhizosphere where densities of fungal hyphae are usually higher than the bulk soil (Henderson & Katznelson 1961; Curl 1982; Curl 1988). Only exceptionally are they found inside roots and these are usually those colonized by pathogenic fungi (Hooper 1974). Possible deleterious effects of nematodes on mycorrhizal plants most likely result from damage to the external mycelium. The sequence in which soil is treated with nematodes or VAM inoculum may thus be important to the outcome of interactions between these organisms (see sections 1.3.1 & 1.3.7). Nematodes may affect plant-fungal complexes to a degree which depends on their numbers (see section 1.3.3). It was therefore decided to start by investigating the effects of inoculation sequence using very high initial numbers of nematodes.

High numbers of nematodes present during the period of the most rapid spread of the fungus, could feed, multiply and restrict or even stop this spread, leading to lower final levels of infection (Fig. 1.1b). Such an effect on the spread of VAM infection was observed when soil with mycorrhizal plants was treated with a fungicide (Hale & Sanders

1982). If the nematodes were introduced at the same time as the VAM inoculum, they could interfere with the primary infection process, leading to a delay in the development of the fungus (Fig. 1.1c). This interference could be caused by grazing of the limited amount of mycelium produced from germinating spores which would reduce the number of spores able to initiate infection.

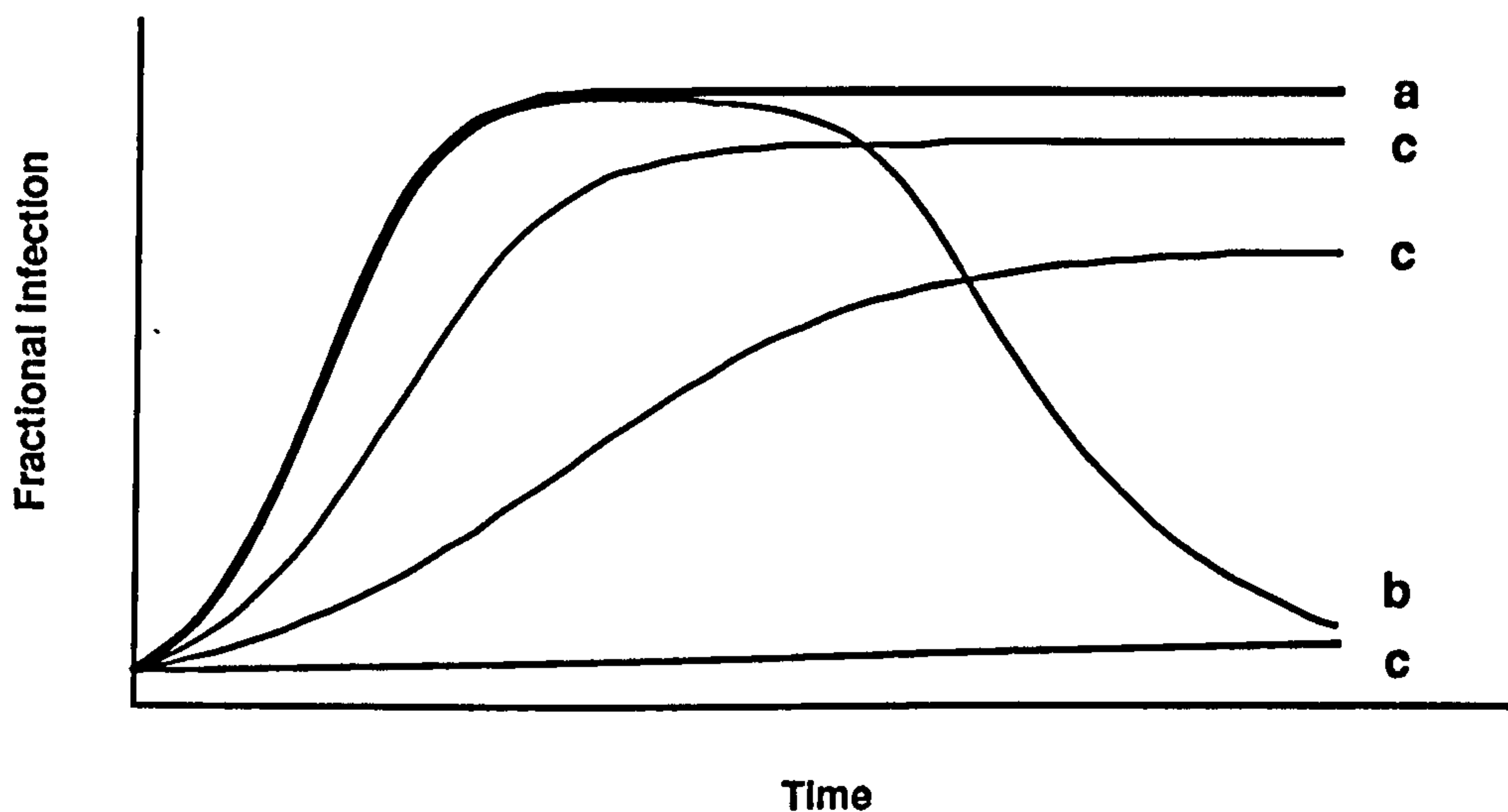


Fig. 1.1. The progress of fractional infection of plant roots by a VAM fungus in the absence of mycophagous nematodes [a], and that predicted under the influence of nematodes present during the period of the most rapid spread of the VAM fungus [b] or during the period when VAM infection first becomes established [c].

The following questions were asked:

1. Is there a relationship between the development of VAM fungi in root systems and mycophagous nematode populations and can such a relationship be revealed using the standard techniques for extraction of nematodes from soil?
2. Do mycophagous nematodes have food preferences among different species of fungi and how are these expressed? Does their feeding significantly damage the mycelia and is this damage related to their multiplication?
3. Do different species of mycophagous nematodes have different effects on the VA mycorrhizal symbiosis?

4. Does the outcome of the effects of these nematodes on VA mycorrhizas depend on the numbers of nematodes present in the soil?

CHAPTER 2. MATERIALS AND METHODS

2.1. CULTURE OF THE ORGANISMS

Table 2.1 lists the organisms used in the various experiments and gives the abbreviations of their scientific names subsequently referred to in the figure legends throughout this thesis.

Table 2.1. The species of organisms used in the experiments described in this thesis.

Plant	Red clover (<i>Trifolium pratense</i> L. cv. Hungaropoly)
Saprotrophic fungi	<i>Agaricus bisporus</i> (Singer) <i>Rhizoctonia solani</i> (Kühn) Unidentified (D) Unidentified (C)
Ectomycorrhizal fungi	<i>Cenococcum graniforme</i> (Sow.) Ferd & Winge <i>Hebeloma crustuliniforme</i> (Bull.: Fr.) Quel. <i>Laccaria laccata</i> (Scop.: Fr.) Berk. & Br. <i>Paxillus involutus</i> (Batsch) Fr. <i>Rhizopogon roseolus</i> (Cda.) Th. Fr.
VAM fungus	<i>Glomus clarum</i> (Nicolson & Schenck)
Mycophagous nematodes	<i>Aphelenchoides composticola</i> (Franklin) <i>Aphelenchus avenae</i> (Bastian) <i>Ditylenchus myceliophagus</i> (Goodey) <i>Ditylenchus triformis</i> (Hirschmann & Sasser)

2.1.1. VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI

Since VAM fungi have not yet been cultured on agar they have to be grown in association with roots of plants in pots of soil which has previously been sterilized.

Cultures of the VAM fungus *Glomus clarum* were prepared using inoculum which consisted of spores, hyphae and mycorrhizal root segments mixed with soil. Layers of inoculum were placed at about 5 cm below the surface of sterile soil in 1.5 Kg pots. The soil was watered to field capacity and red clover seed was sown on the surface. A 2 cm layer of alkathene beads was added to cover the seed and pots were placed in the growth room. Emerging seedlings were later thinned to an eventual 5 per pot. Three months after sowing, soil in these pots was transformed to new mycorrhizal inoculum, often referred to as crude inoculum, usually containing around 100 spores/g soil.

Crude inoculum was collected by harvesting the pots when the moisture content of the soil was sufficiently low for the plants to start wilting. Shoots were cut and discarded and the contents of the pots were sieved through a 1 cm sieve to remove the thicker roots. The inoculum was placed in thick plastic bags, which were sealed and kept at 4 °C. Crude inoculum prepared and stored in this manner remains viable for 3 or 4 years.

2.1.2. ECTOMYCORRHIZAL AND SAPROTROPHIC FUNGI

Pure cultures of these fungi were already available on various media and they were subcultured on a potato dextrose agar medium made by dissolving 39 g of potato dextrose agar powder (4 g potato extract, 20 g dextrose and 15 g agar) in 1000 g of water. Plates were sealed with parafilm in order to avoid contamination and invasion by mites and were kept in cupboards at room temperature. All fungi could be grown successfully on this medium.

The non-mycorrhizal saprotrophs were also grown on moist bran. 15 g of bran and 30 g of water were weighed into a milk bottle, stoppered with a cotton wool plug covered with aluminum foil. The bottles were sterilized in a pressure cooker for 20 minutes, allowed to cool and inoculated with pieces of agar cultures. Bran colonized by mycelium proved ideal for inoculating pot soil.

2.1.2. NEMATODES

Mycophagous nematodes can be grown in agar plates on cultures of a variety of fungal species by inoculating with a block of agar from a mature nematode culture. At room temperature, destruction of the aerial mycelium of the fungus by the nematodes usually occurred within 15-30 days from the time of their introduction (Plate 2.1). Mature cultures of nematodes contained up to 10^6 worms per plate. As shown in Table 2.1, the four species of nematodes that were cultured were *Ditylenchus triformis*, *Ditylenchus myceliophagus*, *Aphelenchoides composticola* and *Aphelenchus avenae*. Eventually these were all grown routinely on *Agaricus bisporus*. Only *D. myceliophagus* and *A. composticola* originated from cultures on this fungus.

D. triformis was initially obtained in culture on *Rhizoctonia solani* and *A. avenae* on an unknown sterile mycelium. It was necessary to grow all species on fungi which were non-pathogenic to plants, so that suspensions of nematodes washed from the cultures and used to inoculate pots would not contain propagules of pathogens. *Agaricus bisporus* was a suitable fungus, being a good nematode host and non-pathogenic to plants, and therefore both *D. triformis* and *A. avenae* were transferred onto this fungus. This was done by surface-sterilizing the nematodes by a method similar to that described by Watrud (1982) for mycorrhizal spores.

The nematodes were washed off the lids of their petri-dish cultures with sterile water and collected in a sterile Eppendorf vial which was spun in a centrifuge for about 1/2 minute. Nematodes settled at the bottom of the vial, the supernatant water was discarded with a sterile pipette and a 5% (w/v) solution of Chloramine-T was added. After 20 minutes the solution was centrifuged again, the supernatant discarded and the nematodes rinsed three times, following the same procedure with pipette and centrifuge. A solution of streptomycin sulphate (0.025%) was added and allowed to act for 20 minutes before rinsing with water 5 more times using centrifugation.

Cultures of *A. bisporus* were inoculated with drops of the clean suspension of surface-sterilized nematodes. Bacterial contamination did occur but was limited to the

areas where drops with nematodes had been applied. It was eventually possible to obtain bacteria-free cultures by transferring uncontaminated blocks of agar with nematodes to other fungal cultures.

2.2. POT EXPERIMENTS

2.2.1. PREPARATION OF SOIL

The soil used was a calcareous sandy clay loam mixed with coarse horticultural sand at a 2:1 ratio, except where otherwise stated. The clay loam was collected from a field of the Leeds University Farm (Wothersome series, Crompton & Mathews 1970) and had the following characteristics (Alloush pers. comm.):

1. Content of Olsen available phosphorus = 19.5 mg/kg except where otherwise stated.
2. Texture: 28.33% sand, 51.04% silt and 20.63% clay.
3. Content of CaCO₃ = 16.9%.
4. pH = 7.7

Soil was partially sterilized by heating at 95 °C in a drying oven for three days.

2.2.2. GROWTH ROOM CONDITIONS

All pot experiments were carried out in a growth room (Plate 2.2). The temperature was set at 25 °C for the 16 hour long light period and at 20 °C for the 8 hours of dark. The photon flux density was 310 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (Incoll, Long & Ashmore 1977) and the relative humidity around 70%.

2.2.3. HARVESTS

Weights of shoots and roots

Shoots were cut off at their base, placed in paper bags and left overnight in a drying oven set at 95 °C. Dried shoots were weighed and kept in sealed plastic bags.

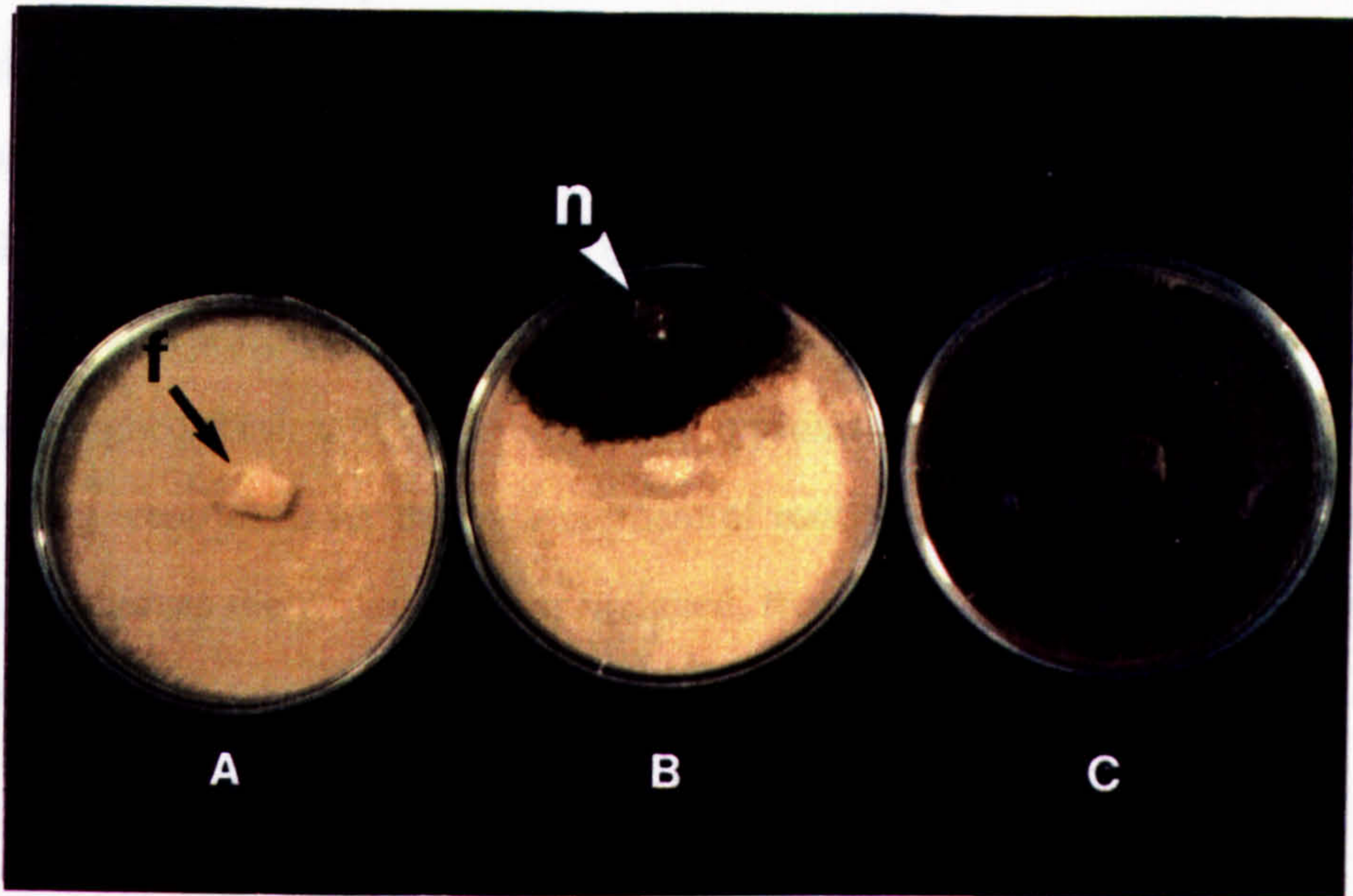


Plate 2.1. The progress of a culture of nematodes (*A. avenae*) in agar plates with a sterile mycelium.

A: The mycelium spreads out from the inoculum of the fungus (f) and occupies the whole surface of the agar. B: The nematodes start to feed on and damage the mycelium in the area around their inoculum (n). C: A mature culture of nematodes. The mycelium is completely destroyed as a result of the feeding of the nematodes.



Plate 2.2. View of the interior of the growth room with randomly arranged pots with red clover plants. Also shown are the black alkathene beads (b) which were used to cover the surface of the soil and the characteristic positive response of the growth of plants to mycorrhizal infection with *G. clarum* (compare c = control to m = mycorrhizal).

Roots had to be separated from the soil and the way that this was done depended on which method was used for the extraction of nematodes as well as on the size of pots. In experiments 1 and 2 the mixture of soil and roots was spread in a tray and roots were picked out with a pair of forceps. In all other experiments, roots were separated from the soil by repeated washing and sieving. Clean root systems were blotted dry using paper towels, weighed fresh and cut in segments approximately 1 cm long. Subsamples of about 0.5 g were taken for the measurement of root length and fractional mycorrhizal infection whereas the remainder was weighed, fresh and dry. Total dry weights could then be calculated.

Root lengths

Root length was measured using a gridline intersect method (see Newman 1966; Marsh 1971; Tennant 1975). 0.5 g samples of root segments were placed in a tray marked with a grid of 14/11 cm squares. Root segments were spread on the grid with the help of an appropriate amount of water and their intersections with the lines of the grid were counted. The number of intersections was equal to the length of the root sample in cm. The total length of the root system in meters (L) was then calculated from the following equation:

$$L = \frac{W l}{w 100}$$

where W is the total fresh weight of the root, l the estimated length of the sample in cm and w the weight of the sample.

The precision and accuracy of the method were tested as follows. A thread which had a known length and had been cut in 1 cm pieces was placed on the grid, the intersects with the grid lines were counted and the pieces were re-arranged before the next count. The procedure was repeated 9 times giving ten replicate counts (Table 2.2) the coefficient of variation of which showed that the method was acceptably precise (Tennant 1975). In addition, the mean estimated length did not differ significantly from the true length of the thread (Bailey 1983) and therefore the method was also sufficiently accurate.

Table 2.2. The efficiency of the gridline intersect method for measuring the length of a string with a known length. CV is the coefficient of variation.

Measured length of string (cm)										True length (cm)		
Replicates										Mean	CV	
287	292	271	287	291	249	283	271	276	280	279	4.62%	286

Mycorrhizal fraction of the root length

Subsamples of root segments were stained with 0.05% trypan blue in lactophenol (Phillips & Hayman 1970) and a modification of the gridline intersect method (Giovannetti & Mosse 1980) was used to measure the fractional infection under a stereomicroscope. It was found, using two threads of equal length and different colour, that the method could give an accurate result and had a precision of $\pm 8\%$ (Table 2.3). It should be noted that the gridline intersect method and its modifications can only provide a measure of infected root length and not of root volume or biomass, because the roots appear as two dimensional rather than three dimensional objects when observed under the microscope.

Table 2.3. A test of the gridline intersect method for measuring the fraction of a string length which had a blue colour. CV is the coefficient of variation.

Measured fraction (with blue colour)										True fraction		
Replicates										Mean	CV	
.51	.43	.54	.51	.49	.43	.52	.52	.51	.49	.50	7.51%	.50

Extraction of nematodes from soil

Two versions of Seinhorst's one-flask method were used initially but were later found to be completely unsatisfactory for the particular experimental requirements and species of nematodes. During the search of a quantitative technique, attention was focused on the Baermann funnel because it has been reported to give satisfactory

extraction of the more mobile and smaller free-living nematodes. Two new modifications of this technique were tested one of which was shown to be quite adequate for the requirements of this work. All extractions were carried out at a room temperature of 17-21 °C.

Seinhorst's flask, version SF1

In order to extract nematodes from pot soil, roots were removed and the soil mixed. A sample of 150 g soil was taken and mixed with about 500 g of water in a beaker. The suspension was passed through a 1 mm sieve and poured in a 2 litre conical flask to whose mouth a funnel could be attached by means of a ground glass joint.

The flask was topped-up with water, shaken and inverted over a beaker of water with the orifice of the funnel just immersed. It was left in that position for 10 min after which the suspension was poured in a 30 µm nylon mesh sieve while gently stroking the underside of the screen to keep it unblocked. The residues were collected from the screen by backwashing the sieve with a weak jet of water and collected in a beaker whose contents were then emptied in the conical flask which was topped-up with water and the procedure was repeated.

The final suspension contained a lot of debris and had to be cleaned by a method based on the principle of the Baermann funnel. Thus, the suspension was poured gently into a 6 cm sieve which rested in a bowl and comprised of a piece of tissue paper (Kleenex Medical Wipes) supported on a nylon screen which was held between two concentric rings of PVC tubing. If necessary, extra water was added in the bowl to ensure that the tissue paper was sufficiently moistened. Nematodes swam through the screen and into the water whereas soil debris was held behind by the tissue. After 48 hours, the nematodes were collected from the bowl and counted in three replicate samples. This method was only used in the first experiment and its efficiency of recovery was not evaluated.

Seinhorst's flask, version SF2

This was a combination and modification of the methods described by Seinhorst (1956) and Hooper (1986) and an improvement of SF1. Roots were removed as before and the soil mixed and sampled. The soil was separated from the roots, mixed and a sample of approximately 200 g was weighed in a large plastic beaker to which 500 g of water were added. The suspension was stirred gently and poured through a domestic hemispherical sieve with an aperture of 2 mm in order to remove stones and other material. The sieve nested in a plastic funnel with a suitable diameter which was supported over the 2 litre conical flask and had its stem closed with a rubber bung attached to the end of a string.

The sieve was run up and down a few times, while immersed in the suspension in the funnel, to allow all particles smaller than 2 mm to pass through, the bung was removed by pulling the string and the suspension allowed to run in the flask which was filled with water. The residues of the sieve were rinsed with a small amount of water which was collected in the flask that was topped-up and the extraction was carried out as in SF1. The difference in this case was that the suspension in the flask was sieved 5 times through a 53 μm instead of once through a 30 μm sieve in order to allow more clay particles to pass through and therefore avoid blockage and obtain cleaner samples. This was also facilitated by the much greater diameter of the sieve which was 20 cm as recommended by Seinhorst (1956). Residues were collected from the sieve each time before the suspension was passed through it again. The cleaner suspensions of these residues did not require processing through the apparatus described in SF1 and as a result the losses of nematodes due to that extra step would not occur if SF2 was used.

In order to determine the efficiency of recovery of the method, 2 treatments with 5 replicates each were employed. In treatment 1, a small suspension containing approximately 5000 *A. composticola*, was added to 2000 g of water which was then simply sieved five times through the 53 μm sieve and the nematodes were collected. In treatment 2, the same number of nematodes was added to 50 g soil which were then suspended in 1000 g of water and the whole extraction procedure was then carried out as

described above. The two treatments were selected in order to differentiate the losses during the first stage of mixing the soil in water and settling in the flask from losses during the second stage of sieving as well as to examine the influence of soil itself on the recovery of the nematodes. All water used was maintained at a constant temperature of 25 °C to avoid any effect of temperature on the settling rates of nematodes.

Results are given on Table 2.4 which shows the great variation between replicates and the low percentage recovery of nematodes from both treatments. Apparently most nematodes were lost by slipping through the sieve. The efficiency of the method was affected very little by the presence of soil in the sieved suspension, since the numbers of nematodes in the two treatments were not significantly different although the sieve became blocked when soil was included.

Table 2.4. Test of Seinhorst's single flask method (SF2) for the extraction of nematodes from soil.

	Replicates					Mean	CV	Recovery
	1	2	3	4	5			
N in inoculum	-	-	-	-	-	5000	-	-
N from water alone	627	1201	980	1359	905	1014 b	27%	20%
N from soil plus water	839	1079	1060	503	470	790 b	37%	16%

N: Number of nematodes.

CV: Coefficient of variation.

Means followed by the same letter do not differ significantly at $p < 0.05$. Statistical analysis was carried-out using the square roots of the numbers of nematodes.

The Baermann funnel (BF)

Tissue paper (Kleenex Medical Wipes) was supported on a 6 cm diameter sieve made of a coarse nylon gauze held between two PVC rings. The sieve was placed in a glass funnel which had a short piece of rubber tubing closed with a pinch clamp attached to the end of its stem. BF was not used in any harvest but its recovery potential was determined with the scope of creating another and perhaps more suitable version.

10 g of sterile soil were moistened slightly and spread on the tissue in the sieve. Approximately 2000 *A. composticola* were added to the soil suspended in a few drops of water. The sieve was placed in the funnel which was filled with water until the soil on the tissue was just saturated. Four replicate funnels were prepared and after 16 hours the clamp was released and about 20 g of water were collected from the bottom of the funnel stem where nematodes had settled. Nematodes were counted by sampling the suspension in triplicate. The efficiency of extraction was 75 % and the coefficient of variation only 1.45% (Table 2.5) which clearly indicated that indirect extraction offered the potential for a much improved and near quantitative extraction of these nematodes from soils.

Table 2.5. Test of the Baermann funnel (BF) for the extraction of nematodes from soil.

	Replicates				Mean	CV	Recovery
	1	2	3	4			
N in inoculum	-	-	-	-	2000	-	-
N recovered	1526	1508	1496	1546	1519	1.45%	75%

N: Number of nematodes.
CV: Coefficient of variation.

Baermann funnel, version BF1

The reliability of funnels as well as screens with tissue papers has been criticized (see 1.2.1). For this reason, the funnel was replaced by a crystallizing dish and the coarse nylon screen and tissue paper of the sieve were replaced by a single 53 μm nylon gauze. The dish had a diameter of 9 cm and was 5 cm deep and inside it, the sieve was supported on three thin rings cut from a rubber tube with a diameter of 1 cm.

Tests of extraction efficiencies by inoculating a soil sample with nematodes and extracting them immediately after are often misleading because in practice, extractions of nematodes may be carried-out quite a long time after these are applied (see 1.2.1). A series of treatments was selected, in order to investigate the effect of time, during which nematodes remained in soil before extraction began (soil time) on the recovery of

nematodes. The effect of extraction duration (extraction time) was also examined by the use of a 12 and a 24 hour period of extraction.

10 g of sterile soil were moistened and spread on the nylon screen and a 4 g suspension containing approximately 1000 *D. myceliophagus* was pipetted on the soil. Nematodes were extracted immediately (treatment 0) or were left in the soil for 12, 24, 48, or 72 hours before extraction was initiated (treatments 12, 24, 48 and 72 respectively). The control was a 4 g suspension of 1000 nematodes made up to 20 g with water and kept in a vial. Every time nematodes extracted from the various treatments were counted, the proportion of dead nematodes in the control was also determined as an estimate of the proportion of nematodes which had died in the soil samples of the treatments at the time of the extraction, and therefore could not be recovered.

Fig. 2.1 shows that for both extraction times of 12 and 24 hours, the recovered numbers declined up to 36 hours after the start of the experiment but remained on a plateau after that time. The means at 60 and 72 hours (treatment 48) were below the plateau because of a very low value of one replicate as shown on Table 2.6. Greater numbers of nematodes were extracted with a 24 hour extraction period.

The time, allowed between introduction and initiation of extraction, had a significant effect on recovery but only up to 36 hours, after which the numbers of nematodes extracted were not significantly smaller (Fig. 2.1).

The way that the method was used in the harvests of experiments 5 and 6 (sections 3.3 & 3.4) is as follows. Soil and roots from the pots was emptied in a tray, cut to four pieces and mixed thoroughly. In order to achieve this the soil had to have a water content at 50% of field capacity. Approximately 13 g of moist soil and roots were placed in the sieve and extraction was carried out for 16 hours.

Baermann funnel, version BF2

Sieves with a 14.5 cm diameter were made out of two concentric uPVC rings with a piece of nylon gauze which had an aperture of 53 μm stretched between them.

The efficiency of this technique was tested by extracting nematodes from 210 g of dry sterile soil, an amount contained in each pot of all experiments with which BF2 was

Table 2.6. Test of a Baermann funnel modification (BF1) for the extraction of nematodes from soil. CV is the coefficient of variation.

Treatments	Extraction period (hours)	Numbers of nematodes					Mean	CV	Recovery
		1	2	3	4	5			
Control	-	980	873	966	1081	983	977	7.56%	-
0	12	951	828	924	765	700	834	12.63%	85.36%
	24	991	845	941	784	738	860	12.26%	88.02%
12	12	895	766	650	661	663	724	14.90%	74.10%
	24	982	833	713	736	754	804	13.63%	82.29%
24	12	690	672	598	714	661	668	6.70%	68.37%
	24	776	730	695	784	724	742	5.02%	75.95%
48	12	379	677	647	570	661	587	21.04%	60.08%
	24	455	742	725	634	728	657	18.40%	67.25%
72	12	522	671	707	585	727	642	13.46%	65.71%
	24	547	771	761	686	765	706	13.50%	72.26%

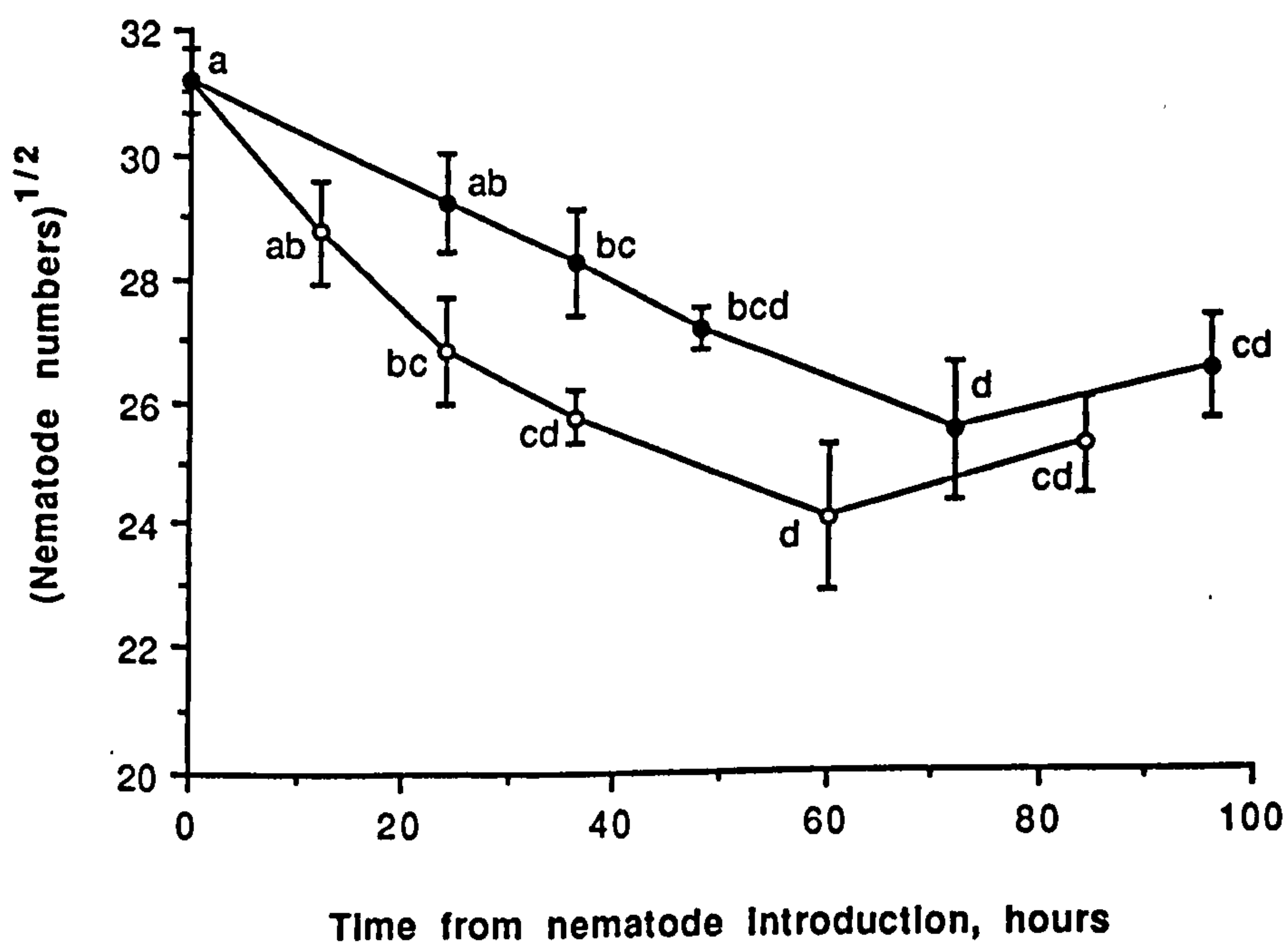


Fig. 2.1. The effect of time after the introduction of nematodes to soil on the numbers (expressed as numbers^{1/2}) of nematodes recovered from soil using a modified Baermann funnel (method BF1) and an extraction time of either 24 (●) or 12 hours (○).

Points along each line which are followed by the same letter, are not significantly different at $p < 0.05$. Bars represent ± 1 standard error.

used. An 8 g suspension of 23000 *A. avenae*, was pipetted onto the soil contained in a pot watered at 50% of field capacity. Three replicate pots were prepared. The soil from the pots was emptied into the sieves described above which were placed in enamel dishes of a slightly greater diameter (Plate 2.3a). Approximately 150 g of water were poured to just saturate the soil. Dishes were left covered with a plastic sheet for 16 hours. The sieves were then removed and the nematode suspensions underneath were poured into beakers allowed to stand overnight. Nematodes settled to the bottom of the beakers and supernatant water was removed by suction, leaving behind a concentrated suspension weighing 25-50 g.

Results are shown on Table 2.7. Percentage recovery was high (83%) and very similar to that of treatment 0 in the test of BF1. Variation was also small even though larger soil samples and volumes of water were involved in BF2 than in BF1. The effect of the settling procedure which followed that of extraction, was examined by pipetting an 8 g suspension with 23000 nematodes in a beaker. Three replicate beakers were prepared and left undisturbed for 12 hours which was chosen as a minimum overnight period. After that time suspensions were concentrated by sucking out the supernatants with the water pump. Numbers recovered were not significantly smaller than those applied.

Table 2.7. Test of a Baermann funnel modification (BF2) for the extraction of nematodes from soil.

	Replicates			Mean	CV	Recovery
	1	2	3			
N in inoculum	22720	24240	22880	23280 a	3.59%	-
N after settling	22570	22687	22629	22629 a	0.26%	97.20%
N after extraction + settling	19720	19530	18450	19233 b	3.56%	82.62%

N: Number of nematodes.

CV: Coefficient of variation.

Means followed by the same letter do not differ significantly at $p < 0.05$. Statistical analysis was carried-out using the square roots of the numbers of nematodes.

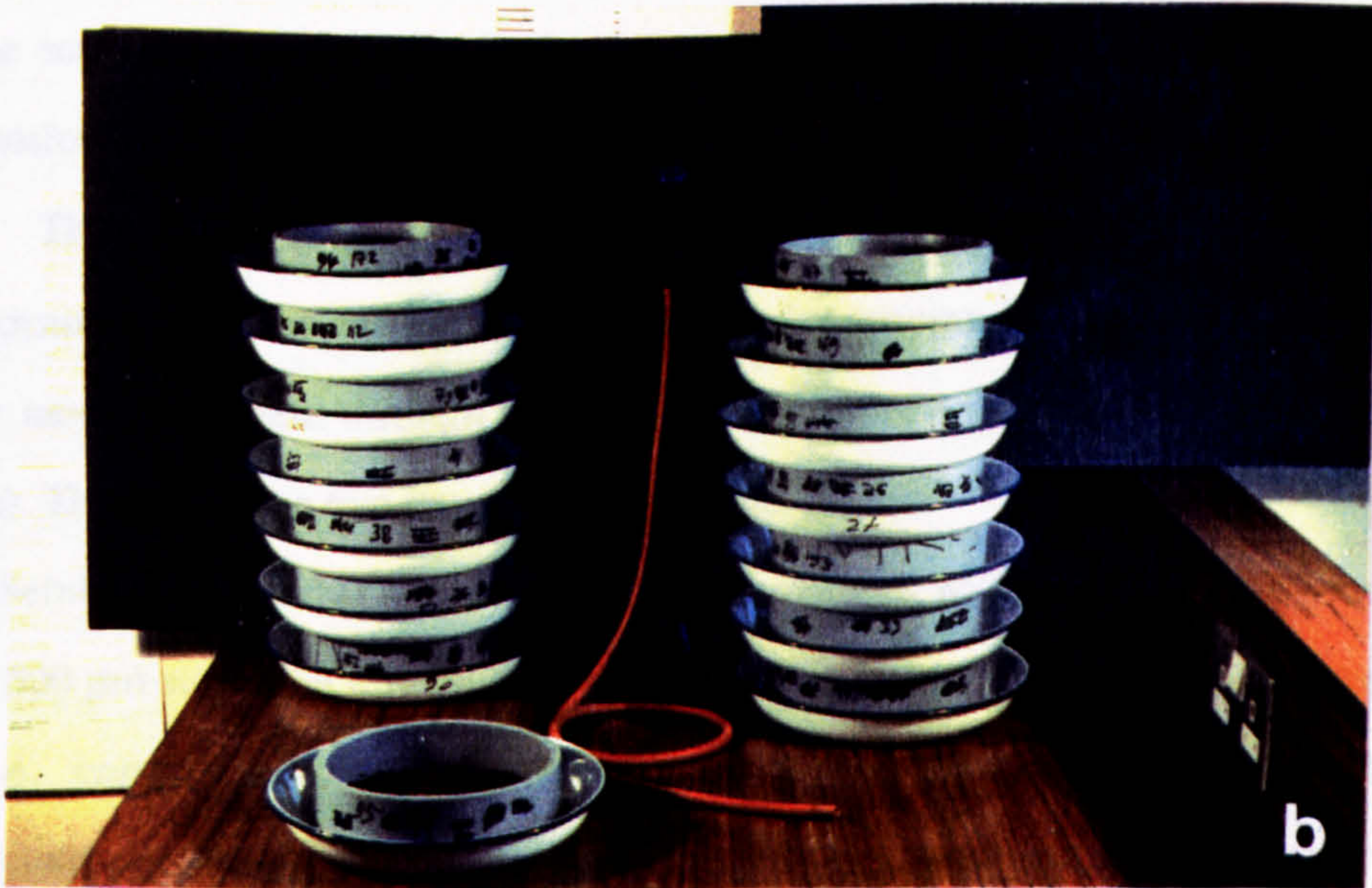
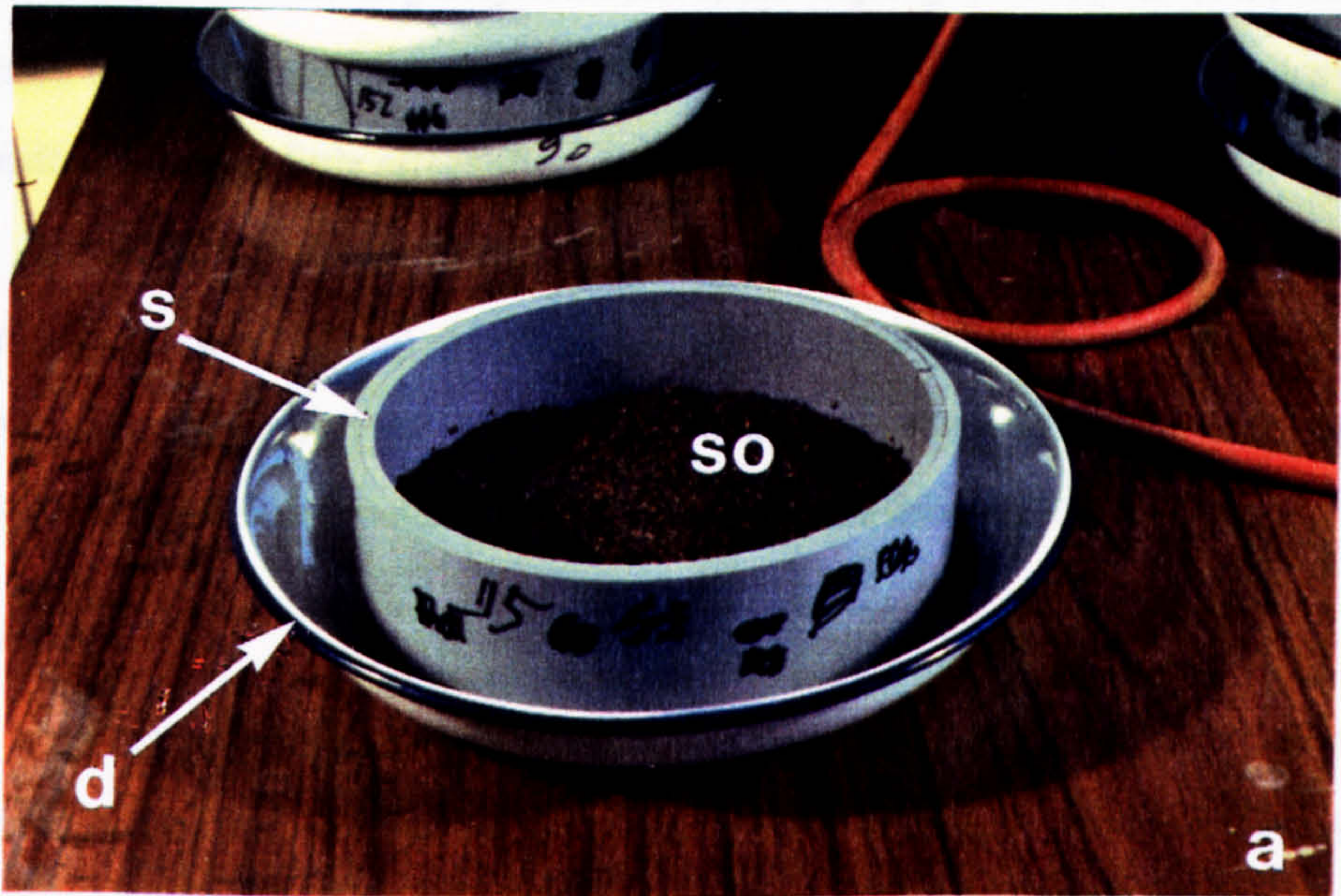


Plate 2.3. The modification of the Baermann funnel BF2 (a) in use for the extraction of nematodes from soil. Dishes could be stacked (b) to save space.

s = sieve, so = soil, d = enamel dish.

The number of nematodes which were recovered by extraction and settling was smaller than the number after settling only. Therefore, the majority of losses occurred during the extraction from the soil whereas the settling procedure which was used to concentrate the suspensions had very little effect on recovery.

To extract nematodes from pots with growing plants, pots were emptied into large plastic beakers, and the soil and roots were broken apart and mixed thoroughly. The mixture was then emptied into sieves and nematodes extracted following the procedure described above. Dishes could be stacked to save space (Plate 2.3b).

Conclusion

The 30 μm sieve of SF1 became blocked very easily. McSorley & Parrado (1981), suggested that when sieves get blocked and as a consequence the time needed for the suspension to pass through increases, nematodes which would otherwise be collected on the screen may have enough time to settle and crawl through its pores while the suspension is still slowly draining.

The variable and low recoveries of SF2, clearly demonstrated that this technique was unsuitable for the extraction of the particular species of nematodes from the soil. Many nematodes went through the sieve in contradiction of the findings of Seinhorst (1956). There could be two possible explanations for this:

1. Seinhorst found that almost 99% of the nematodes with an average length of 500 μm stayed on a sieve with an aperture of 50 μm but the larval stages of *A. composticola* are smaller than this. The age structure of the *A. composticola* population which was tested may have been such that only a small proportion of nematodes were full-length adults.
2. Seinhorst's method depended on the skill of the operator which highlights the need for simpler extraction procedures involving less human intervention.

The Baermann funnel (BF), gave much better and more consistent results with the same species of nematode and recoveries were further improved when the method was modified (BF1 and BF2).

BF1, required subsampling as only 15 g could be extracted in each sieve. Since nematodes are usually not uniformly distributed in soil (Barker & Campbell 1981) and may congregate around their food source producing a clumped distribution (Thorp 1987) one small subsample from each pot will give variable results and may not allow accurate estimation of the total population (see Fig. 3.10).

BF2 avoided sampling errors by allowing the extraction from the whole of the soil contained in a 7 cm square pot. This was very convenient and saved considerable time. The extraction time was chosen as a compromise between a long period which would give high recoveries but also opportunity for eggs to hatch and a short period which would yield fewer nematodes but allow less hatching and deterioration of roots in the soil sample. Since the majority of nematodes were extracted between 12 and 16 hours after the initiation of extraction an extraction time of 16 hours was selected, allowing extractions to be carried out overnight. These nematodes are most active at temperatures of around 20 °C and extractions carried out at room temperature (17-22 °C) were therefore satisfactory.

The incomplete recovery of nematodes during the tests of BF, BF1 or BF2 cannot be explained. The nematodes may have been damaged mechanically during their introduction to the soil or during the handling of the soil samples in the process of extraction. Alternatively, trapping of nematodes has been suggested as a cause of recovery errors (Harrison & Green 1976). Moulting may be another important factor that could affect extraction efficiency since moulting larvae may be less active (Flegg 1967).

The efficiency of BF2 was measured by extracting nematodes immediately after their introduction to a soil sample. Since recovery was found to decrease slightly with the time between introduction and initiation of extraction of the nematodes (see Fig. 2.1; Table 2.6), it is possible that the efficiency of BF2 is actually smaller. However, the results of the tests clearly showed that the Baermann funnel and its modifications were more reliable than the Seinhorst flask, for the extraction of the small but active free-living nematodes from soil. Method BF2 fitted well with the general methodology of the experiments and harvest schedules.

Nematode counting techniques

After their extraction from soil, nematodes were collected and suspended in water. The suspension was weighed, mixed thoroughly with a dropping pipette and samples were transferred to a 1 cm³ Eelworm Counting Slide (Peters 1952). Nematodes were counted under the stereomicroscope using a 25x magnification. According to Peters (1952), one count of a thoroughly mixed suspension is enough and replicate counts are not required particularly in experiments with replicated treatments.

Counts should follow a Poisson rather than normal distribution (Peters 1952) which meant that analyses of variance could only be carried out on the square roots of the nematode numbers (Elliott 1971).

2.3. MICROSCOPY, PHOTOGRAPHY AND STATISTICAL ANALYSIS.

Microscopical examination was carried out using a Wild M8 Zoom Stereomicroscope with magnifications ranging from 6x to 75x and with both incident and transmitted illumination. A Wild Photoautomat MPS50, MPS551 shutterpiece and MPS55 electronic control unit was fitted to the microscope and pictures were taken using Kodak Ectachrome (160 ASA) film. Standard photographs were taken with a Canon T70 camera with an 80B filter and tungsten lamps for illumination. Nematode feeding behaviour was observed and photographed with a microscope and camera (Jenamed, Carl Zeiss Jena).

Statistical analyses were carried out on the Amdahl, a Leeds University Mainframe Computer, using the SAS package, version 5.18.

CHAPTER 3. PRELIMINARY EXPERIMENTS ON THE INTERACTIONS BETWEEN NEMATODES AND MYCORRHIZAS OF RED CLOVER

3.1. EXPERIMENTS 1 AND 2.

3.1.1. INTRODUCTION

In the preliminary stage of the work, attention was focused on introducing the nematode factor to an experimental system similar to that used in previous work with VAM fungi and plants in the growth room (Sheikh 1984; Al-Nahidh 1985). The aim was to investigate the hypothesis that if high numbers of nematodes were added to plants being colonized by a VAM fungus at the stage of its most rapid growth, they could stop the spread of mycorrhizal infection and reduce the mycorrhizal response of the plant. A simple factorial design was employed.

3.1.2. MATERIALS AND METHODS

Experiment 1

Mycorrhizal plants were grown in a mixture of 200 g of *Glomus clarum* inoculum and 1300 g of sterile soil whereas non-mycorrhizal plants were grown in 1500 g of sterile soil. Each treatment was replicated 6 times. Pots were completely randomized in the growth room and the soil was watered from below. Clover seed was sown on the soil surface which was then covered with a layer of black alkathene beads to minimize evaporation and prevent the growth of algae or mosses.

Seedlings were progressively thinned to an eventual 5 per pot. *Ditylenchus trififormis* was applied around the seedlings at a rate of 40000 per pot (27/g of soil) as soon as the mycorrhizal growth response was evident at 47 days after sowing. Pots were harvested on day 68. Fresh and dry weights of shoots and roots, root lengths and levels

of infection of roots were measured. Nematode extractions were carried out using Seinhorst's flask, version SF1.

Experiment 2

Pots were prepared in the same way as before, except that the mycorrhizal inoculum was not mixed with the soil but added as a layer approximately 4 cm under the soil surface. Also non-mycorrhizal pots received 10 g of filtrate from crushed roots to standardize the contaminant microflora (see Abbott & Robson 1984) and each treatment had 4 replicates.

The numbers of *D. triformis* in the inoculum were increased to 70000 per pot (47/g soil) and since addition of *G. clarum* inoculum in a layer form resulted in an earlier mycorrhizal response nematodes were applied at the earlier time of 27 days after sowing. Pots were harvested 62 days after sowing in the same way as for experiment 1. Leaf area was also measured using a Delta-t area meter. Nematodes were extracted from soil with Seinhorst's flask, version SF2.

3.1.3. RESULTS AND DISCUSSION

Inoculation with *G. clarum* increased the dry weights of the shoots, roots and whole plants as well as root lengths and leaf areas (Figs 3.1a–d & 3.2a–c). *D. triformis* did not affect mycorrhizal infection, nor did it influence the mycorrhizal response of the plant (Figs 3.1a–e & 3.2a–d). Nematodes had no effect on the growth of non-mycorrhizal plants either.

In experiment 1, the numbers of nematodes which were recovered from the pots were extremely variable but apparently did not differ between mycorrhizal and non-mycorrhizal treatments (Fig. 3.1f). In experiment 2 there was less variation and numbers were higher in mycorrhizal than in non-mycorrhizal soils (Fig. 3.2e). Only around 5% or 10% of the nematodes applied initially were recovered from the soil at the end of the first and second experiments respectively. This was not surprising since the methods of extraction which were used probably recovered no more than 16% of the nematodes added to a soil (see Table 2.4, section 2.2.3). However, the numbers extracted were

much lower than expected, on the basis of the probable per cent recovery during extraction. Other factors may therefore ^{have} been involved:

1. If the numbers of nematodes added initially, were too high in relation to the fungal substrate available in the soil, the majority of nematodes may then have died of starvation, disintegrated and not therefore have been recovered.
2. Heat treatment of the soil may release toxins which are detrimental to the growth of microorganisms or plants (Abbott & Robson 1984; Rovira & Bowen 1966). Such toxic substances may have contributed to the loss of nematode numbers. Salawu & Estey (1979) recovered only 12.5% of *A. avenae* which were applied to steam sterilized soil treated with VAM inoculum and Hussey & Roncadori (1981), attributed similar low recoveries of *A. avenae* from soil with mycorrhizal plants to an unidentified soil factor.
3. If *D. triformis* was not able to feed on *G. clarum*, it could only maintain its population by feeding on the smaller biomass of non-mycorrhizal soil fungi. This would explain why numbers did not differ between mycorrhizal and non-mycorrhizal treatments and their little effect on the level of mycorrhizal infection.

In the following experiments in this chapter, the above possibilities were further investigated. At the same time efforts were made to find a better method for the quantitative extraction of nematodes from soil (see section 2.2.3).

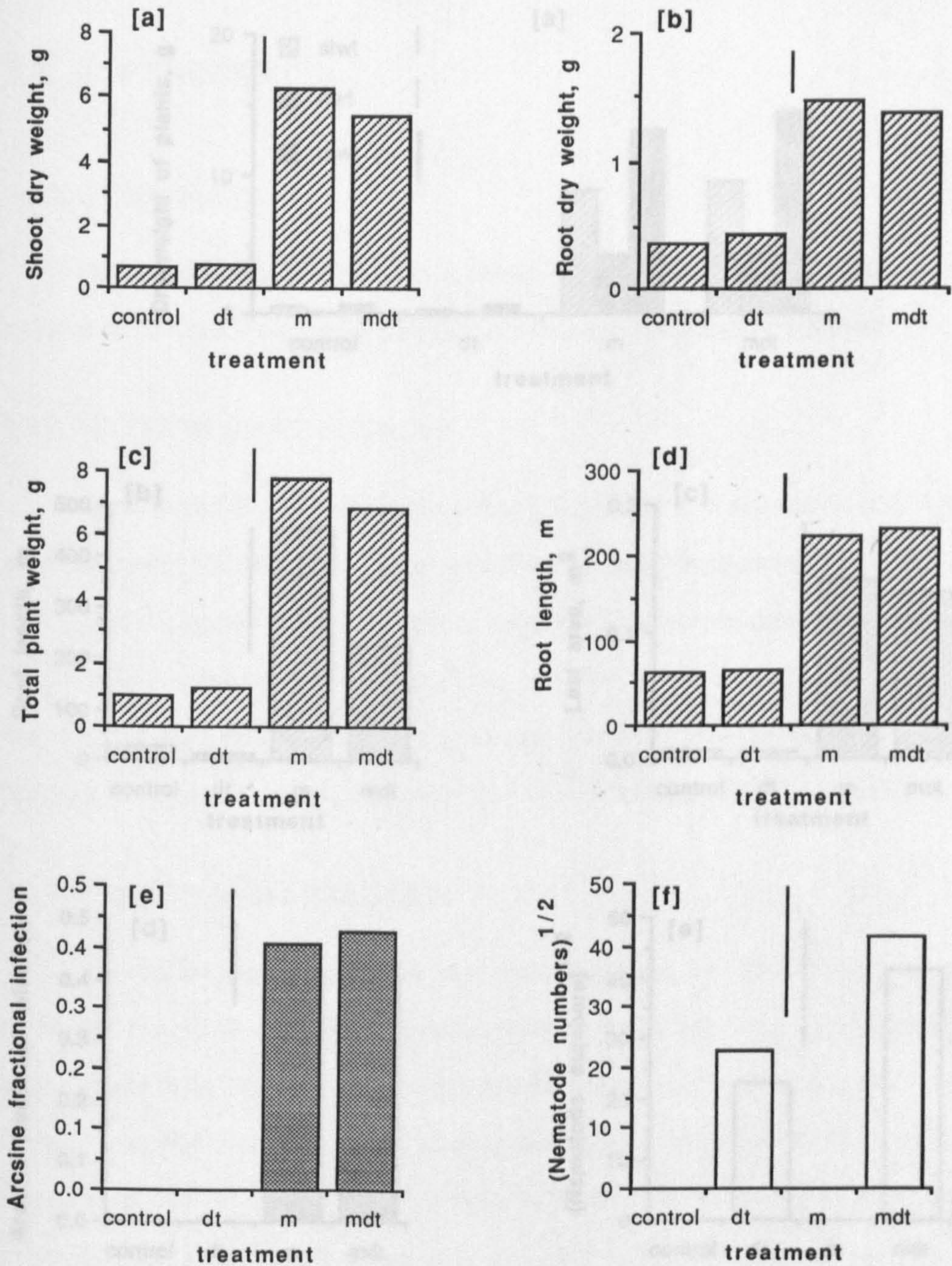


Fig. 3.1. [a], [b], [c] & [d] The effects of *D. triformis* and of *G. clarum* on some measured growth parameters of red clover.

[e] The effect of *D. triformis* on infection (expressed as arcsine fractional infection) of roots by *G. clarum*.

[f] The effect of *G. clarum* on the numbers (expressed as numbers^{1/2}) of *D. triformis* recovered from soil.

dt: treatment with *D. triformis* alone; m: treatment with *G. clarum* alone; mdt: treatment with *G. clarum* and *D. triformis* in combination. Columns are means per pot and bars represent LSDs at p=0.05.

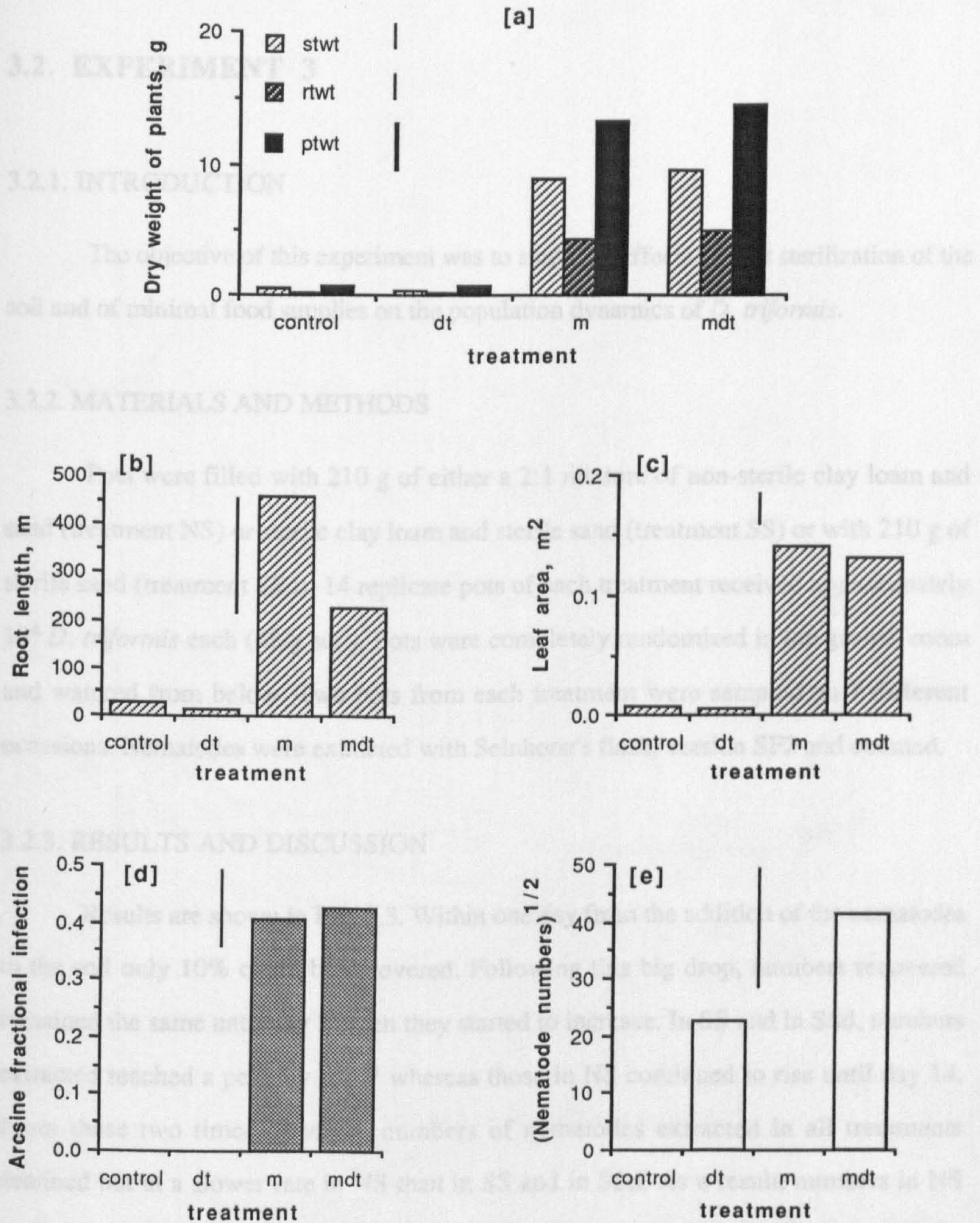


Fig. 3.2. [a], [b] & [c]. Dry weights of shoots (stwt), roots (rtwt), total plant weights (ptwt), root lengths and leaf areas of red clover as affected by *D. triformis* and *G. clarum*.

[d] The effect of *D. triformis* on infection (expressed as arcsine fractional infection) of roots by *G. clarum*.

[e] The effect of *G. clarum* on the numbers (expressed as numbers^{1/2}) of *D. triformis* recovered from soil.

dt: treatment with *D. triformis* alone; m: treatment with *G. clarum* alone; mdt: treatment with *G. clarum* and *D. triformis* in combination. Columns are means per pot and bars represent LSDs at $p=0.05$.

3.2. EXPERIMENT 3

3.2.1. INTRODUCTION

The objective of this experiment was to study the effects of heat sterilization of the soil and of minimal food supplies on the population dynamics of *D. triformis*.

3.2.2. MATERIALS AND METHODS

Pots were filled with 210 g of either a 2:1 mixture of non-sterile clay loam and sand (treatment NS) or sterile clay loam and sterile sand (treatment SS) or with 210 g of sterile sand (treatment SSd). 14 replicate pots of each treatment received approximately 10^4 *D. triformis* each (48/g soil). Pots were completely randomized in the growth room and watered from below. Two pots from each treatment were sampled on 7 different occasions. Nematodes were extracted with Seinhorst's flask, version SF2 and counted.

3.2.3. RESULTS AND DISCUSSION

Results are shown in Fig. 3.3. Within one day from the addition of the nematodes to the soil only 10% could be recovered. Following this big drop, numbers recovered remained the same until day 3 when they started to increase. In SS and in SSd, numbers extracted reached a peak by day 7 whereas those in NS continued to rise until day 14. From these two times onwards, numbers of nematodes extracted in all treatments declined but at a slower rate in NS than in SS and in SSd. As a result, numbers in NS remained higher than in the other two treatments. 27 weeks after the beginning of the experiment, hardly any nematodes were recovered from pots of SS or SSd and less than 10% of the numbers added from pots of NS.

The most significant change in numbers of nematodes throughout the whole experiment occurred right at the start. Within the same day that the nematodes were applied to the soil, only 10% were recovered, a figure which was very near the variable

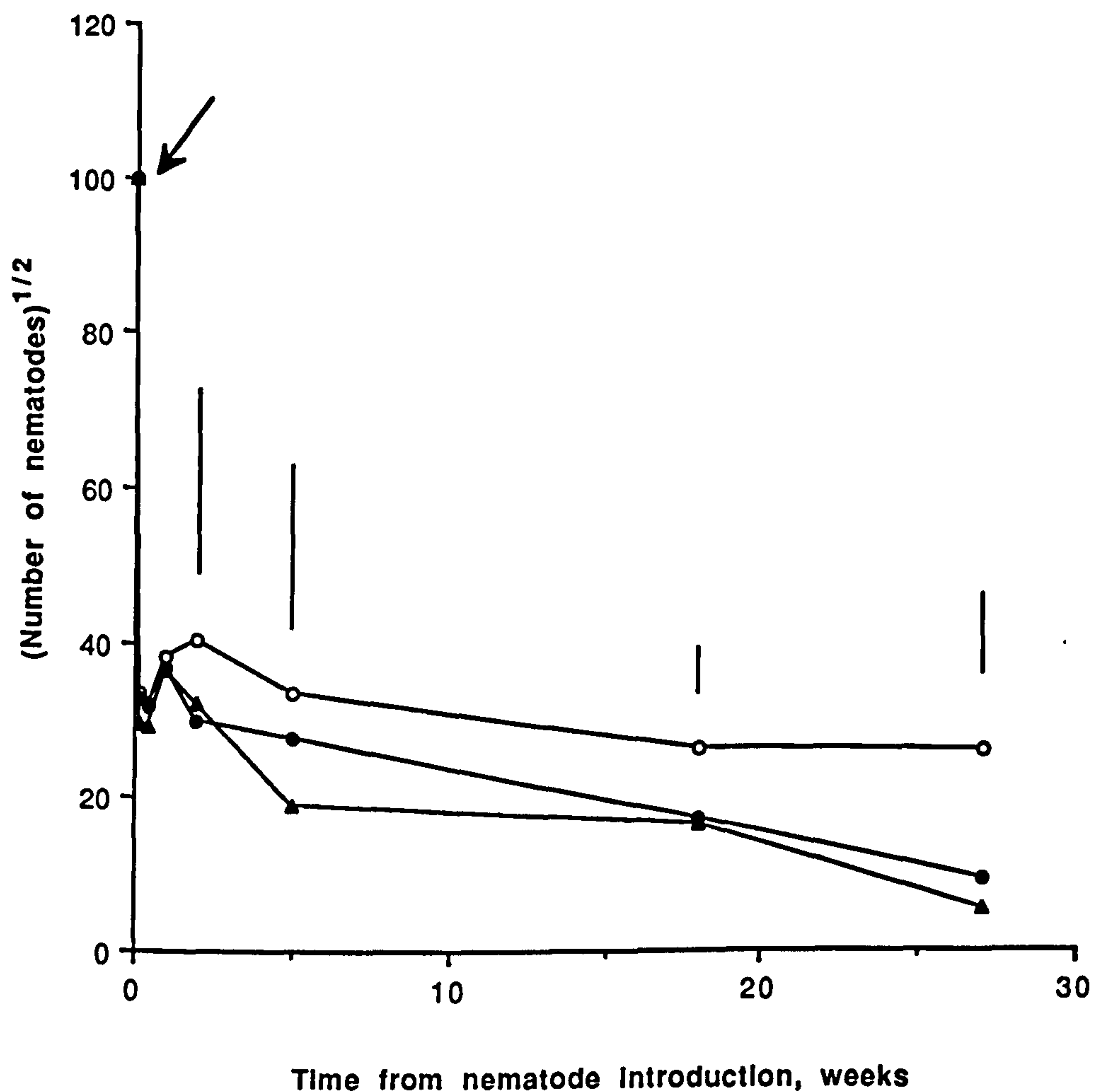


Fig. 3.3. The effects of sterilizing soils containing minimal food supplies for nematodes on the numbers of *D. triformis* recovered from the soils.

O: non sterile clay loam plus sand (NSS).

●: sterile clay loam plus sterile sand (SS)

▲: sterile sand (SSd).

Points are means of the square roots of the numbers of nematodes per pot. Bars represent LSDs at $p = 0.05$. The arrow shows the square root of the number of nematodes added to each pot.

17% recovery potential of Seinhorst's flask. The apparent loss may therefore have been due to the poor recovery of the extraction technique.

The failure of the nematode numbers to increase subsequently was probably related to a shortage of food. The soil did not contain live roots or significant amounts of organic material which are the main sources of substrate for microbial growth in soil (Lynch 1983), limiting development of fungi and hence fungal-feeding nematodes.

It has been suggested by Walker (1984b) that nematode females transferred from a medium with a suitable fungal host to a medium without, will respond by laying a small number of eggs, the larvae from which may not reach maturity. Hirschmann (1962), observed that *D. triformis* larvae hatched from the eggs 4-6 days after their deposition. If this also happens in soil, the small increase of nematode numbers 3 days after addition, was most likely due to the hatching of eggs transferred with the nematode inoculum or laid by the females, in response to the limited food supply.

Heat treatment of the soil before introducing the nematodes did not greatly affect their development. The higher numbers recovered from NS towards the end of the experiment (between 18 and 27 weeks) were probably due to the greater amounts of fungal food available to the nematodes in that soil rather than to any toxic factor released by heating.

35 days after their application to the pots of treatment SS, the density of the nematodes was 3.81/g soil which is very similar to that of 3.95 measured in the soils of the mycorrhizal treatment in experiment 2, carrying the implication that the biomass of fungi on which the nematodes could feed were similar in these two situations. However, the soil of the mycorrhizal treatment of experiment 2 must have contained a greater fungal biomass than the sterile soil of treatment SS of experiment 3 which had not been inoculated with *G. clarum* and did not contain mycorrhizal or non-mycorrhizal roots. It is probable, therefore that *D. triformis* had similar densities in the soil of the mycorrhizal treatment of experiment 2 and in that of treatment SS of experiment 3 because it had not fed on the mycelium of *G. clarum*.

3.3. EXPERIMENTS 4 AND 5

3.3.1. INTRODUCTION

Experiment 4 was set up to examine the possibility that different species of mycophagous nematodes might vary in their effect on the mycorrhizal symbiosis between *G. clarum* and red clover. Furthermore, the effect of the combined, instead of sequential, introduction of VAM fungus and nematodes was examined. Experiment 5 was essentially similar to experiment 4 but with extra replicates and harvests and only one species of nematode.

3.3.2. MATERIALS AND METHODS

Experiment 4

Pots were filled with 210 g of soil which was a mixture of a calcareous clay loam (Wothersome series, Olsen available phosphorus 31 mg/kg, Alloush pers comm.) and sand. *G. clarum* inoculum was added in the form of a 10 g layer below the surface of the soil (final average density, 3 spores/g soil). Approximately 5000 nematodes (either *D. triformis*, *Aphelenchus avenae*, *Aphelenchoides composticola* or *Ditylenchus myceliophagus*) were added directly on the inoculum layer in mycorrhizal or on the sterile soil in non-mycorrhizal treatments to give 24 nematodes/g soil. There were 2 replicates of each treatment. Pots were placed in the growth room in a completely randomized design and watered from below. Clover seed was sown on the surface of the soil which was covered with alkathene beads.

Germination was poor and seedlings had to be thinned to an eventual 1 per pot to achieve homogeneity between replicates. Harvest was at 29 days after sowing. Fresh and dry weights of shoots and roots, root lengths and the mycorrhizal infection were measured and nematodes counted after extraction by method SF2.

Experiment 5

The methods and materials were the same as before with the following exceptions:

1. The clover seed came from a different batch.
2. *D. myceliophagus* was the only nematode species.
3. Treatments were replicated 10 times to allow for 2 harvests of 5 replicates each, carried out 29 and 48 days after sowing.
4. Nematodes were extracted with the Baermann funnel, version BF1.

3.3.3. RESULTS

Experiment 4

Results are shown in Figs 3.4 & 3.5. *G. clarum* did not enhance the growth of clover as it had done in experiments 1 and 2. The final (extracted) numbers of nematodes were again lower than the initial (applied) ones. However, there was an indication that nematodes reproduced even in the non-mycorrhizal pots since recoveries of up to 43% were recorded. This enhanced reproduction of the nematodes in both mycorrhizal and non-mycorrhizal pots probably indicated that they found a sufficient amount of food, not necessarily mycorrhizal in origin.

Non-mycorrhizal plants treated with nematodes were smaller than the control (Fig. 3.4a–c & Plate 3.1a). The total root length was consistently reduced by all species of nematode (Fig. 3.5a). With the other parameters differences were more pronounced with *D. myceliophagus* and *A. composticola* than with *D. triformis* or *A. avenae*. In the case of mycorrhizal plants, neither the fractional infection nor plant growth were affected by the nematodes. This indicated that *G. clarum* was able to counteract the adverse effects of all nematode species. Similar ameliorating effects are well documented with plant-parasitic but not with mycophagous nematodes (see section 1.3.1).

The nematode-treated non-mycorrhizal plants had brown necrotic areas on their leaves (Plate 3.1b) which are typical symptoms of phosphorus deficiency (Sutcliffe &

Baker 1981). This may have been due to some adverse effect of the nematodes on the roots because of the obvious differences in root length between the control and nematode treatments. Microscopical examination of roots from the non-mycorrhizal plants treated with nematodes revealed severe stunting of lateral roots (Plate 3.2a) which had swollen tips resembling *Rhizobium* nodules (Plate 3.2b). Dark lesions were also clearly visible along portions of the root system which in some cases had completely rotted, indicating possible involvement of fungal and bacterial pathogens (Plate 3.2c). In contrast roots in the control and mycorrhizal treatments were without any visible signs of damage.

Experiment 5

In contrast to the previous experiment, microscopical examination of the roots did not reveal infections with pathogens. Non-mycorrhizal root systems were not damaged by the nematodes and plant yield was generally not affected by neither *D. myceliophagus* or *G. clarum* (Fig. 3.6a–c). The only exception was that in the first harvest, where dry weights of roots of mycorrhizal plants treated with nematodes were smaller than those of untreated mycorrhizal plants (Fig. 3.6b) and in the second harvest where root lengths were greater in mycorrhizal than in non-mycorrhizal treatments (Fig. 3.7a). Mycorrhizal infection in harvest 2, was significantly lower in the presence of nematodes ($p < 0.01$) indicating a possible reduction in the growth of the mycorrhizal mycelium (Fig. 3.7b). The numbers of nematodes extracted by method BF1 were highly variable (Fig. 3.7c) but no obvious cause for this was apparent.

3.3.4. DISCUSSION

The small mycorrhizal responses of the plants in experiments 4 and 5, were probably due to the moderately high level of available phosphorus in the soil (31 mg/kg and see also section 1.1.1).

Rovira & Bowen (1966) reported symptoms similar to those shown in Plate 3.2, in the roots of subterranean clover growing in heat sterilized soil which they attributed to a phytotoxic factor released from the soil after heating. The soil in experiment 4 had been heat sterilized but it is unlikely that this was the cause of root damage because roots of the

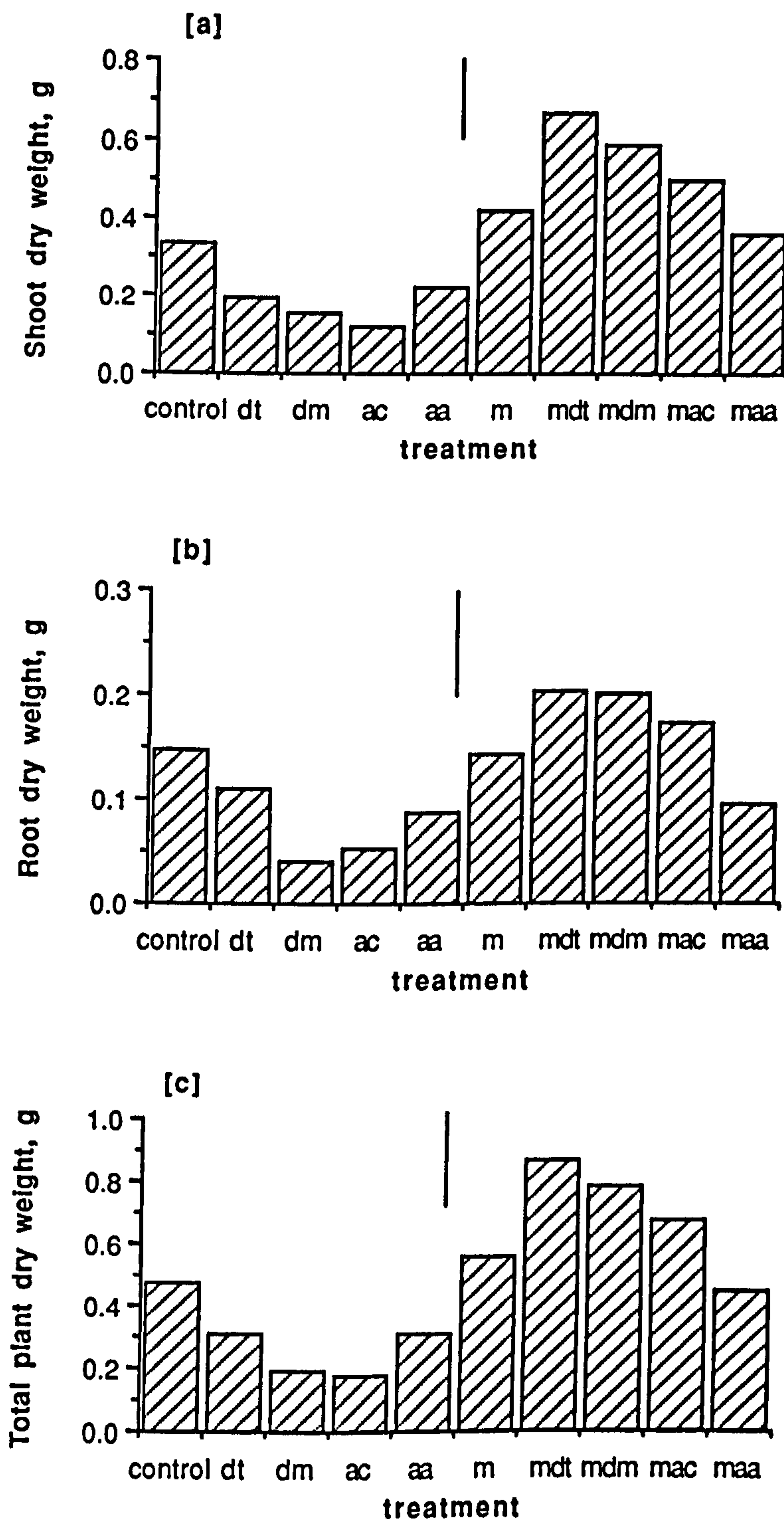


Fig. 3.4. [a], [b], [c]. The effect of four species of nematodes and of *G. clarum* on some measured growth parameters of red clover.

dt, dm, ac and aa: treatments with *D. triformis*, *D. myceliophagus*, *A. composticola* or *A. avenae* alone respectively; m: treatment with *G. clarum* alone; mdt, mdm, mac and maa: treatments with *G. clarum* and the nematodes in combination. Columns are means per pot and bars represent LSDs at $p=0.05$.

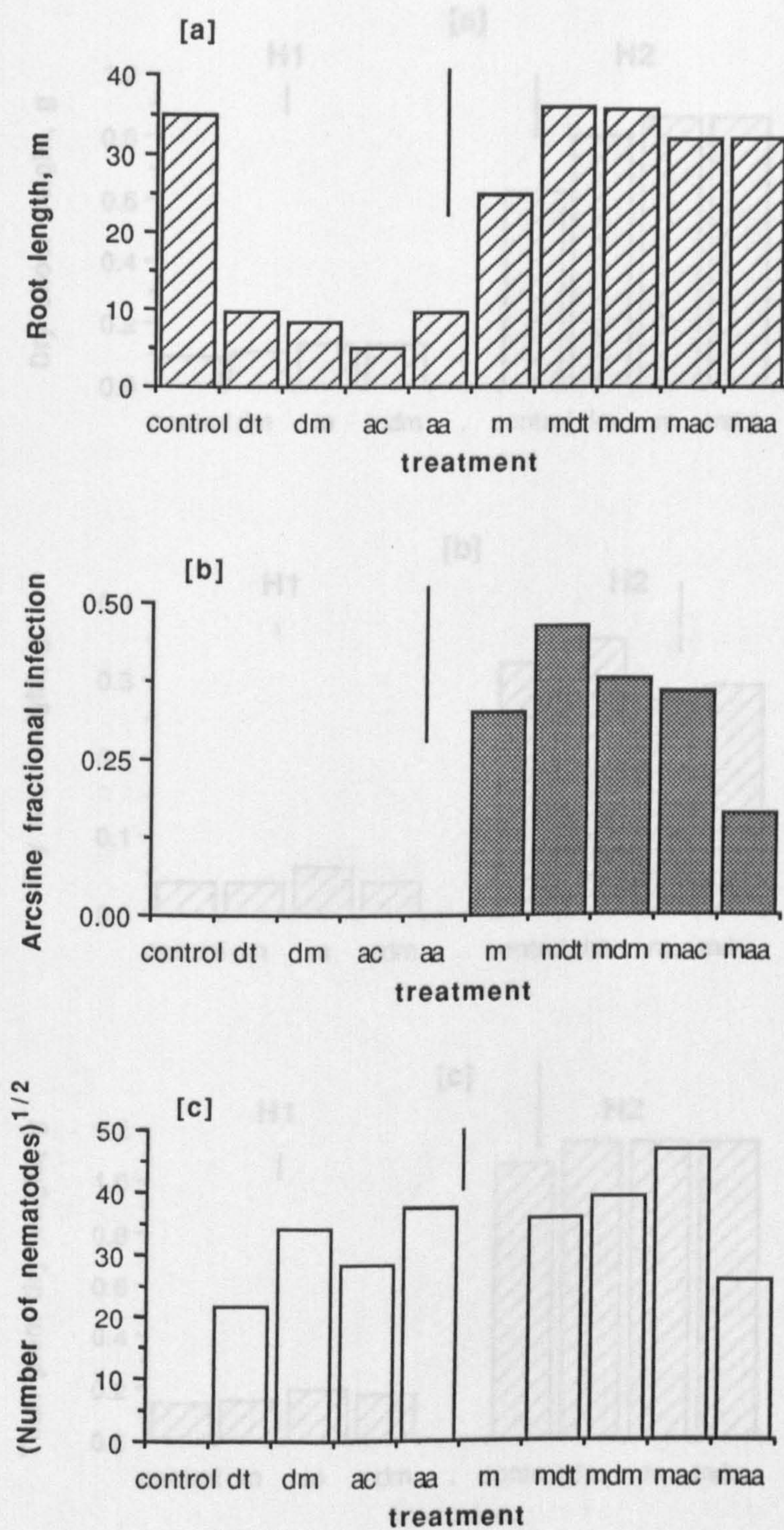


Fig. 3.5. [a] The effect of four species of nematodes and of *G. clarum* on the length of roots of red clover. [b] The effect of the nematodes on infection (expressed as arcsine fractional infection) of roots by *G. clarum*.

[c] The effect of *G. clarum* on the numbers (expressed as numbers^{1/2}) of different species of nematodes recovered from soil.

Treatment abbreviations as in Fig. 3.4. Columns are means per pot and bars represent LSDs at $p=0.05$.

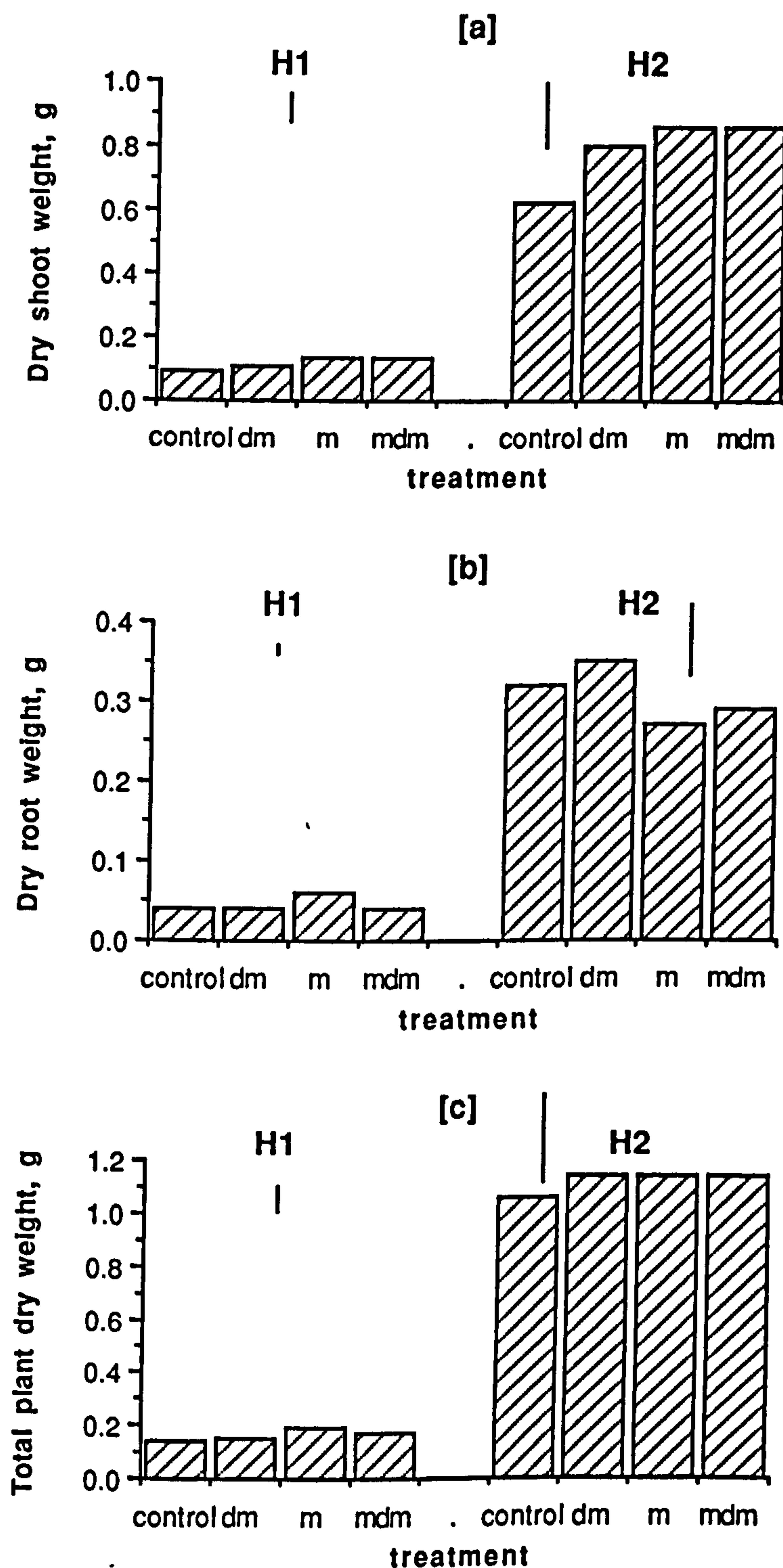


Fig. 3.6. [a], [b], [c]. The effect of *D. myceliophagus* and of *G. clarum* on some growth parameters of red clover measured on two occasions, H1 (29 days after sowing) and H2 (48 days after sowing).

dm: treatment with *D. myceliophagus* alone; m: treatment with *G. clarum* alone; mdm :treatment with *G. clarum* and *D. myceliophagus* in combination. Columns are means per pot and bars represent LSDs at $p=0.05$.

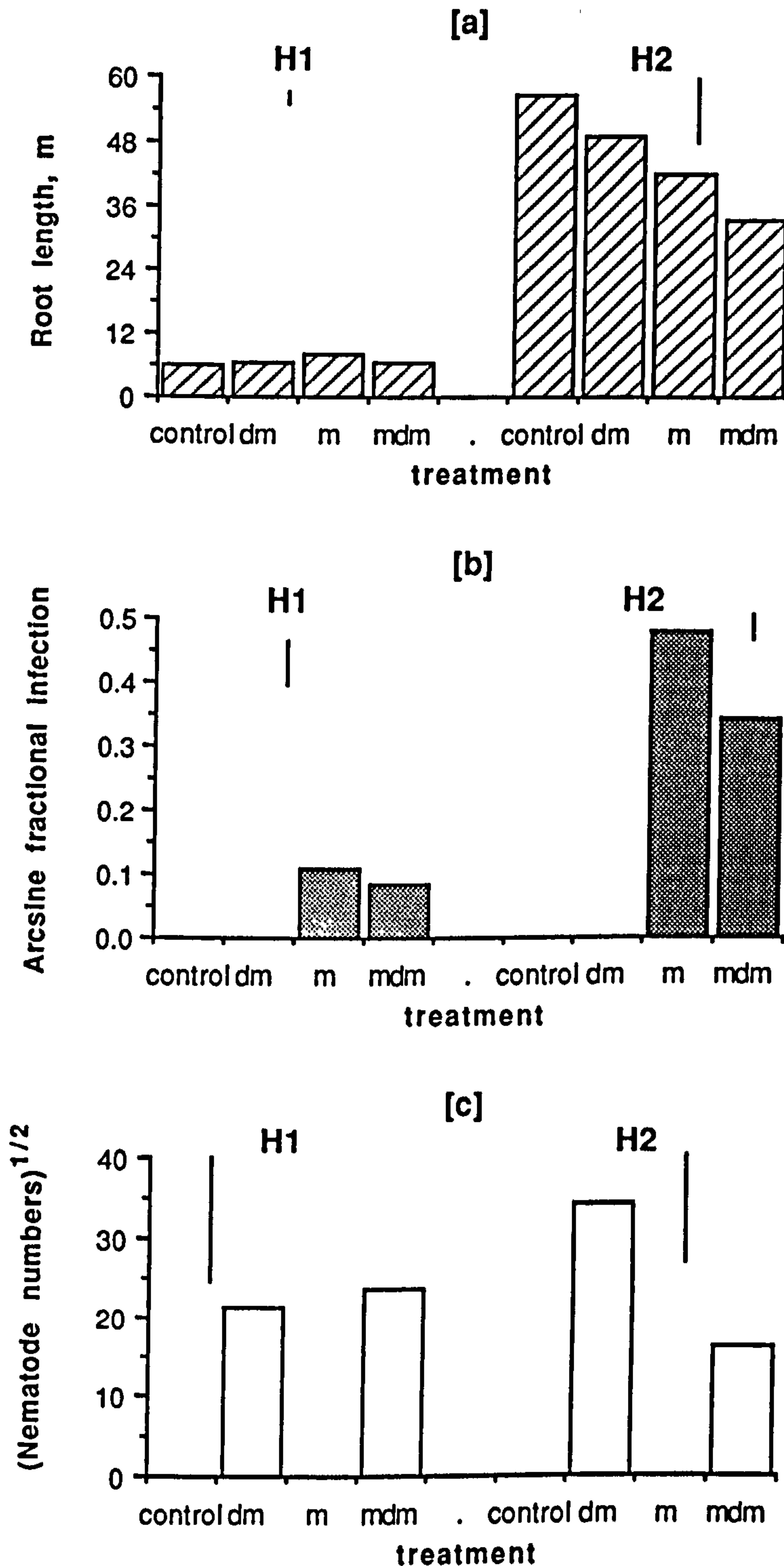


Fig. 3.7. [a] The effect of *D. myceliophagus* and of *G. clarum* on the length of roots of red clover. [b] The effect of *D. myceliophagus* on infection (expressed as arcsine fractional infection) of roots by *G. clarum*. [c] The effect of *G. clarum* on the numbers (expressed as numbers^{1/2}) of *D. myceliophagus* recovered from soil.

Treatment abbreviations as in Fig. 3.6. Columns are means per pot and bars represent LSDs at $p=0.05$. H1: harvest 1 (29 days after sowing); H2: harvest 2 (48 days after sowing).

control plants were not affected. Roots invaded by pathogens may also be stunted and have swollen tips. For example, Inserra *et al.* (1983) in an experiment with an ectoparasitic species of *Paratrichodorus*, observed symptoms similar to those shown in Plate 3.2, and measured severe reductions in shoot weight when nematodes were present. Since root malfunction and reduced growth of the plants in experiment 4 occurred only in the treatments with nematodes it is possible that the nematodes were the direct cause. However, this effect of the nematodes could never be repeated, indicating a probable role of another factor, namely the presence of a root pathogen whose effects were made severe by the presence of the nematodes.

In experiment 4, the soil surface in the pots of all treatments was covered with the mycelia of several unidentified fungi which appeared to be colonizing the seeds while they were germinating and root rotting was also evident (Plate 3.2c). The possibility that fungal pathogens were involved in the root damage was therefore considered. The root pathogenic fungus *Thielaviopsis basicola* also causes seedling damping-off, stunting of root growth and black root rots that are diagnostic of the disease (Agrios 1978) and all these symptoms were observed in the current experiment (Plate 3.2). Some *Fusarium* species are also commonly associated with root rots of red clover and are generally considered to be more damaging to young seedlings or plants weakened by other factors (Fulton & Hanson 1960; Chi, Childers & Hanson 1964; Rufelt 1987). The spermosphere flora very often includes root pathogens (Lynch 1983) which may have been the case for the seed used in experiment 4. In experiment 5, the seed was from a different source and there was little fungal contamination which may explain why no damaged roots were found.

The most likely explanation for the growth depressions of red clover in the presence of the nematodes observed in experiment 4 is that the latter formed a disease complex with seed-borne pathogenic fungi, whose entry to the roots was facilitated by the slight damage to the root hairs and cortex caused by nematode probing. Such interactions are frequent in the case of plant-parasitic nematodes but have rarely been reported in the case of mycophagous nematodes (see 1.3.2).

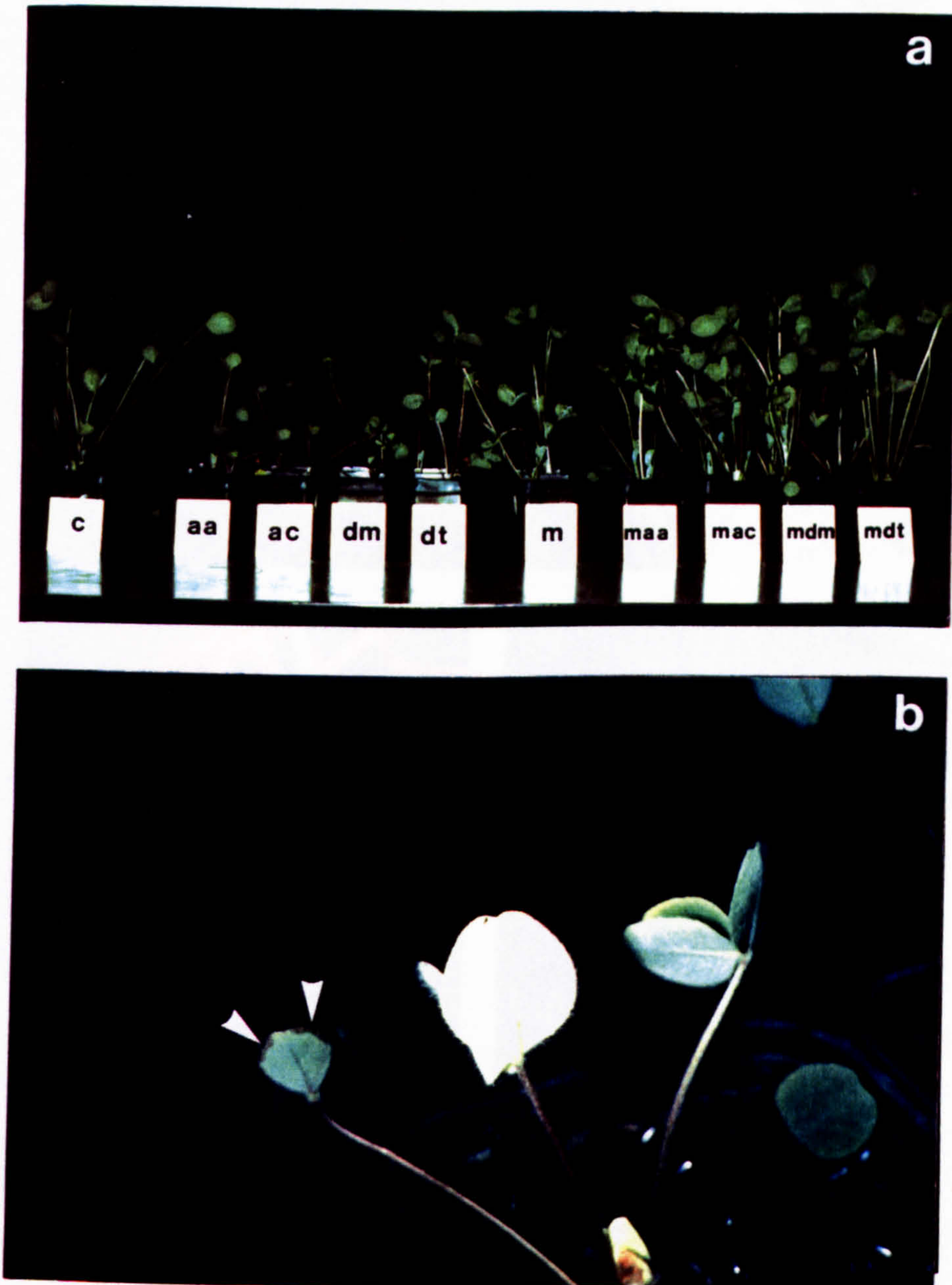


Plate 3.1. [a] The growth of plants of red clover with either *A. avenae*, *A. composticola*, *D. myceliophagus* or *D. triformis* present in soil alone (aa, ac, dm or dt respectively), and in soil with added inoculum of *G. clarum* alone (m) or in combination with the above species of nematodes (maa, mac, mdm or mdt). c: control.

[b] Characteristic signs of phosphorus deficiency (arrows) on the leaves of non-mycorrhizal red clover growing in soil to which nematodes had been added.

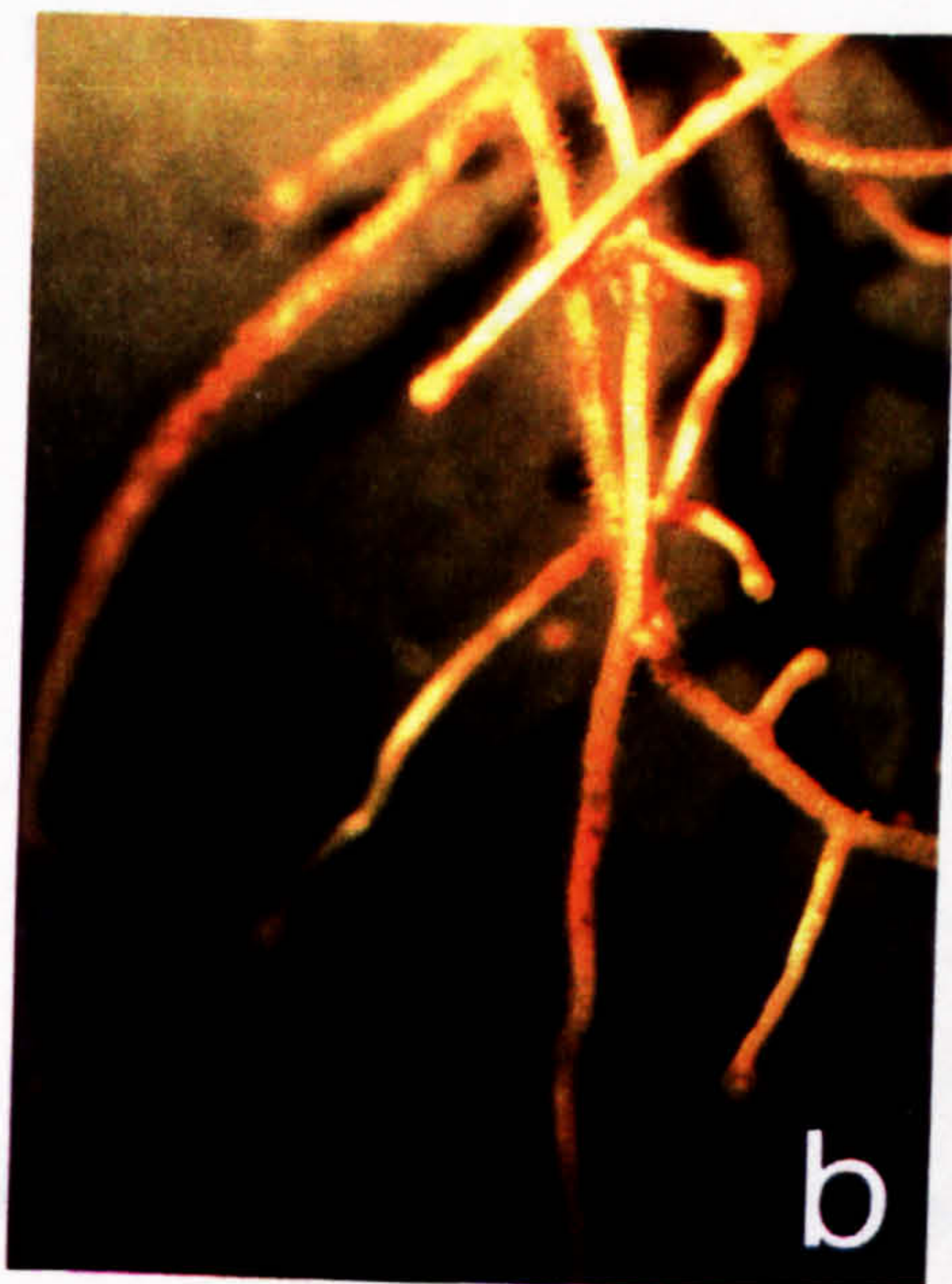


Plate 3.2. Roots of red clover removed from soil containing nematodes (*D. myceliophagus*). Roots show stunted lateral growth (a), swollen tips (b) and dark rotted portions (c).

3.4. EXPERIMENT 6

3.4.1. INTRODUCTION

In this experiment, sequential harvests were used to study in greater detail the effects of nematodes on the development of clover roots and the spread of mycorrhizal infection. The seed was treated with fungicide in order to avoid the introduction of pathogens.

The following questions were asked:

1. Are mycophagous nematodes alone capable of damaging the roots and reducing plant yield?
2. If nematodes are applied to soil simultaneously with mycorrhizal inoculum, but some time before the seed is sown, can they feed on and kill the hyphae originating from germinating spores? Will this reduce the potential of the VAM fungus to infect the roots of the plants which subsequently grow in that soil?

3.4.2. MATERIALS AND METHODS

Pots were filled with 210 g of soil which included a layer of autoclaved inoculum of *G. clarum* in the non-mycorrhizal or live inoculum (final average density of 3 spores/g soil) in the mycorrhizal treatments. Non-mycorrhizal pots also received leachings from the live mycorrhizal inoculum to ensure contamination with the same bacteria. In pots treated with nematodes, *D. myceliophagus*, *A. composticola*, or *A. avenae* were added at a rate of approximately 10^4 per pot. Pots were arranged in the growth room in a randomized block design and watered from below. Each block had 10 pots comprising 2 controls, 2 with mycorrhizal inoculum only, 1 of each species of nematodes alone and 1 with each species in combination with mycorrhizal inoculum.

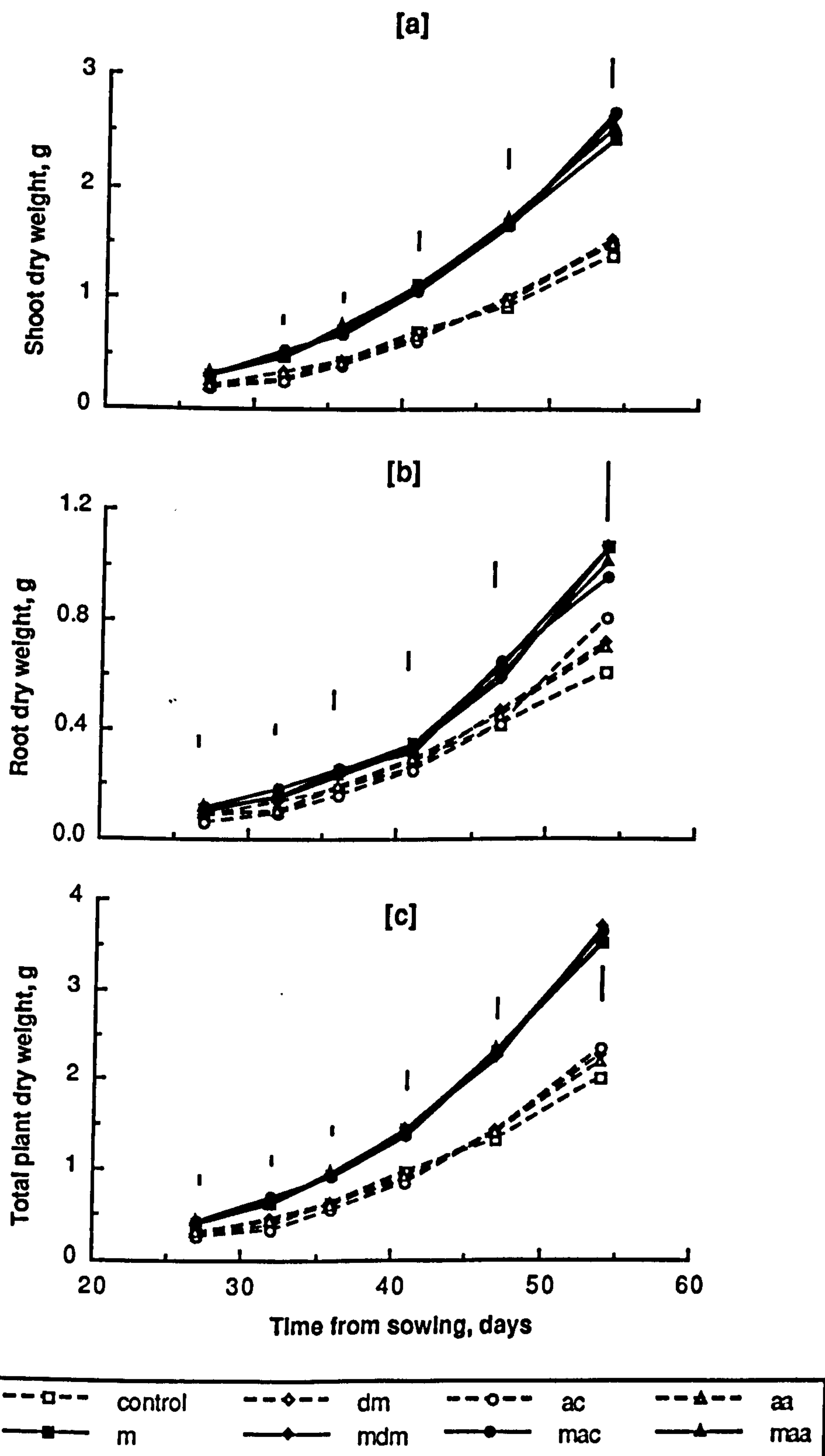


Fig. 3.8. [a], [b], [c]. The effect of three species of nematodes and of *G. clarum* on some measured growth parameters of red clover.

dm, ac and aa: treatments with *D. myceliophagus*, *A. composticola* or *A. avenae* alone respectively; m: treatment with *G. clarum* alone; mdm, mac and maa: treatments with *G. clarum* and the nematodes in combination. Points represent means per pot and bars are LSDs at $p=0.05$.

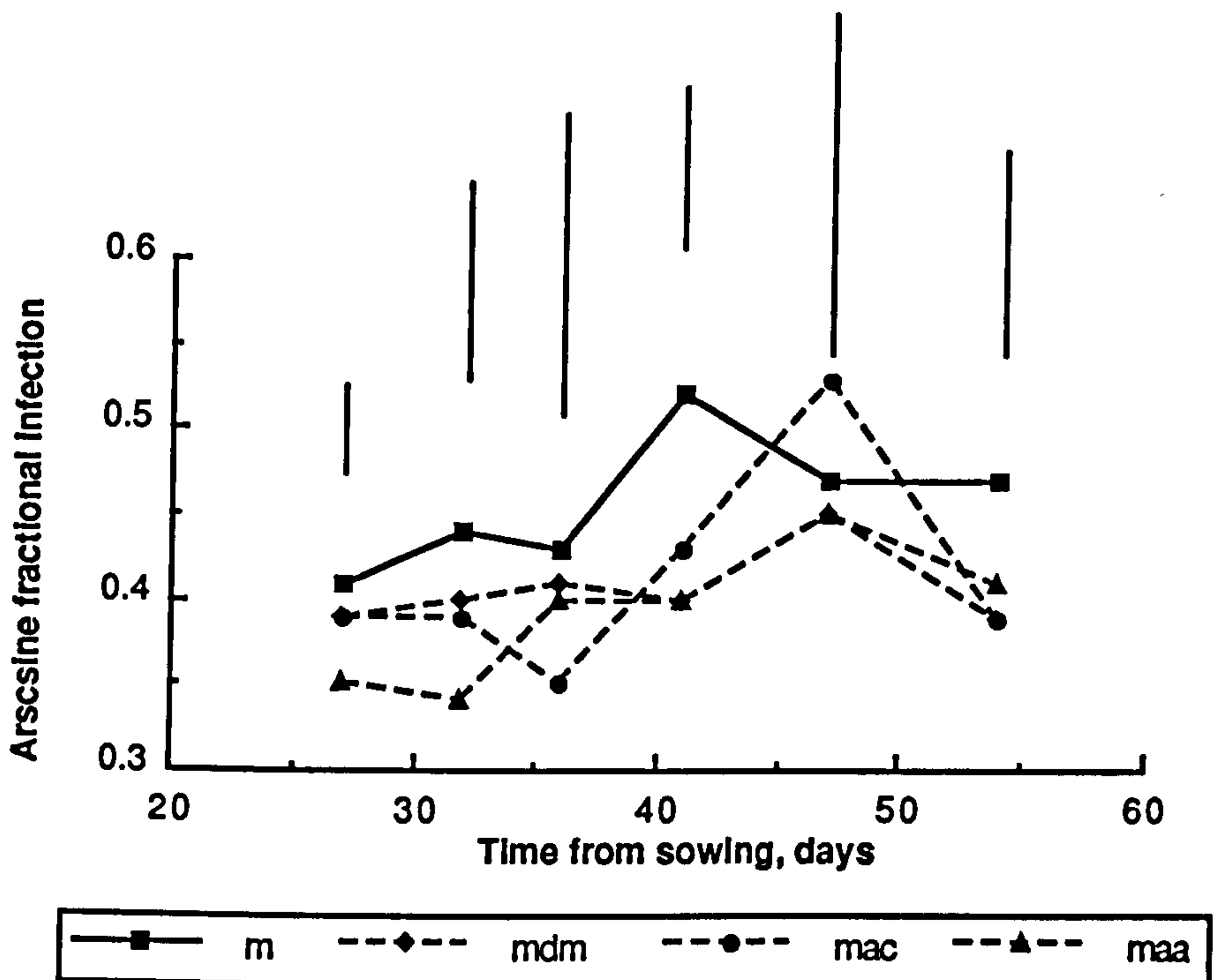


Fig. 3.9. The effect of three species of nematodes on infection (expressed as arcsine fractional infection) of roots by *G. clarum*. Points are means per pot and bars represent LSDs at $p=0.05$. Treatment abbreviations as in Fig. 3.8.

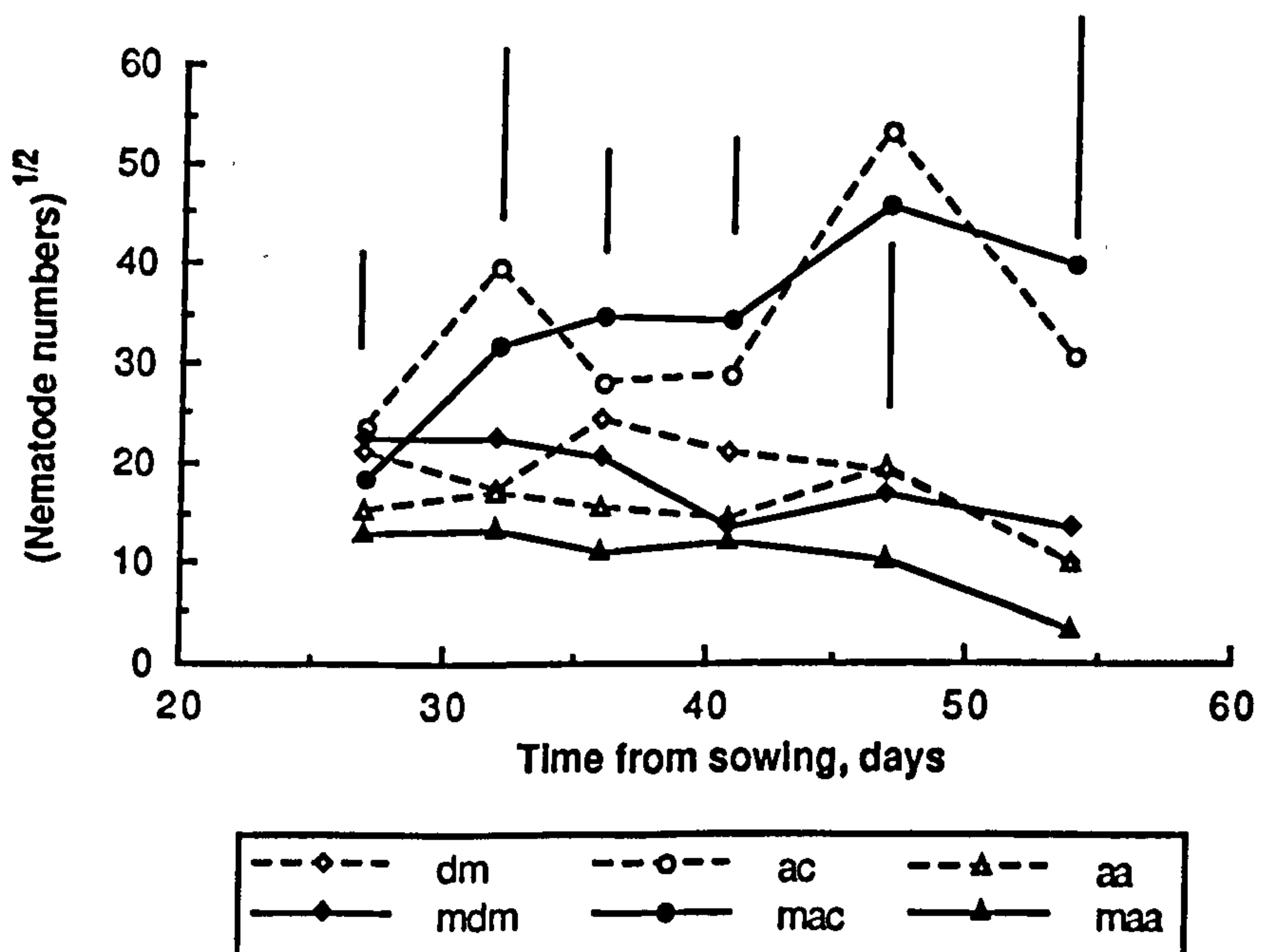


Fig. 3.10. The effect of *G. clarum* on the numbers (expressed as numbers^{1/2}) of three species of nematodes recovered from soil. Points are means per pot and bars represent LSDs at $p=0.05$. Treatment abbreviations as in Fig. 3.8.

Before sowing, the seed had been treated with the fungicide captan which had been shown not to be toxic to the nematodes. Seedlings were progressively thinned to 4 per pot. The six harvests began on day 27, ended on day 54 after sowing and involved measurements of dry weights of shoots and roots, fractional mycorrhizal infection and nematode numbers after their extraction with BF1.

3.4.3. RESULTS AND DISCUSSION

The treatment of the seed with captan was effective in suppressing the growth of soil fungi but did not affect *G. clarum*. Mycorrhizal inoculum had increased shoot and total dry weight by the time of the first harvest and this growth response continued throughout (Fig. 3.8a,c). Although differences in root weights between mycorrhizal and non-mycorrhizal treatments were not significant at every harvest, mycorrhizal root weights tended to be greater (Fig. 3.8b). The yield of the plants was not affected by any of the nematode species irrespective of their mycorrhizal status.

Mycorrhizal infection did not significantly increase with time in any of the mycorrhizal treatments (Fig. 3.9) indicating that infection had reached the plateau phase by the time of the first harvest. The more rapid spread of infection in experiment 6 compared to experiments 4 and 5 can be explained by the inoculation of the soil with *G. clarum* 7 days prior to sowing (Smith, Nicholas & Smith 1979; Ocampo & Hayman 1981; Jakobsen & Andersen 1982) and possibly by the greater planting density (Al-Nahidh 1985). The presence of nematodes led to lower levels of mycorrhizal infection (Fig.3.9), in agreement with the results of experiment 5. This probably indicated that the nematodes were able to damage the mycorrhizal mycelium. However, these differences in mycorrhizal infection were significant only at the first (m vs maa) and at the fourth harvest (m vs maa or mdm or mac). Salawu & Estey (1979) measured reduced sporulation of mycorrhizal fungi in the presence of *A. avenae*, but experiment 6 appears to be the first occasion on which the effects of mycophagous nematodes on the development of a mycorrhizal fungus have been measured in terms of reduced fractional infection.

The numbers of nematodes extracted were consistently lower than those applied, and were very variable (Fig. 3.10). Numbers of *A. composticola* appeared to increase over the period of the harvests while the other nematodes slowly declined. However numbers per pot were not influenced by the presence of *G. clarum*. It is therefore possible that none of these nematodes were able to feed on *G. clarum*.

CHAPTER 4. FEEDING OF MYCOPHAGOUS NEMATODES ON FUNGI GROWN IN PURE CULTURE

4.1. INTRODUCTION

Mycophagous nematodes are capable of reproduction when fed on a variety of fungal species in pure culture (see section 1.2.1). Townshend (1964) categorized the suitability of 59 fungi as hosts for *A. avenae* and *Bursaphelenchus fungivorus*, according to rate of increase of nematodes produced on pure cultures of each fungus. Riffle (1971) demonstrated food preferences of the nematode *Aphelenchoides cibolensis* which fed on and reproduced on in cultures of 53 out of 58 ectomycorrhizal fungi, increasing its population within a range of 0 to 2353 times. He suggested that fungi on which the nematode did not multiply well produced a substance which was toxic to the nematodes. According to Sutherland & Fortin (1968), some ectomycorrhizal fungi were better than others in their ability to support reproduction of *A. avenae*. They also suggested that production of toxic substances may explain the food preferences of mycophagous nematodes.

In order to decide whether a given fungus is a good host for nematodes, the reduction in fungal biomass caused by nematode feeding can be quantified. Various workers have measured differences between diameters of fungal colonies in the presence or absence of nematodes (Arrold & Blake 1966; Sutherland & Fortin 1968). Other workers (Mankau & Mankau 1963) have measured differences in lengths of hyphae growing in tubes. Cooke & Pramer (1968) were unable to detect any reduction in the radial growth of fungal colonies caused by nematodes, even though the population of nematodes increased. Therefore, measurements of fungal growth alone may not be reliable indices of whether a given fungus is suitable as a host for a given nematode.

Wasilewska, Jakubczyk & Paplinska (1975) measured reductions in the dry weight of mycelium caused by *A. avenae*. They found that the nematode reduced the weight of the mycelium by up to 40% after 22 days. This reduction was attributed not

only to consumption of the fungus but also to another effect of the nematode in limiting fungal growth. This may have been the production by the nematodes of a metabolite toxic to the fungus as suggested by Cooke & Pramer (1968).

Since earlier experiments showed no clear-cut quantitative effect of nematodes on the development of mycorrhizas (see Chapter 3), an attempt was made to observe qualitatively these nematodes feeding on hyphae of the VAM fungus *G. clarum* in pure culture. Spores of the fungus were selected under the microscope and surface sterilized with chloramine-T and streptomycin sulphate (Watrud 1982). However, all attempts to germinate spores of *G. clarum* on water agar failed.

As an alternative, it was decided to examine the food preferences of the nematodes and the effects of their feeding on mycelia of various saprotrophic and ectomycorrhizal fungi growing in pure culture. The purpose of the experiment was to provide general information on the host range of the nematodes.

4.2. MATERIALS AND METHODS

Plates containing equal volumes of potato dextrose agar, were inoculated with one of the following fungi (1 colony per plate): Ectomycorrhizal -*Cenococcum graniforme*, *Hebeloma crustuliniforme*, *Laccaria laccata*, *Paxillus involutus*, *Rhizopogon roseolus*; Saprotrophic -*Agaricus bisporus*, *Rhizoctonia solani*, C and D, two unidentified species isolated from non-sterile soil.

Once radial growth of the fungi had started, (2 days after inoculation with C, D and *R. solani* and 28 days after inoculation with the remaining slower growing fungi) three replicate plates were inoculated with approximately 500 *A. composticola*, *A. avenae*, *D. myceliophagus* or *D. triformis*. Three replicate control plates received no nematodes. All plates were kept in the dark and at room temperature (17-22 °C).

Plates with C, D and *R. solani* were sampled 43 days after introduction of the nematodes. With other fungi plates were sampled 28 days after introduction of the nematodes. This gave nematodes more time to feed on the mycelium of the faster growing fungi. At sampling, mycelia and nematodes were extracted from the plates by

melting the agar in water at 100 °C followed by sieving (250 µm) to separate mycelium from the nematodes. The mycelium was then dried at 95 °C and weighed while nematodes were counted in sub-samples of the sieved suspensions.

The efficiency of this method of extraction was not tested quantitatively. Nevertheless, the nematode suspensions contained very few hyphal fragments while the numbers of nematodes trapped in the mycelium were judged to constitute an insignificant fraction of the total number collected and counted.

Direct observations of nematode feeding

The feeding of *A. composticola* on the mycelium of the ectomycorrhizal fungus *P. involutus* was directly observed through the microscope and photographed (x630 magnification). In order to do this, a sterile glass slide was placed inside a sterile petri dish. A small quantity of Pachlewska's nutrient agar medium (20 g glucose, 5 g maltose, 0.5 g aqueous magnesium sulphate, 1 g potassium dihydrogen phosphate, 0.5 g ammonium tartrate, 0.05 g thiamine hydrochloride and 0.005 g iron citrate dissolve in water and made up to 1000 cm³) was poured in each petri dish to just cover the surface of the slide. The agar was inoculated with one colony of *P. involutus* in a position away from the slide. As soon as the hyphae of the fungus grew over a slide, it was inoculated with nematodes. The slide was then removed from the dish, covered with a cover slip and observed and photographed under a microscope.

4.3. RESULTS

Direct observations of nematode feeding

Under the microscope, *A. composticola* was seen to search continuously for live hyphae on which it could feed. Occasionally, a nematode pressed its lips against a hypha and successfully penetrated the hyphal wall with its stylet (Plate 4.1a,b,c). As soon as this happened the nematode remained still, pulsating its median bulb and thus directing the contents of the hypha towards its mouth (Plate 4.1b). Each feeding episode lasted for only a few seconds and in this respect this nematode is similar to *Aphelenchoides*

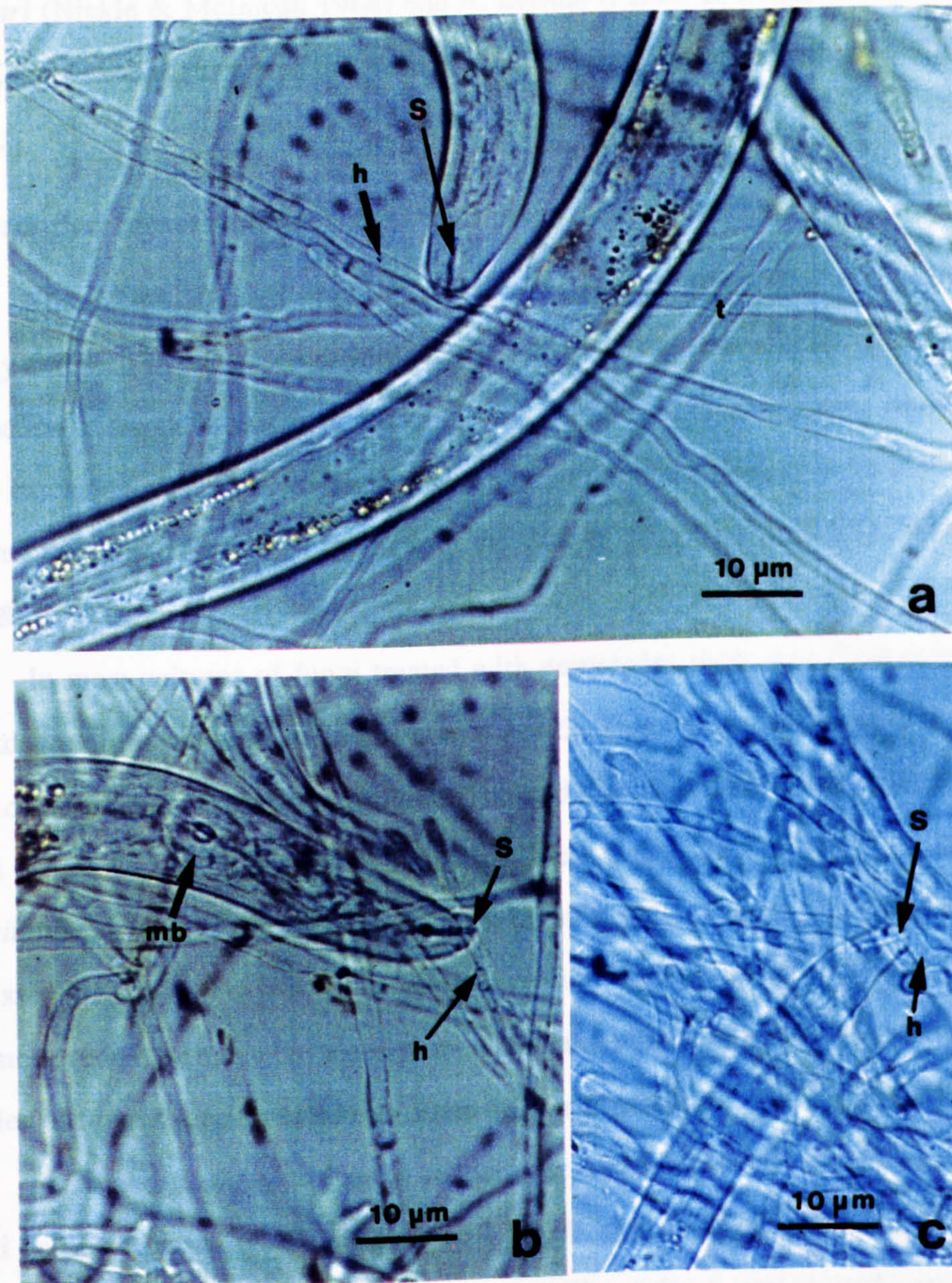


Plate 4.1. Nematodes (*A. composticola*) feeding on hyphae of the ectomycorrhizal fungus *P. involutus*.

[a] The nematode has positioned its head vertically against a hypha (h) on which it is feeding. s: stylet.

[b] The head region of a nematode feeding on a hypha (h). mb = median bulb and s = stylet, slightly protruding.

[c] The stylet of a nematode (s) has penetrated a hypha (h).

sacchari (Nickle & McIntosh 1968) and *A. avenae* (Fisher & Evans 1967) but differs from *D. myceliophagus* which has been observed to feed for hours each time (Doncaster 1966).

Quantitative observations

All species of fungi grew on the potato dextrose agar. The saprotrophs grew rapidly and their hyphae soon covered the plates. Radial growth was not correlated with production of fungal biomass. For example, the colonies of *C. graniforme* whose rate of radial extension was small, weighed significantly more than those of C or D which had covered the whole surface of the agar at the time of sampling (Fig. 4.1a; Plates 4.2b, 4.3a & 4.6a).

In most cultures of fungi treated with nematodes there was clear evidence of breakdown of the aerial mycelium (Plates 4.2–4.6). This was most extensive in the case of *A. composticola* which was able to feed on and reproduce in cultures of all species of fungi except C, and its populations reached levels higher than those of *A. avenae*, *D. myceliophagus* or *D. triformis* (Fig. 4.1b). With most species of fungi, the differences in the extent of visible breakdown of aerial mycelium caused by the different species of nematodes could be related to the measured reductions in the weight of mycelium of each species of fungus (e.g. *P. involutus*, Plate 4.4b & Fig. 4.2).

A. composticola significantly reduced the weights of recovered mycelium of all fungi except *R. roseolus*, C and D (Fig. 4.1a), *H. crustuliniforme*, *A. bisporus* and *R. solani* being the fungal species most affected (Fig. 4.2). *A. avenae* only reduced the weights of *A. bisporus*, *C. graniforme*, *H. crustuliniforme* and *R. solani* (Fig. 4.1a). *D. myceliophagus* reduced the weights of *A. bisporus* and *C. graniforme* only, although some damage to the mycelium of *P. involutus*, *R. roseolus* and *R. solani* was also visible (Plate 4.4a, & 4.5a,b). The preference of this species of nematode for *A. bisporus* was quite pronounced in terms of both numbers produced and per cent reduction in weight of dry mycelium (Fig. 4.1b & Fig. 4.2).

All cultures of the ectomycorrhizal species to which *D. triformis* was added, were contaminated with another fungus and could not be sampled. The contaminant was

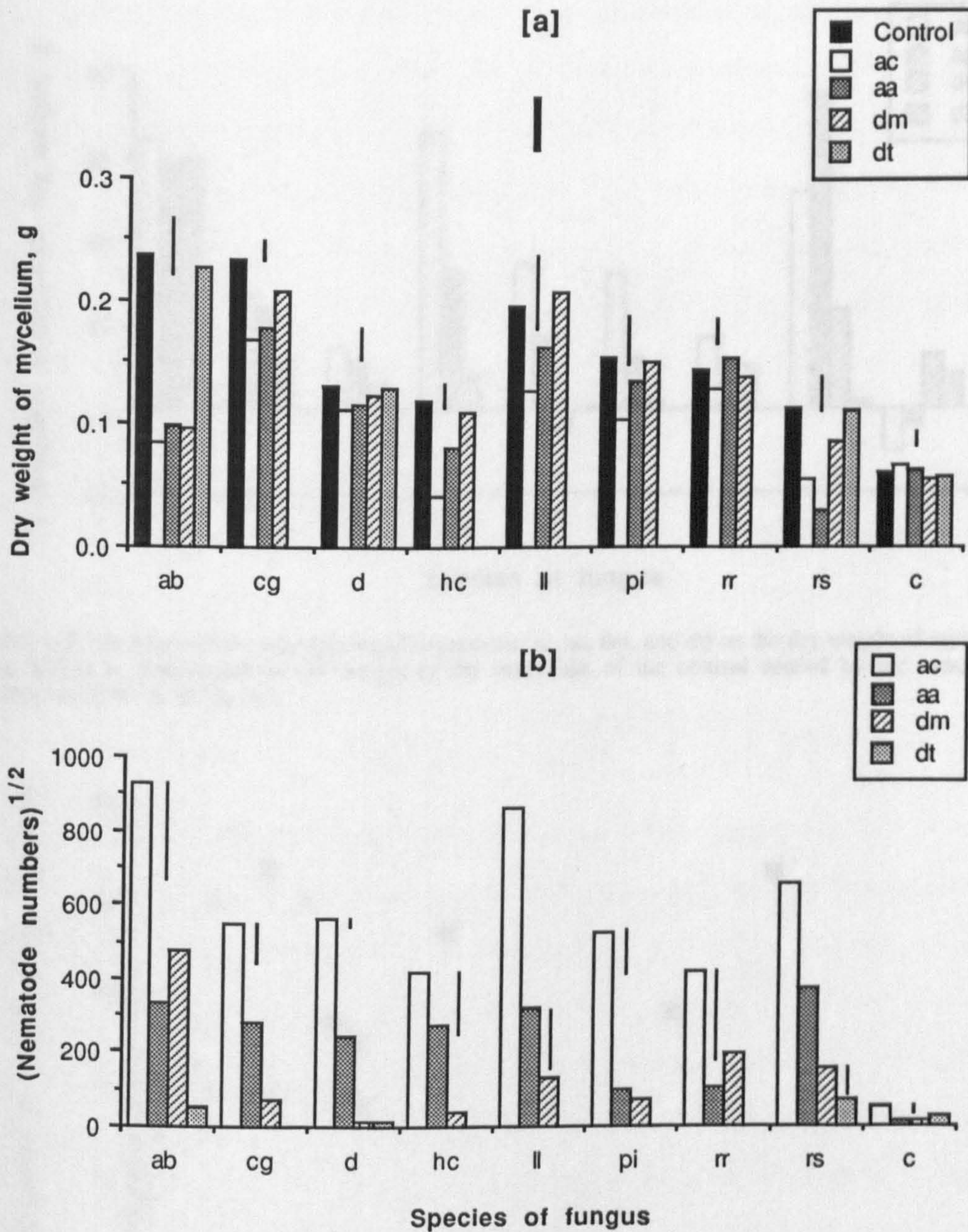


Fig. 4.1.[a] The effect of four nematode species, *A. composticola* (ac), *A. avenae* (aa), *D. myceliophagus* (dm) and *D. trifurmis* (dt), on the dry weight of mycelium of the fungi *A. bisporus* (ab), *C. graniforme* (cg), *D* (d), *H. crustuliniforme* (hc), *L. laccata* (ll), *P. involutus* (pi), *R. roseolus* (rr), *R. solani* (rs) and *C* (c). Bars are LSDs at $p=0.05$. The thin bars are for the comparison of the effect of nematodes within each of the fungal species and the thick bar is for comparison of the controls between fungi.

[b] Numbers of the four species of nematodes in the fungal cultures expressed as (nematode numbers)^{1/2} 43 days after their introduction into cultures of *C*, *D* and *R. solani* or 28 days after their introduction into cultures of the other species of fungi. Bars are LSDs at $p=0.05$ for the comparison of transformed nematode numbers within each species of fungus. Abbreviations are as in [a].

Columns show means of three replicates.

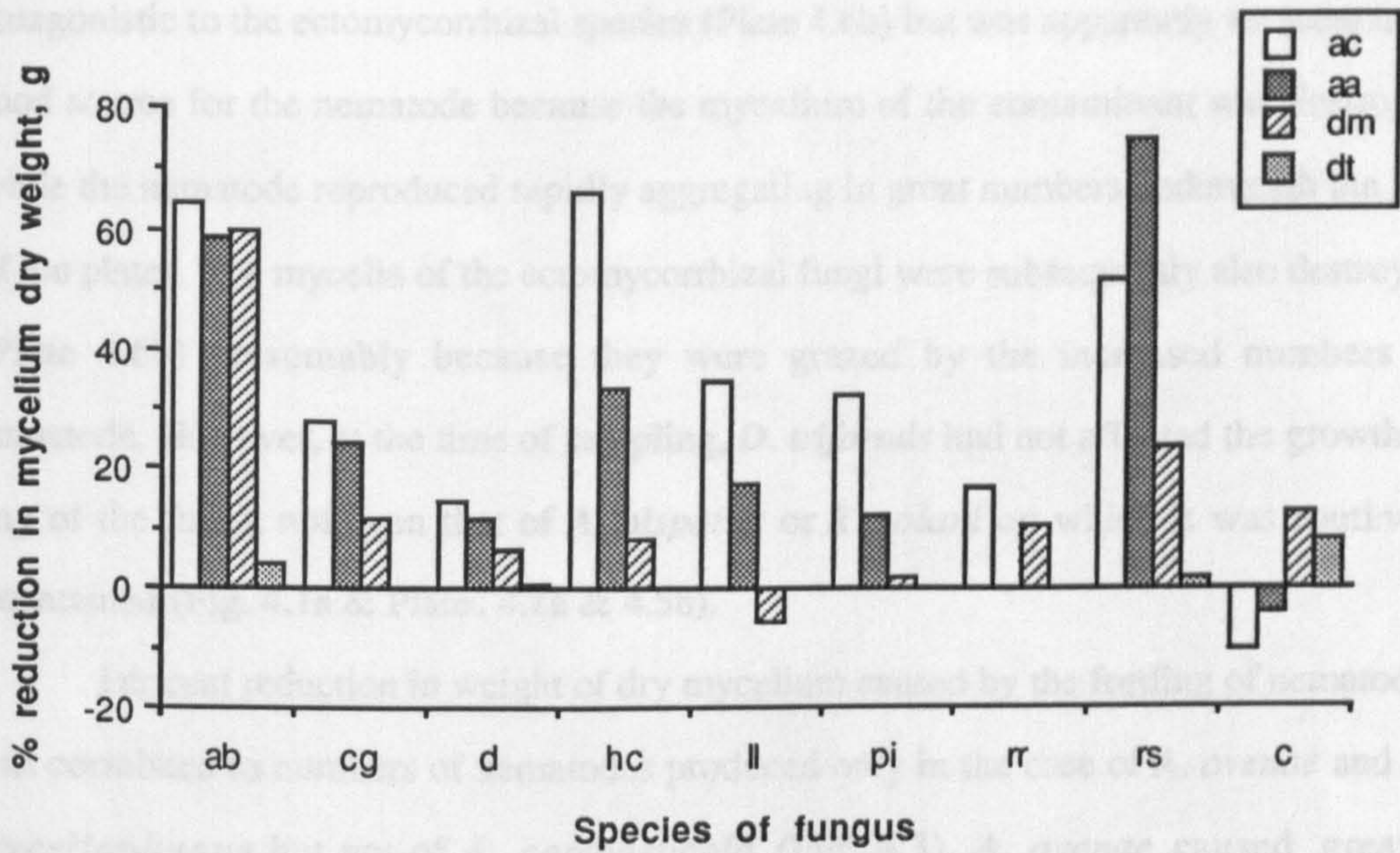


Fig. 4.2. The effect of the four species of nematodes (ac, aa, dm, and dt) on the dry weight of mycelium, measured as %reduction in the weight of dry mycelium of the control caused by the nematodes. Abbreviations as in Fig. 4.1.

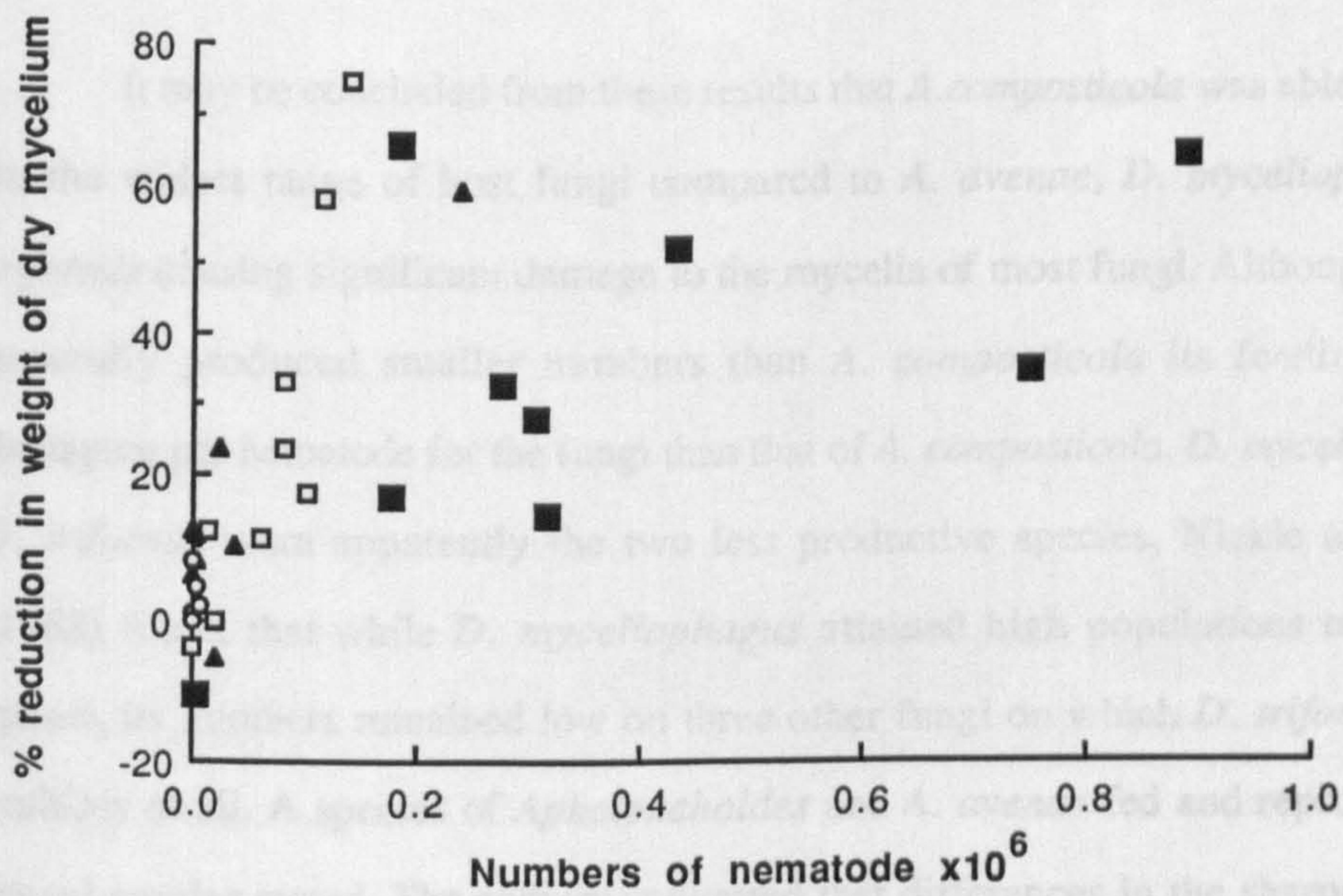


Fig. 4.3. The relationship between %reduction in weight of dry mycelium of the control caused by the feeding of nematodes and numbers of nematodes. *A. composticola*: ■ $r^2=0.330$ at $p=0.1064$; *A. avenae*: □, $r^2=0.763$ at $p=0.0021$; *D. myceliophagus*: ▲ $r^2=0.817$ at $p=0.0008$; *D. triformis*: ○ $r^2=0.061$ at $p=0.5234$.

antagonistic to the ectomycorrhizal species (Plate 4.6b) but was apparently an acceptable food source for the nematode because the mycelium of the contaminant was destroyed while the nematode reproduced rapidly aggregating in great numbers underneath the lids of the plates. The mycelia of the ectomycorrhizal fungi were subsequently also destroyed (Plate 4.6b) presumably because they were grazed by the increased numbers of nematode. However, at the time of sampling, *D. triformis* had not affected the growth of any of the fungi, not even that of *A. bisporus* or *R. solani* on which it was routinely maintained (Fig. 4.1a & Plates 4.2a & 4.5b).

Per cent reduction in weight of dry mycelium caused by the feeding of nematodes was correlated to numbers of nematodes produced only in the case of *A. avenae* and *D. myceliophagus* but not of *A. composticola* (Fig. 4.3). *A. avenae* caused greater reductions to the weights of dry mycelia per nematode than did *A. composticola*.

4.4. DISCUSSION

It may be concluded from these results that *A. composticola* was able to reproduce on the widest range of host fungi compared to *A. avenae*, *D. myceliophagus* or *D. triformis* causing significant damage to the mycelia of most fungi. Although, *A. avenae* generally produced smaller numbers than *A. composticola* its feeding was more damaging per nematode for the fungi than that of *A. composticola*. *D. myceliophagus* and *D. triformis* were apparently the two less productive species. Nickle and McIntosh (1968) found that while *D. myceliophagus* attained high populations on mushroom spawn, its numbers remained low on three other fungi on which *D. triformis* failed to multiply at all. A species of *Aphelenchoides* and *A. avenae* fed and reproduced on all fungal species tested. The authors suggested that differences in the sharpness of stylet and differences in the parts of the fungi attacked by the different species of nematodes could explain their results.

Reproduction of *A. avenae* and *D. myceliophagus* has previously been shown to be inversely proportional to the age of their fungal host and this effect was more significant with *D. myceliophagus* probably because of its longer life-cycle

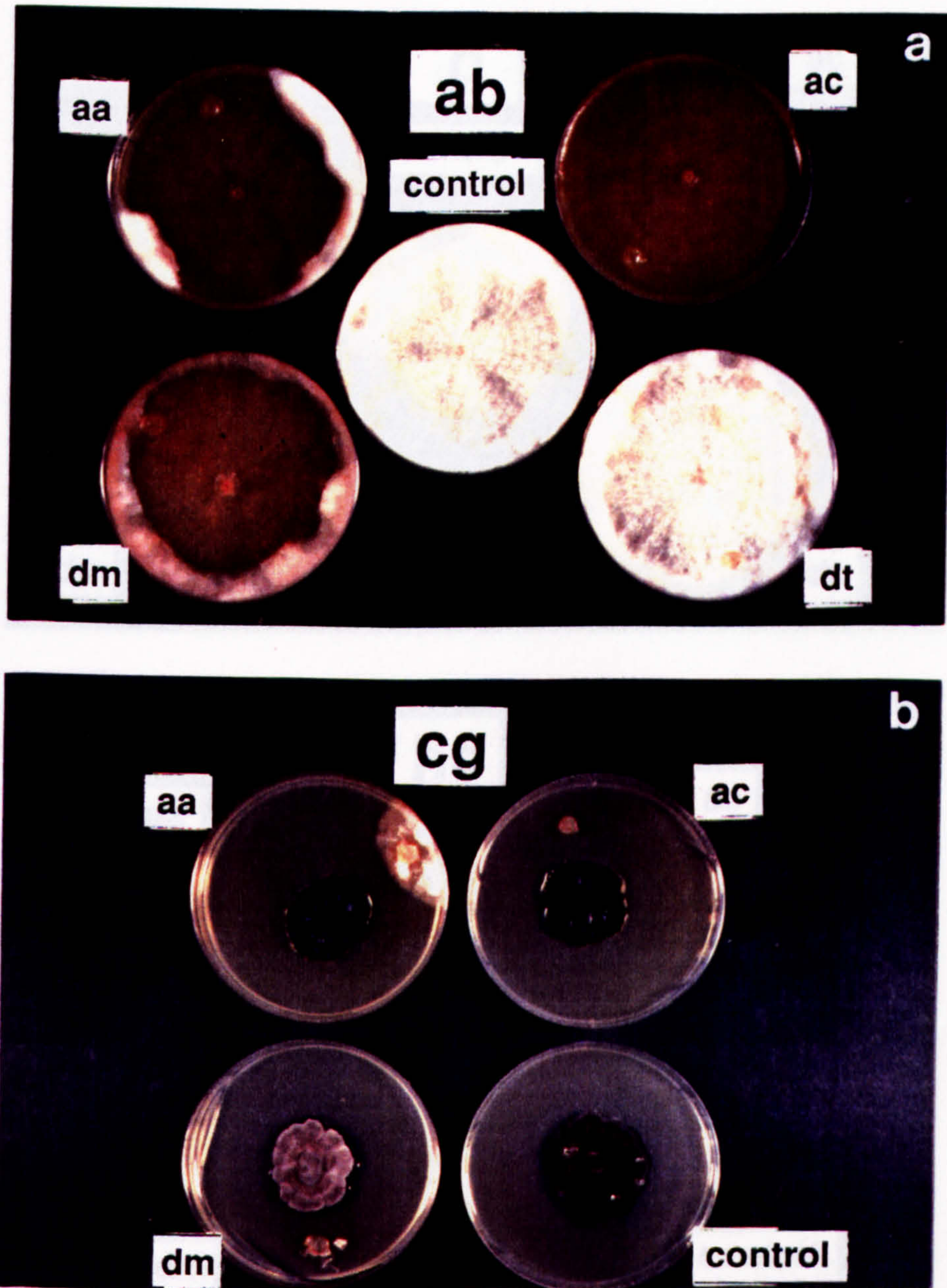


Plate 4.2. [a] The effect of the nematodes *A. composticola* (ac), *A. avenae* (aa), *D. myceliophagus* (dm) and *D. triformis* (dt) on the growth of mycelium of *A. bisporus* (ab). The damaged hyphae of the fungus have lost their contents and their characteristic white colour as a result of grazing of the nematodes. Damage is most extensive in the case of *A. composticola* whereas there is no visible effect of *D. triformis*.

[b] The effect of *A. composticola* (ac), *A. avenae* (aa) and *D. myceliophagus* (dm) on the growth of mycelium of the ectomycorrhizal fungus *C. graniforme* (cg). Both *A. composticola* and *A. avenae* have completely destroyed the mycelium of the fungus as shown by the shiny and darker surface of the colonies of the fungus. The effect of *D. myceliophagus* is beginning to show at the periphery of the fungal colony but is not yet substantial.

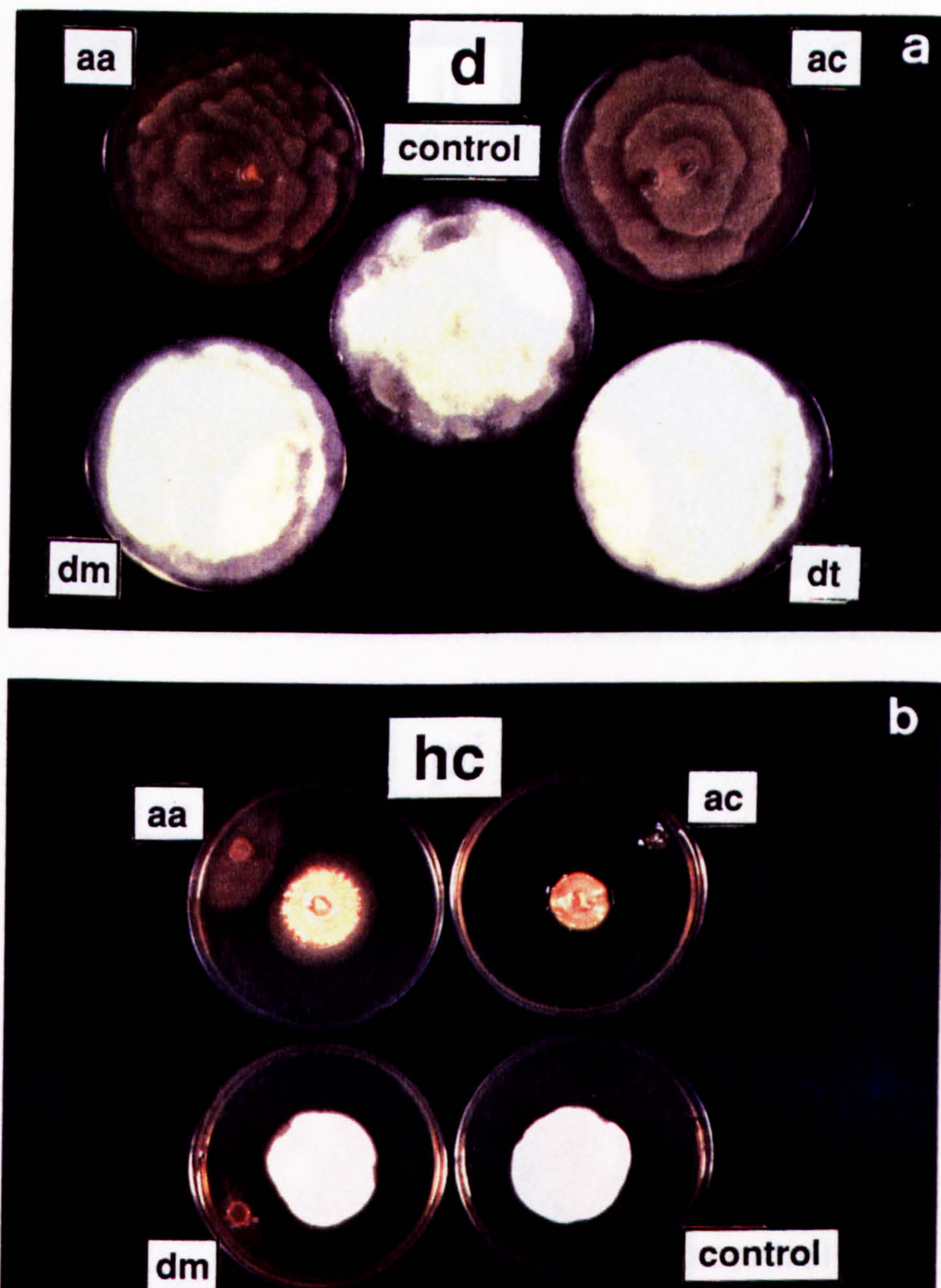


Plate 4.3. [a] The effect of the nematodes *A. composticola* (ac), *A. avenae* (aa), *D. myceliophagus* (dm) and *D. triformis* (dt) on the growth of mycelium of fungus D (d). *A. composticola* and *A. avenae* have totally destroyed the mycelium whereas there is no visible effect of either *D. myceliophagus* or *D. triformis*. Damaged hyphae lost their contents and their characteristic white colour as a result of grazing by the nematodes.

[b] The effect of *A. composticola* (ac), *A. avenae* (aa) and *D. myceliophagus* (dm) on the growth of mycelium of the ectomycorrhizal fungus *H. crustuliniforme* (hc). Both *A. composticola* and *A. avenae* have completely destroyed the mycelium of the fungus as shown by the shiny surface of the colonies of the fungus. There is no visible effect of *D. myceliophagus*.

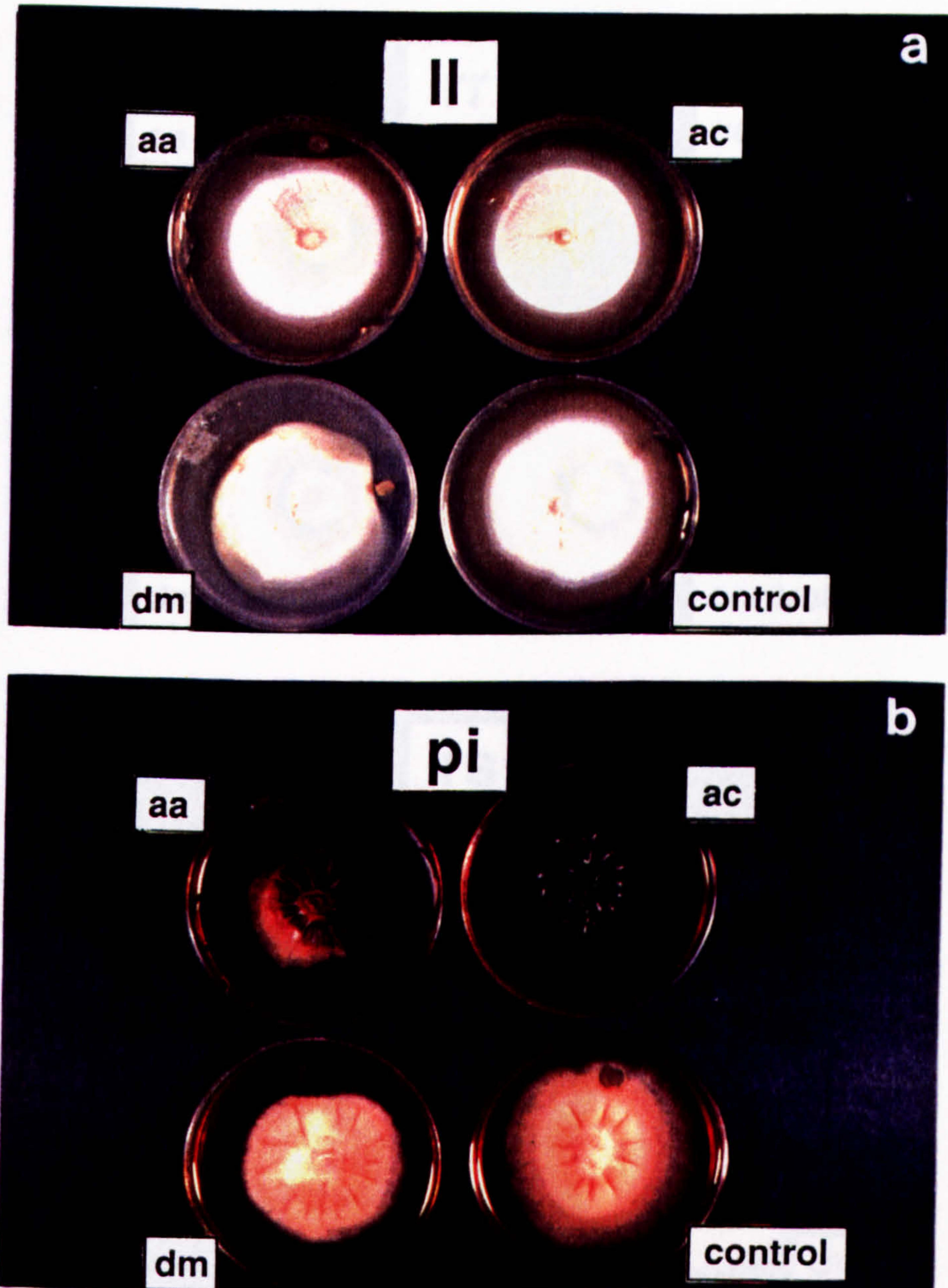


Plate 4.4. [a] The effect of the nematodes *A. composticola* (ac), *A. avenae* (aa), and *D. myceliophagus* (dm) on the growth of mycelium of the ectomycorrhizal fungus *L. laccata* (II). *A. composticola* and *A. avenae* have destroyed the mycelium completely as shown by the shiny surface of the fungal colonies. There is no visible effect of *D. myceliophagus*.

[b] The effect of *A. composticola* (ac), *A. avenae* (aa) and *D. myceliophagus* (dm) on the growth of mycelium of the ectomycorrhizal fungus *P. involutus* (pi). *A. composticola* has completely destroyed the mycelium which has lost its characteristic colour. *A. avenae* has also caused considerable but not complete damage to the mycelium whereas there is only a slight visible effect of *D. myceliophagus*.

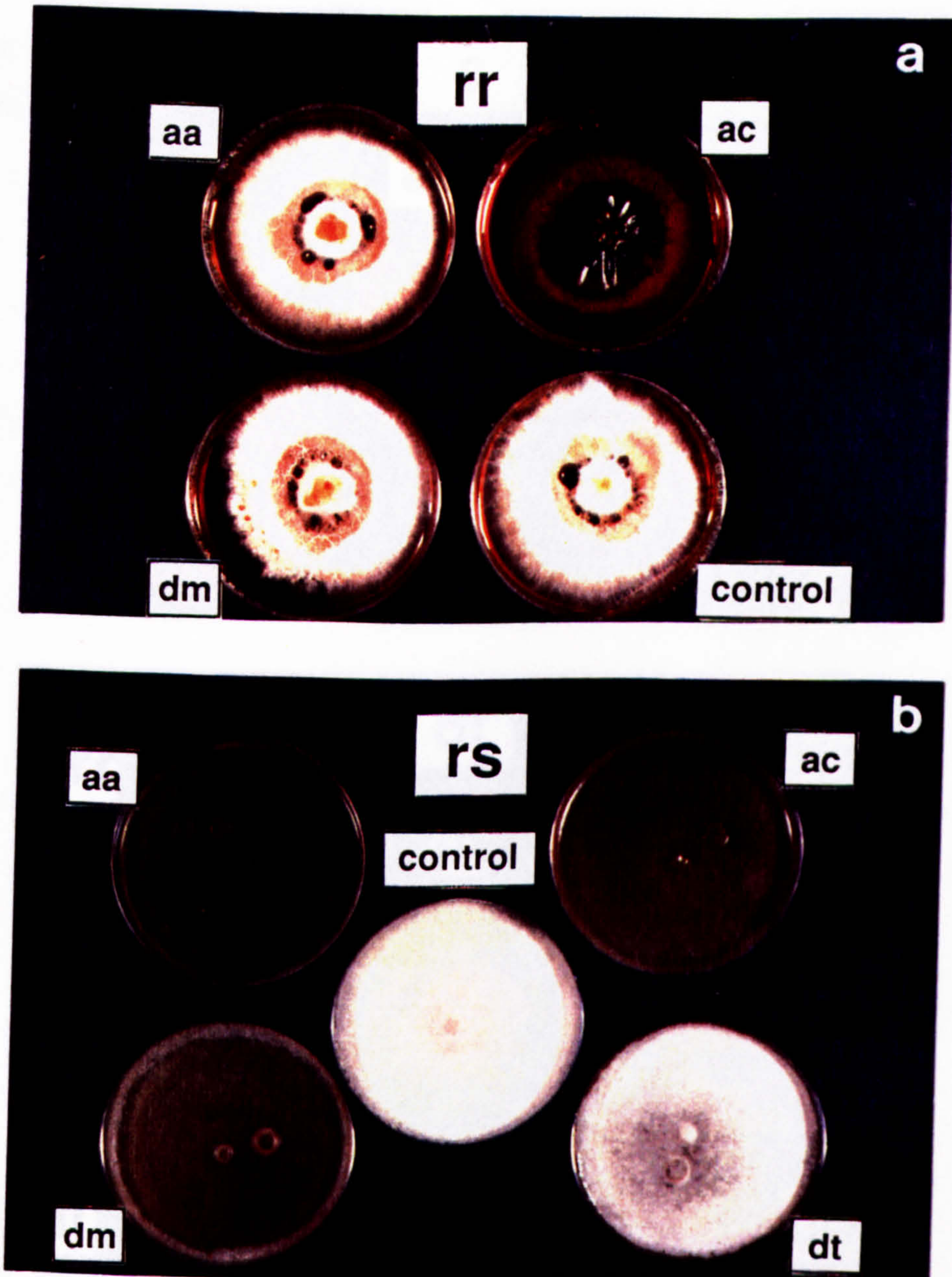


Plate 4.5. [a] The effect of the nematodes *A. composticola* (ac), *A. avenae* (aa), and *D. myceliophagus* (dm) on the growth of mycelium of the ectomycorrhizal fungus *R. roseolus* (rr). *A. composticola* has completely destroyed the mycelium as shown by the loss of its characteristic white colour. There is no visible effect of *A. avenae* and only a small effect of *D. myceliophagus*.

[b] The effect of *A. composticola* (ac), *A. avenae* (aa), *D. myceliophagus* (dm) and *D. triformis* (dt) on the growth of mycelium of the phytopathogenic *R. solani* (rs). *A. composticola* and *A. avenae* have both completely destroyed the hyphae which have lost their characteristic colour. Damage by *D. myceliophagus* is also nearly complete whereas there is no visible effect of *D. triformis*.

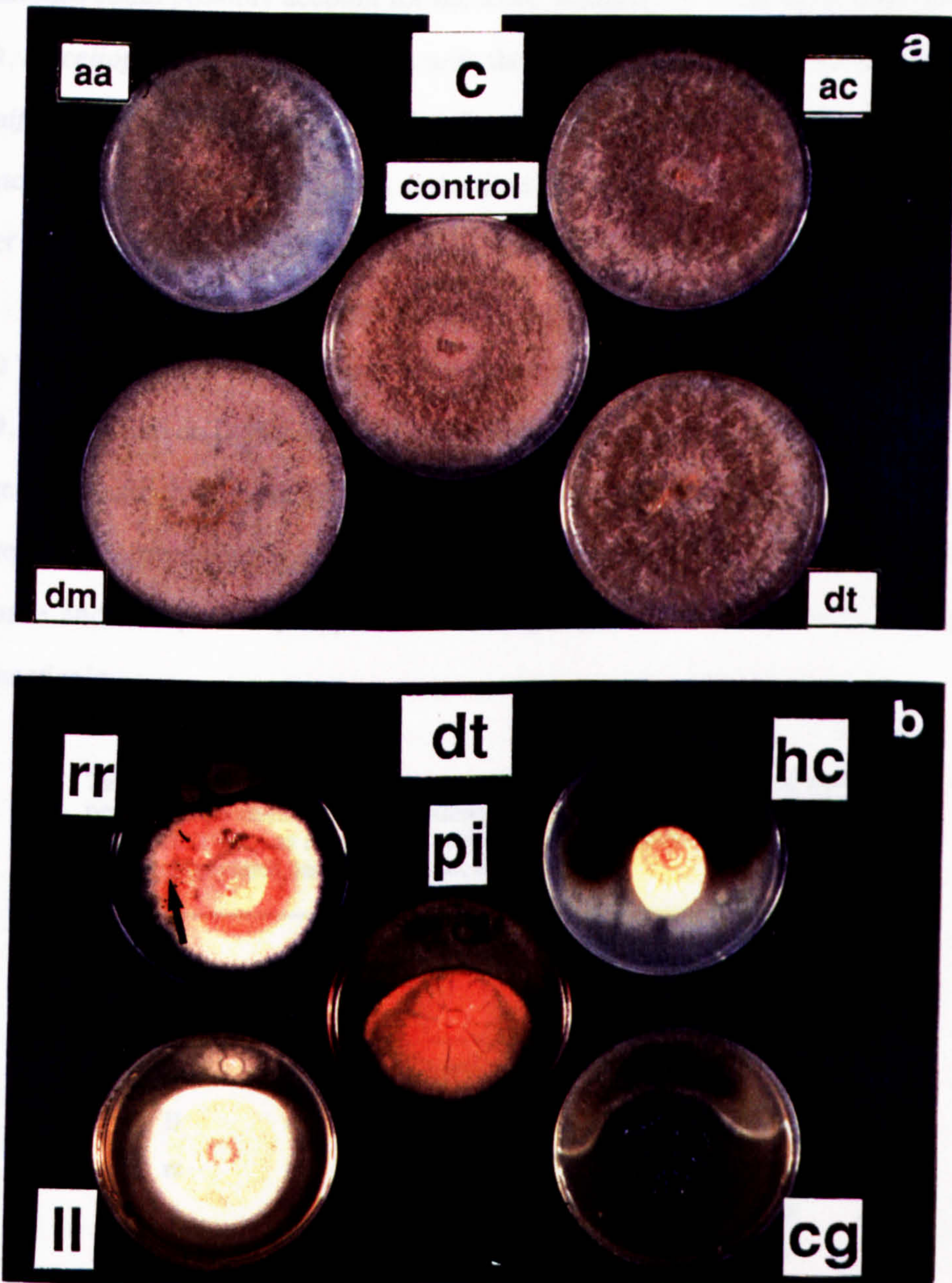


Plate 4.6. [a] Neither *A. composticola* (ac), *A. avenae* (aa), *D. myceliophagus* (dm) nor *D. triformis* (dt) have affected the growth of fungus C (c).

[b] The effect of *D. triformis* (dt) on the growth of mycelium of the ectomycorrhizal fungi *H. crustuliniforme* (hc), *P. involutus* (pi), *R. roseolus* (rr), *L. laccata* (ll) and *C. graniforme* (cg).

The contaminant fungus (f) introduced with the inoculum of the nematodes was antagonistic to all mycorrhizal fungi. The colonies of *H. crustuliniforme* and *P. involutus* grew less on the side facing the colony of the contaminant whereas a demarcation zone was formed between the colonies of *R. roseolus*, *L. laccata* or *C. graniforme* or the contaminant fungus. The contaminant fungus was grazed by the nematodes which subsequently completely destroyed the mycelia of all fungi except *R. roseolus* whose mycelium was only partly damaged (arrow).

(Kondrollochis 1977). This influence of the age of mycelium on the reproduction of the nematodes could possibly account for the lower numbers of nematodes, especially those of *D. myceliophagus*, found in plates with the slower growing ectomycorrhizal fungi *C. graniforme*, *H. crustuliniforme*, *P. involutus* and *R. roseolus* which would thus have a greater proportion of old mycelium at the time of introduction of the nematodes than the faster growing *L. laccata* and the saprotrophs.

The life cycles of *A. composticola*, *A. avenae*, *D. myceliophagus* and *D. triformis* at 20 °C are of length 9, 12, 19 and 25 days respectively (Hesling 1977; Evans & Fisher 1969, 1970; Pillai & Taylor 1967a). In addition, eggs of *D. triformis* take at least 3 times longer to hatch than those of *A. composticola*, *A. avenae* and *D. myceliophagus*. Therefore, *D. myceliophagus* and *D. triformis* have a much slower rate of population increase and this probably explains why they reproduced less than *A. avenae* or *A. composticola*.

Table 4.1. Ranking of the species of fungi according to numbers of nematodes produced (N) or according to per cent reduction in weight of dry mycelium (%R), as a result of feeding of the nematodes. See also Fig. 4.1 for the abbreviated names of the species of fungi.

<i>A. composticola</i>		<i>A. avenae</i>		<i>D. myceliophagus</i>	
N	%R	N	%R	N	%R
ab	hc	rs	rs	ab	ab
ll	ab	ab	ab	rr	rs
rs	rs	ll	hc	rs	c
d	ll	hc	cg	ll	cg
cg	pi	cg	ll	pi	rr
pi	cg	d	pi	cg	hc
hc	rr	rr	d	hc	d
rr	d	pi	rr	d	pi
c	c	c	c	c	ll

Different orders of species of fungi were derived when these were ranked in terms of mean numbers of nematodes produced, per cent reduction in weight of dry mycelium or mean numbers of nematodes per g mycelium (Table 4.1). This made it

difficult to rank the species of fungi tested in terms of their suitability as hosts for nematodes. Consequently an attempt was made to model mathematically the relationship between the increase in numbers of nematodes and their effect on the growth of fungal mycelia on which they manage to feed.

4.5. MODELLING DYNAMICS OF NEMATODES AND THEIR HOST FUNGI

In an attempt to interpret the experimental results, a 'Lotka-Volterra' model which describes plant-herbivore dynamics (see Crawley 1983) was used. This model was modified by assuming that no significant death of nematodes will occur over the time-span of an experiment such as that described in this chapter and that weighed fungal mycelium grazed by nematodes includes both dead and live hyphae.

When nematodes are absent, the fungus is assumed to grow to the carrying capacity (K) of an agar plate in a logistic fashion. The rate of increase in weight of mycelium $\frac{dV}{dt}$ is assumed to be proportional to the weight of the existing live mycelium (V) and the fraction of carrying capacity ^{utilized} not by the fungus $\left[1 - \frac{V+B}{K}\right]$ where B is the weight of dead mycelium:

$$\frac{1}{V} \frac{dV}{dt} = a \left[1 - \frac{V+B}{K} \right] \dots \dots \dots 4.1$$

Parameter a , assumed to be constant, may be termed the 'intrinsic growth rate' of the fungus, which is the proportional growth rate $\left[\frac{1}{V} \frac{dV}{dt} \right]$ when $\frac{V+B}{K}$ is very small.

When nematodes are also present, a factor, bN , which accounts for decreased rate of growth of the fungus as a result of grazing is also included:

$$\frac{1}{V} \frac{dV}{dt} = a \left[1 - \frac{V+B}{K} \right] - bN \dots \dots \dots 4.2$$

where N is the total number of nematodes and b is a constant termed the 'growth depression coefficient' which relates depression in the proportional growth rate of the fungus to numbers of nematodes.

The rate of change of nematode numbers is given by the following equation, assuming that no nematodes die:

$$\frac{1}{N} \frac{dN}{dt} = \frac{d(\ln N)}{dt} = cV \dots\dots\dots 4.3$$

where c is a third parameter, assumed to be constant and termed the 'nematode increase coefficient', which in biological terms relates the reproduction of nematodes to availability of biomass of food.

The equations were simulated numerically by a simple computer program written in C, which could be supplied with appropriate values for starting numbers of nematodes (N_0), starting weight of mycelium (V_0), a , b , and c (Fig. 4.4). The computer program was then able to calculate daily increments ΔN , ΔB and ΔV and to add these increments to accumulated totals of N , B and V thus generating a list of the predicted values of V , B , $V+B$, per cent of live mycelium and N in an iterative manner up to 100 days. The simulation provides only approximate solutions to the equations and the iteration can lead to cumulative errors.

Since the present experiment included only one sampling date, parameters N_0 , V_0 , a , b , and c were estimated using experimental data obtained by serial sampling of *A. composticola* feeding on the fungus *P. involutus* in agar culture (Hickson pers comm.). The calculated values of these parameters were subsequently adjusted to give best possible agreement between experimental ^{data} and predictions (Table 4.2).

When $\frac{V+B}{K}$ is very small, $\left[1 - \frac{V+B}{K}\right]$ is practically equal to 1 and equations 4.1

and 4.2 become:

$$a = \frac{1}{V} \frac{dV}{dt} = \frac{d(\ln V)}{dt} \dots\dots\dots 4.4$$

$$a - bN = \frac{1}{V} \frac{dV}{dt} = \frac{d(\ln V)}{dt} \dots\dots\dots 4.5$$

a is equal to the differentiated natural logarithm of the weight of dry live mycelium ($\ln V$) with respect to time in the absence of nematode feeding. The value of a was therefore

Fig. 4.4. The C program written according to the modified 'Lotka-Volterra' model (see Crawley 1983) to simulate the effects of grazing of nematodes on some fungi grown in pure culture.

```

main ()
{
    double V, B, N, V0, N0, K, a, b, c, dV, dB, dN, dt;
    int day, t;

    printf ("Starting number of nematodes?\n");
    scanf ("%lf", &N0);
    printf ("Starting wt of fungus?\n");
    scanf ("%lf", &V0);
    printf ("Intrinsic rgr of fungus?\n");
    scanf ("%lf", &a);
    printf ("Max wt of fungus?\n");
    scanf ("%lf", &K);
    printf ("Growth depression coefficient?\n");
    scanf ("%lf", &b);
    printf ("Nem increase coefficient?\n");
    scanf ("%lf", &c);

    V = V0; N = N0; t = 0; B = 0;
    for (day = 0; day < 100; day++)
    {
        1 dN = c*N*V;
        dB=b*dN/c;
        dV = a*V*(1 - (V+B)/K) - dB;
        N += dN;
        V += dV;
        B += dB;
        t += 1;
        printf ("%3d %6.31f %6.31f %6.31f %6.31f %6.31f\n", t, V, B, V+B, V*100/(V+B), n);
    }
}

```

¹: d is a symbol used here instead of Δ

estimated by plotting $\ln V$ against time and fitting a straight line, whose slope gave an estimate of the value of a , equal to 0.1 day^{-1} (Fig. 4.5a & Table 4.2).

Parameter b was calculated by solving equation 4.2 for b and using the appropriate values of $\frac{dV}{dt}$, a and N . From equation 4.5:

$$b = \frac{1}{N} \left[a - \frac{d(\ln V)}{dt} \right]$$

where $a = 0.1 \text{ day}^{-1}$ (see Fig. 4.5a). $\frac{d(\ln V)}{dt}$ was calculated by plotting the natural logarithm of the weight of dry mycelium from plates of *P. involutus* with *A. composticola* against time and differentiating the equation that described the best fitted line (Fig. 4.5b). This gave $\frac{d[\ln(V+B)]}{dt} = 0.0784$. Depending on N which varied with time, b had a value in the range 1.7×10^{-5} to $0.08 \times 10^{-5} \text{ gg}^{-1} \text{ nematode}^{-1} \text{ day}^{-1}$. The value which was actually used in the model was $10^{-5} \text{ gg}^{-1} \text{ nematode}^{-1} \text{ day}^{-1}$ corresponding to a time when B was small, as this produced the closest fit between experimental and predicted results.

Table 4.2. The parameters and their standard values used in the simulation program.

Parameter	Value
Starting numbers of nematode, N_0	1276 nematodes
Starting weight of the dry weight of the fungus, V_0	0.004 g
Intrinsic proportional growth rate of the fungus, a	0.1 day^{-1}
Maximum weight of dry mycelium allowed, K	100 g
Growth depression coefficient, b	$0.00001 \text{ gg}^{-1} \text{ nematode}^{-1} \text{ day}^{-1}$
Nematode increase coefficient, c	$20 \text{ nematode nematode}^{-1} \text{ g}^{-1} \text{ day}^{-1}$

Parameter c was estimated by solving equation 4.3. The values of $\frac{d(\ln N)}{dt}$ were derived by plotting the logarithms of numbers of nematodes against time and differentiating the equation of the fitted straight line (Fig. 4.5c). V , the other variable

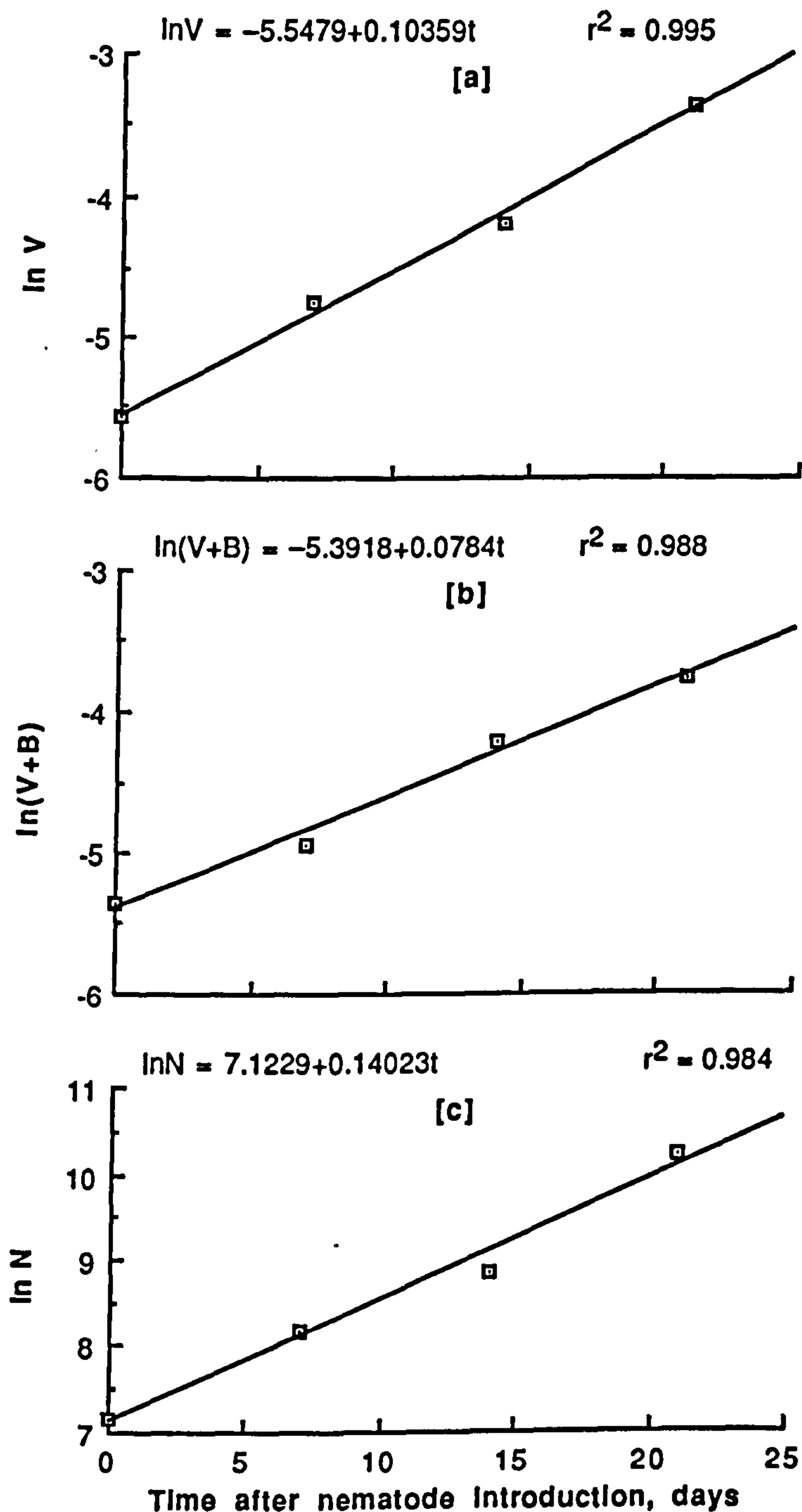


Fig. 4.5. [a] The natural logarithm of V , the mean dry weight of the mycelium of *P. involutus* in plates without nematodes, plotted against time. The slope of the line is equal to the value of parameter a of the model (see equation 4.4).

[b] The natural logarithm of $V+B$, the mean dry weight of the mycelium of *P. involutus* in plates with added nematodes. The slope of the line is equal to $\frac{d(\ln V)}{dt} = 0.0784$, see equation 4.5.

[c] The natural logarithm of N , the mean number of *A. composticola* produced in plates of *P. involutus*, plotted against time. The fitted equation served to calculate the value of c .

(Data of Hickson).

required for the solution of equation 4.3, was only known at $t=0$ because the mycelium recovered from plates with nematodes consisted of both live and dead hyphae. To overcome this problem the greater values of $V+B$, the weights actually measured had to be used in equation 4.3 in order to calculate c . This probably caused its value to decline from 21 at $t=1$ day to 8 at $t=21$ days instead of being constant as had been assumed. The value of c which was optimum for the model was 20 nematode $\text{nematode}^{-1}\text{g}^{-1}\text{day}^{-1}$ and was close to the value of 21 estimated at $t=0$. Finally it was assumed that the fungus never reached the carrying capacity of an agar plate and therefore K was given the relatively large value of 100.

The values of either V_0 , N_0 , a , b or c , were halved or doubled while keeping the others at their standard values, in order to determine the sensitivity of the model to changing values of these parameters, in terms of prediction of weight of dry mycelium and numbers of nematodes extracted at the time when practically all mycelium had been killed by the nematodes (Fig. 4.6a,b).

Changes in numbers of nematode and weight of dry mycelium were greatest with changes in a . Changing b strongly influenced nematode numbers whereas changing c influenced the weight of the fungus more than numbers of nematode. When N_0 was halved or V_0 doubled, production of mycelium was increased by about 50% whereas halving V_0 or doubling N_0 had little effect. The effect of V_0 and N_0 on the final nematode numbers was relatively small.

Dynamics of the weight of live mycelium and of the numbers of nematodes predicted by the model when supplied with the values of a , b , c etc estimated from data (Table 4.2) are shown in Fig. 4.7. The effect of nematodes on the rate of increase in weight of mycelium is also shown. There was reasonable agreement between prediction and the experimental data of Hickson indicating that the dynamics of *A. composticola* and its host *P. involutus* on agar may have been adequately described by the model.

The model predicts that at the time when almost all mycelium has been killed by the nematodes, the ratio R of the number of nematodes (N) to the weight of live plus dead

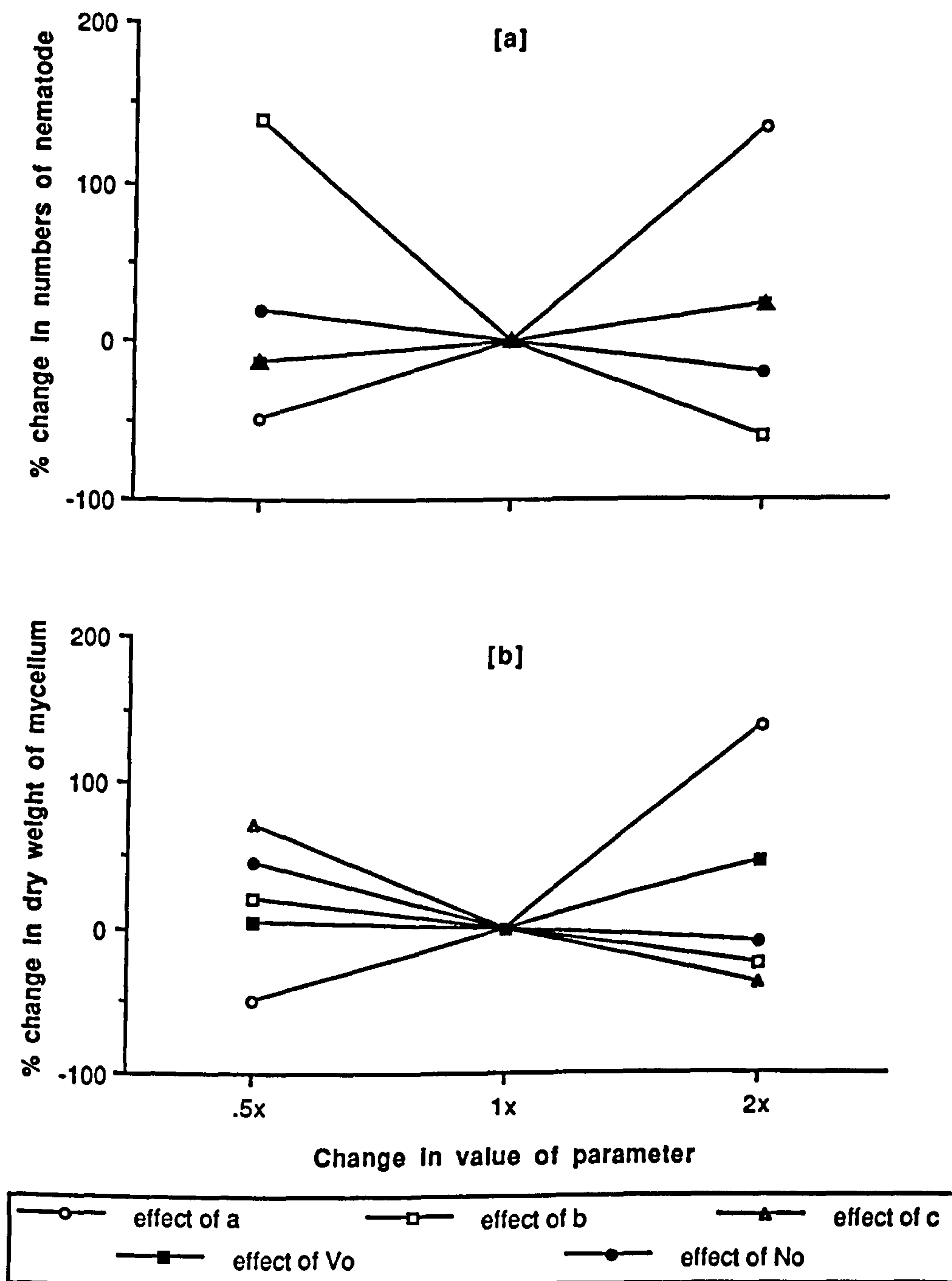


Fig. 4.6. The effect of changing the values of parameters a , b , c , V_0 and N_0 on the predicted number of nematodes [a], and the dry weight of live plus dead mycellium [b] produced at the time when almost all mycellium has been killed by the nematodes.

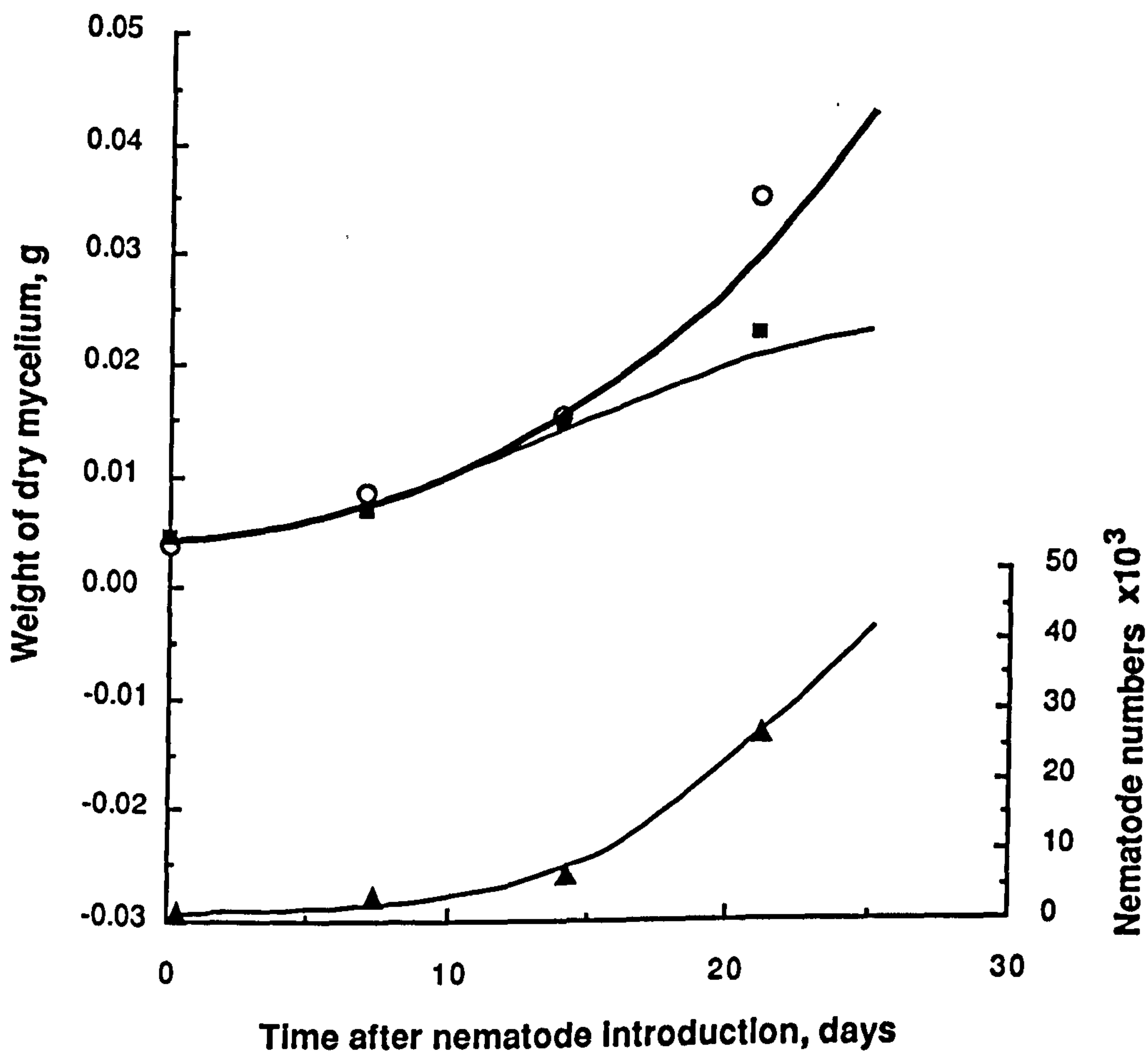


Fig. 4.7. The closeness of fit between experimental data of the dry weight of mycelium of *P. involutus* and numbers of *A. composticola* recovered from plates (points), and predictions (lines) for these data obtained with the simulation program shown in Fig. 4.4.

○: mycelium from the control, thick line: prediction.

■: live plus dead mycelium from the plates treated with nematodes, thin line: prediction.

▲: actual numbers of nematodes recovered; thin line: numbers of nematodes predicted.

(Experimental data of Hickson).

mycelium ($V+B$) equals $\frac{c}{b}$ and is nearly independent of V_0 , N_0 or a (Fig. 4.8a,b,c). The calculated value of R was 2×10^6 nematodes/g mycelium and agrees reasonably well with the figure of 2.7×10^6 , derived from values measured in plates of *A. composticola* feeding on *P. involutus* in the experiment described in this chapter.

As mentioned previously in this section, this type of computer simulation by its nature cannot give an exact solution of the differential equations. Nevertheless, the simulation aids understanding of some aspects of these nematode-fungus interactions, especially in agar plates. Fig. 4.9 shows that as the weight of live mycelium (V) in the plates increases, so do numbers of nematodes become sufficiently large to interfere seriously with the growth of the fungus. The weight of live mycelium then declines until most has been killed, while the numbers of nematodes approach a maximum value. If the fungus grows relatively fast, equivalent to greater values of a (e.g. 2x the standard value of a given in Table 4.2), its weight of live mycelium increases at a faster rate. This results in a faster increase in numbers of nematodes and earlier death of the mycelium. Slower growing fungi produce smaller quantities of mycelium but also produce smaller numbers of nematodes thus surviving for a longer time (.5x standard value of a).

Species of nematodes which are capable of significantly damaging fungal mycelia should have greater values of b . At 2x the standard value of b , the mycelium grows little with relatively small numbers of nematodes. In contrast, with small values of b (e.g. .5x its standard value), the mycelium grows more but its growth eventually decreases sharply as a result of very rapidly increasing numbers of nematodes (Fig. 4.9).

Situations where a small production of fungal mycelium leads to a relatively fast increase in numbers of nematode will occur when c is large. When c is small, numbers of nematodes increase slowly at the beginning, allowing the mycelium to grow more and survive longer. This eventually leads to the production of numbers of nematodes similar to those for the standard value of c given in Table 4.2 (Fig. 4.9).

Riffle (1971) grouped a series of fungal species according to the effect that the nematode *Aphelenchoides cibolensis* had on the viability and the linear growth of the fungal colonies and according to the numbers of nematodes produced in the cultures of

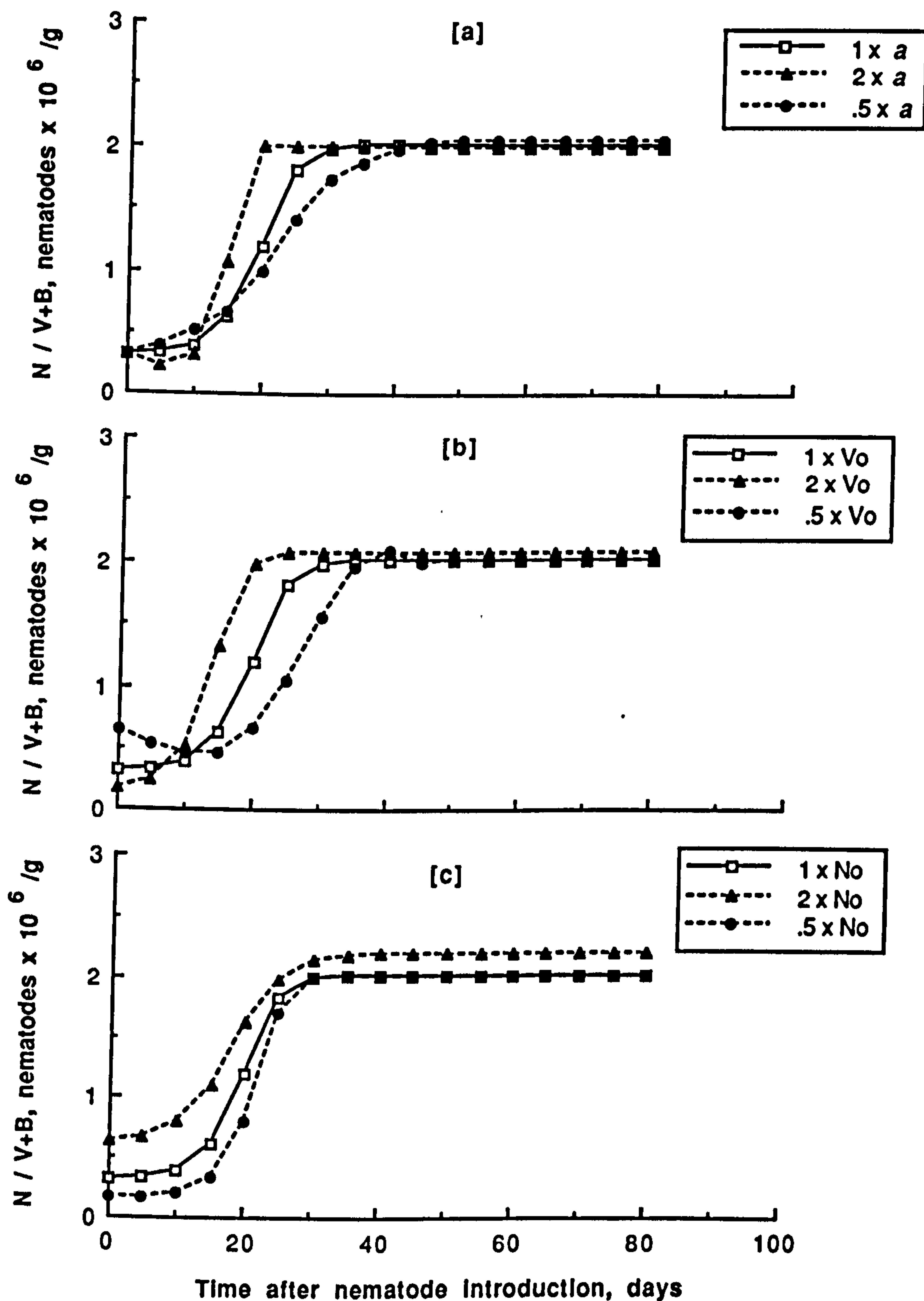


Fig. 4.8. The effect of doubling or halving the values of a , V_0 and N_0 , graphs [a], [b] and [c] respectively, on the predicted ratio of the number of nematodes (N) to the total weight of mycelium ($V+B$) recovered from agar plates at the time when almost all mycelium had been killed by the nematode.

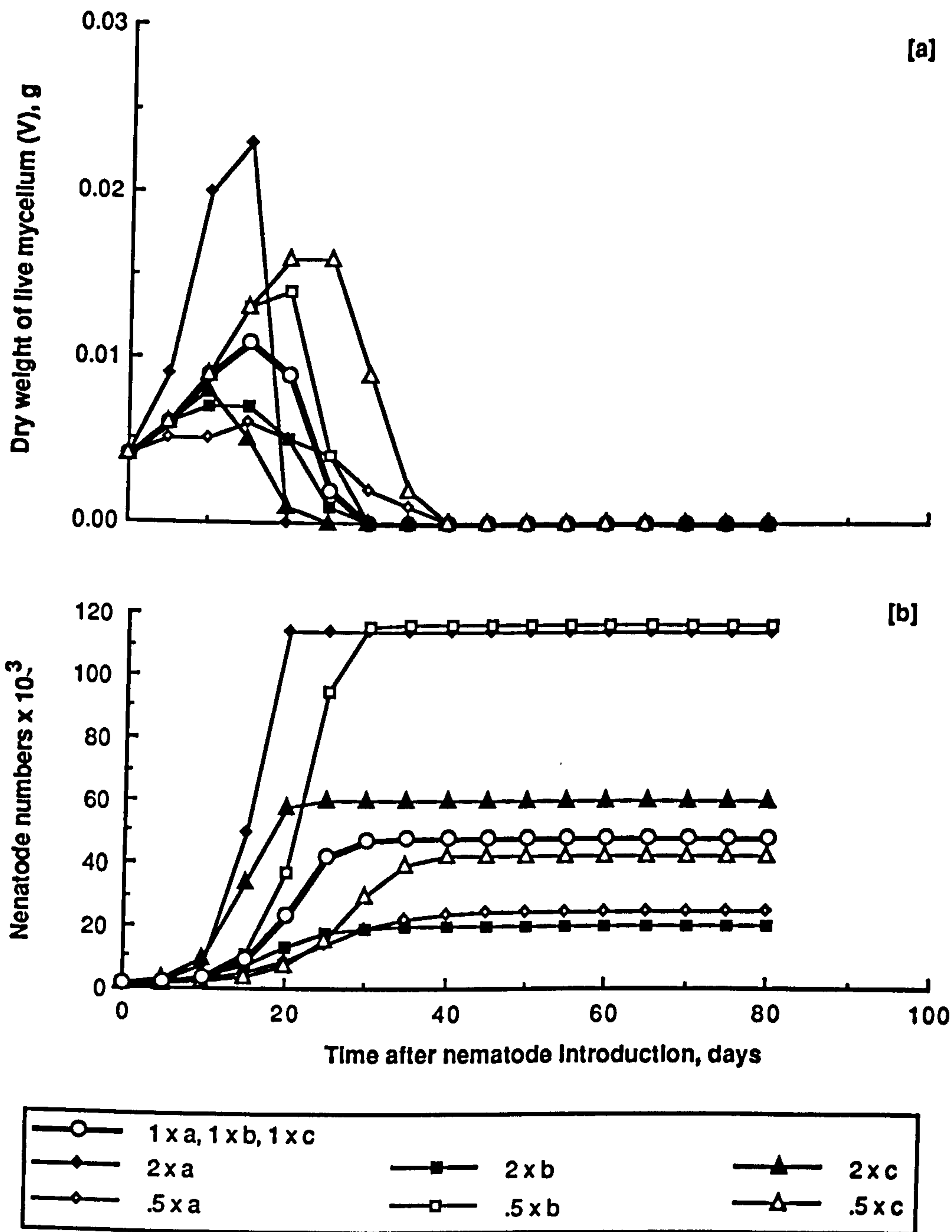


Fig. 4.9. The influence of different values of a , b or c on: [a] The effect of nematodes on the dry weight of live mycelium of a fungus and [b] the increase of the numbers of nematodes in response to feeding. Data were calculated by the simulation program shown in Fig. 4.4 using the standard values of parameters a , b and c (1x) as well as the doubles or halves of each parameter while keeping the standard values of the other two parameters.

each fungus. These groups which were described by Riffle seem to correspond to the predictions which were obtained using the simulation model while varying either *a*, *b* or *c*.

Townshend (1964) fed *A. avenae* on a variety of species of fungi and ranked these in terms of their suitability as hosts for the nematode according to final numbers of nematodes produced. Mankau & Mankau (1963) and Pillai & Taylor (1967b) also used information on the increase in numbers of nematodes in a discussion of the food preferences of the nematodes but did not link these food preferences to reductions in the linear growth of fungal colonies caused by the nematodes which they also measured. Sutherland & Fortin (1968) found that reduction in growth of the fungi was more dependent upon the number of nematodes added initially than upon the final number of nematodes in the plates, although in cases where fungal growth was substantially impaired, high numbers of nematodes had usually been produced.

The present experiment showed that the increase in numbers of *A. composticola* and the reduction in weight of mycelium were not well correlated (Fig. 4.3). As a result, two different orders were obtained when the species of fungi were ranked according to numbers of *A. composticola* produced or according to per cent reduction in weight of dry mycelium (Table 4.1). For example, *H. crustuliniforme* is the species of fungus whose mycelial weight was reduced most by the nematode but also the species of fungus which produced only a small population of nematodes. In contrast, the growth of *L. laccata* was reduced less but the nematode reached greater numbers on this species compared to *H. crustuliniforme*.

Two different orders of species of fungi were also obtained when these were ranked according to numbers of *A. avenae*, or *D. myceliophagus* produced or according to per cent reduction in weight of dry mycelium which was caused by the feeding of either one of these species of nematodes (Table 4.1).

The most useful outcome of the simulation model was to predict that the ratio (*R*) of numbers of nematode to weight of dry mycelium recovered at the time when almost all mycelium had been killed by the nematode was largely independent of fungal growth

rate, the biomass of fungus at the time of introduction of the nematodes and initial numbers of nematodes. Therefore *R*, might be an appropriate index of the suitability of different species of fungi as food sources for a given species of nematode. In this experiment, the mycelium of a fungus was considered to be dead as a result of the feeding of nematodes only when all of its aerial mycelium had been destroyed and its dry weight reduced significantly in relation to the control. On this basis only certain fungi presented to *A. composticola* and *A. avenae* could be ranked in terms of their suitability as hosts for each species of nematode.

It was concluded that *A. bisporus* was the favourite host of *A. composticola*, followed by *R. solani*, *L. laccata* and *H. crustuliniforme* (Fig. 4.10a) and *R. solani* was the favourite host of *A. avenae*, followed by *A. bisporus* and *H. crustuliniforme* (Fig. 4.10b). All these fungi shared the characteristic of having fine, non-pigmented hyphae (Plates 4.2a, 4.3b, 4.4a & 4.5b). *P. involutus* and *C. graniforme* were the least favoured hosts of *A. composticola*. *C. graniforme* was the least favoured host of *A.avenae*. *C. graniforme* had heavily pigmented hyphae and *P. involutus* exuded metabolic products which stained the agar and may have been toxic to the nematodes (Plates 4.2b, 4.4b).

For example, Sutherland & Fortin (1968) found that the younger mycelium of *R. roseolus*, growing in agar culture, produced an exudate which was toxic to *A. avenae*.

4.6. CONCLUSION

When a species of nematode manages to feed on a species of fungus grown in pure culture, it increases in numbers and causes a reduction in the growth of the fungus which is best measured in terms of reduced dry weight. Of the four species of nematodes whose food preferences were examined, *A. composticola* usually reproduced most and caused the greater reductions in weight of dry mycelium at the time of sampling. It is likely that its generally faster rate of reproduction as well as its short period of feeding at each site causing more widespread damage to mycelia at an earlier stage than other species of nematode (e.g. *D. myceliophagus*) which feed for longer times at each site, could account for its superior productivity.

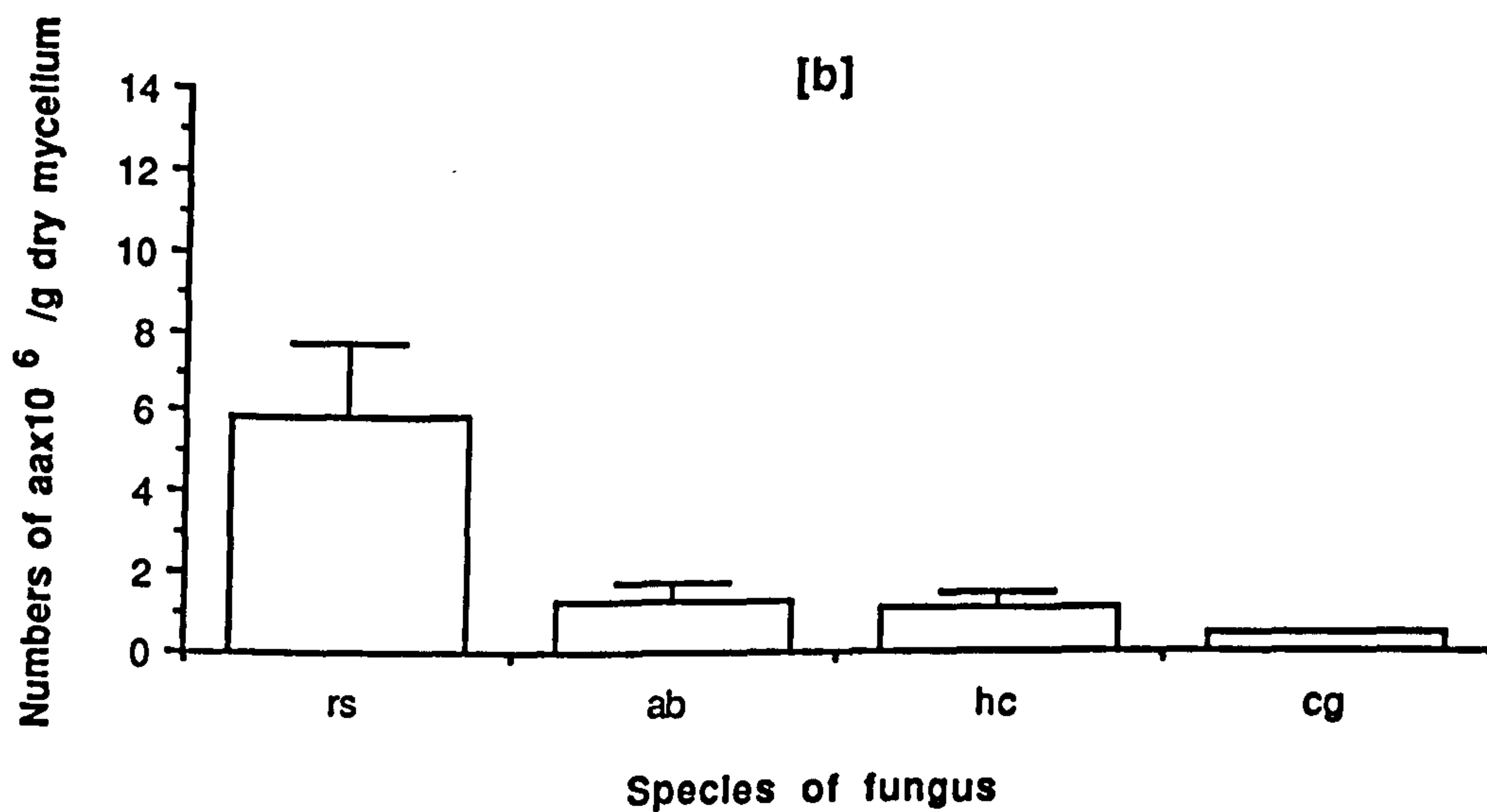
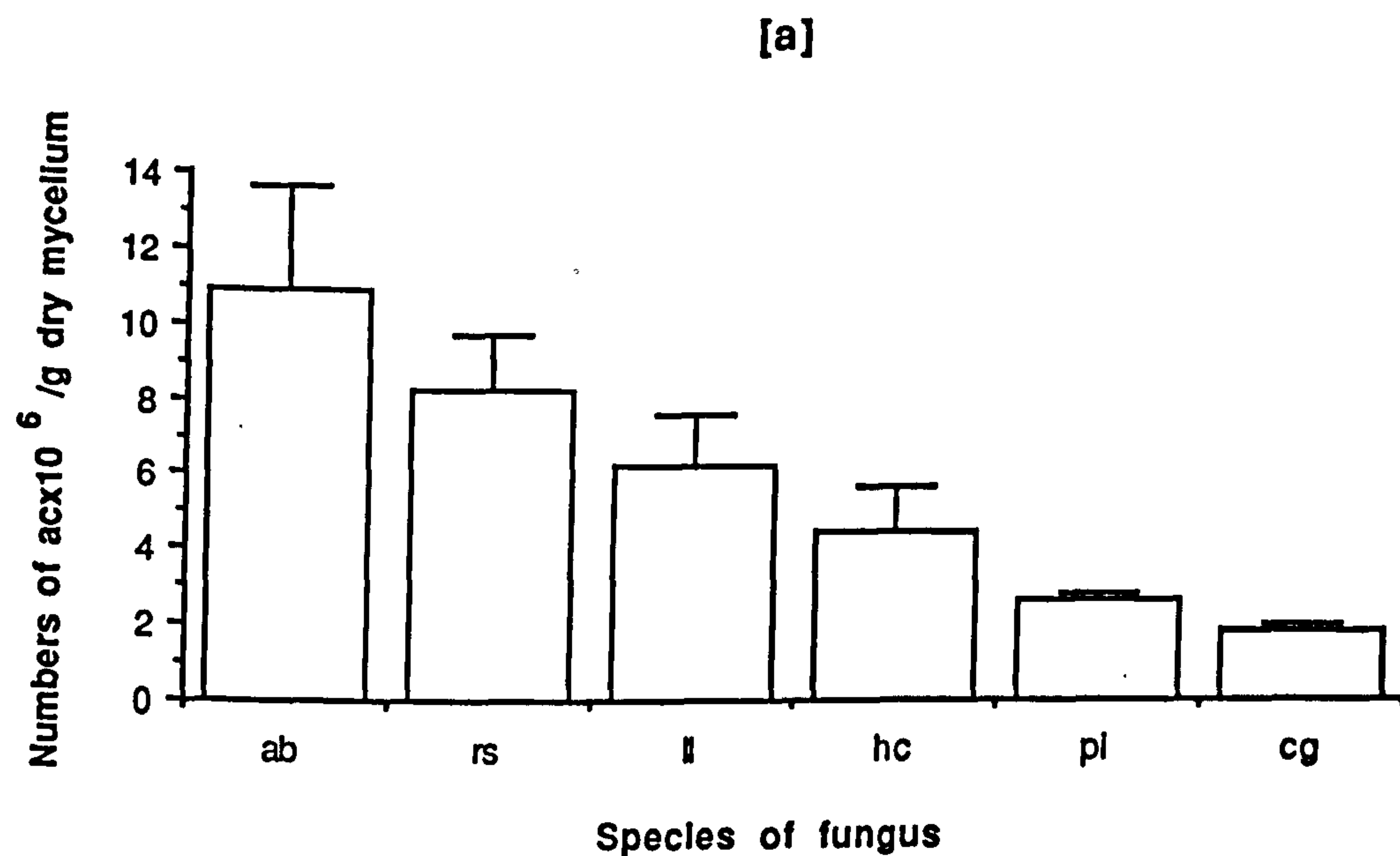


Fig. 4.10. Species of fungi ranked according to their suitability as hosts for the nematodes [a] *A. composticola* (ac) and [b] *A. avenae* (aa), in terms of the ratio of numbers of nematodes to weight of dry mycelium recovered from agar plates.

Columns show means of three replicates and bars represent ± 1 standard error. See also Fig. 4.1 for the abbreviated names of the species of fungus.

C, the only fungus that produced large numbers of conidia, was also the only fungus not damaged by any of the nematodes. Riffle (1971) attributed the fact that *Aphelenchoides cibolensis* did not reduce the viability of the root pathogenic fungus *Leptographium* sp. to its large production of conidia. He observed that germination of the conidia was unaffected by the nematode. The same could have happened in the present experiment, thus allowing the fungus to spread, while at the same time suppressing reproduction of *A. composticola*. Thus in general, the ability of soil fungi to avoid being damaged by nematodes may be considerably enhanced by rapid formation of conidia which are resistant to grazing. Such fungi are the r-selected species whose main characteristics are the rapid germination of spores and high rates of extension of mycelium that permit them to rapidly utilize available resources and produce large numbers of non-dormant spores (see Cooke & Rayner 1984). In contrast, the k-selected fungi, such as the ectomycorrhizal species, devote only a small proportion of their resources to reproduction, have a slow rate of growth and long vegetative phases which probably make them more susceptible to damage by fungal-feeding nematodes.

The dynamics of numbers of nematodes and of fungal biomass could be reasonably described with the use of the simulation model based on the 'Lotka-Volterra' equations. The model involved parameter R , which related the effect of nematodes on the growth of fungal mycelia to the increase of numbers of the nematodes in response to the food source. Parameter R , by being independent of the 'intrinsic growth rates' of fungi, their initial weights and the initial numbers of the nematode present, could be used to rank several species of fungi in terms of their suitability as hosts for a given species of nematode.

CHAPTER 5. INTERACTIONS BETWEEN *APHELENCHUS AVENAE* AND VA MYCORRHIZAS OF *GLOMUS CLARUM* IN RED CLOVER.

5.1. INTRODUCTION

The measurement of numbers of nematodes and biomass of mycelium in agar plates made it possible to assess the suitability of several species of fungi as hosts for different species of mycophagous nematodes. However, the lack of a quantitative technique for the measurement of the numbers of nematodes in soil caused difficulty in the interpretation of the results of experiments with VAM fungi growing symbiotically with plant roots in soil (see Chapter 3). The aim of the experiment described in this chapter was to employ the more satisfactory technique BF2 (see section 2.2.3) to:

1. Investigate whether the sequence in which nematodes and *G. clarum* were added to the soil could have any effect on the outcome of the plant-VAM fungus-nematode interaction.
2. Relate the development of the VAM fungus to the population of nematodes.

Although *A. composticola* seemed to have the widest range of suitable hosts and reproduced more than the other species of nematodes, it had a smaller effect on fungal growth in relation to its numbers than *A. avenae* (see section 4.3, Fig. 4.3). In addition, *A. avenae* had the most detrimental effect on mycorrhizal infection (section 3.4.3, Fig. 3.9). It has been suggested that the mycophagous habit of *A. avenae* may be more important in agricultural soils where VAM fungi are also more abundant, whereas the presence of *A. composticola* may be more significant in forest soils (Walker 1984a). For all these reasons *A. avenae* was chosen for the present experiment.

5.2. MATERIALS AND METHODS

Each pot was filled with soil (210 g) which included either a layer of live inoculum of *G. clarum* (final average density of 3 spores/g soil) in mycorrhizal treatments

(m, mt₁, mt₂, mt₃) or the same amount of sterile inoculum in non-mycorrhizal treatments (control, t₁, t₂, t₃). Filtered leachings of live mycorrhizal inoculum were also added to the pots of non-mycorrhizal treatments.

The control consisted of pots to which neither mycorrhizal inoculum nor nematodes were added. Pots of treatment m received mycorrhizal inoculum but no nematodes. Where appropriate, pots received *A. avenae* at the rate of approximately 100/g soil applied to the soil surface. Pots were arranged in the growth room in a completely randomized design and the soil was watered from below. Pots which received nematodes were of three types depending on when *A. avenae* was applied in relation to the sowing date (day 0). Those in treatments mt₁ and t₁ received 22500±1984 *A. avenae* 11 days prior to sowing (day -11). On day 0, pots in treatments mt₂ and t₂ received 22073±2100 *A. avenae*, and red clover seed was sown in all pots. The weight of each pot, watered to field capacity, was adjusted to a common value by adding black alkathene beads. Subsequent watering was by weight, with water added to the pot surface to maintain the water content of the medium at 50% of field capacity. By following this watering regime, it was possible to avoid loss of nematodes into the saucers in which the pots stood. This loss had often been observed during previous experiments in which soil was watered from below. Seedlings were gradually thinned to 4 per pot. 11 days after sowing treatments mt₃ and t₃ received 23280±482 *A. avenae* per pot. The three doses of nematodes, introduced at times t₁, t₂ and t₃, came from the same batch of cultures, but from different dishes.

Three replicate pots from each treatment were harvested on 7 occasions (days 12, 17, 22, 25, 37, 46 and 60 after sowing) with the exception of treatments mt₃ and t₃ which were harvested only on days 12, 22, 37 and 60. The dry weights of shoots, fresh weights of roots and fractional infection were measured. The dry weights of roots were estimated by multiplying the fresh weights of roots by 0.104 which was the mean value of the ratio of dry to fresh weight of roots from a previous experiment. Total dry weights of the plants were then calculated. The numbers of nematodes were counted following their extraction from soil by method BF2 (see section 2.2.3).

5.3. RESULTS

Roots had become mycorrhizal by the first harvest (day 12 after sowing) and a significant response of dry weights of shoots, roots and whole plants to mycorrhizal infection was first measured 22 days after sowing (Fig. 5.1a,b,c). In all subsequent harvests, dry weights of shoots, roots and whole plants in the mycorrhizal treatments were significantly greater than those in non-mycorrhizal treatments.

There was no effect of the nematodes on the increase in dry weight of shoot, root or whole plant caused by mycorrhizal infection irrespective of the time when nematodes were added to the soil (Fig. 5.1a,b,c). Furthermore, nematodes did not damage the roots and did not reduce the growth of plants in the non-mycorrhizal treatments. The rate of increase and final level of fractional infection were not affected by the nematodes (Fig. 5.2). This result differed from that of experiment 6 (see section 3.4.3).

Changes in numbers of *A. avenae* in the soil are shown in Fig. 5.3. Numbers of nematodes extracted from soils of treatments t_3 and mt_3 were about 50% of those added 1 day previously. After 10 days (day 22), only around 20% of the initial number could be recovered. Numbers recovered on subsequent occasions showed little further change. Numbers of *A. avenae* introduced at the two earlier times (treatments t_1 , mt_1 , t_2 and mt_2), declined from their initial levels in a similar fashion to treatments t_3 and mt_3 . Mycorrhizal infection had no effect on the numbers of nematodes.

5.4. DISCUSSION

When the nematodes feed on the mycelium of a host fungus they multiply and may significantly reduce fungal biomass (see section 4.3). In the present experiment, however, neither was spread of mycorrhizal infection in the roots reduced by the nematodes nor did the numbers of nematodes increase. The question remains as to whether *A. avenae* could have been expected to reduce fractional infection and multiply significantly if it was able to feed on the mycorrhizal mycelium. This question was addressed by modelling the spread of infection and possible effects of nematodes using a

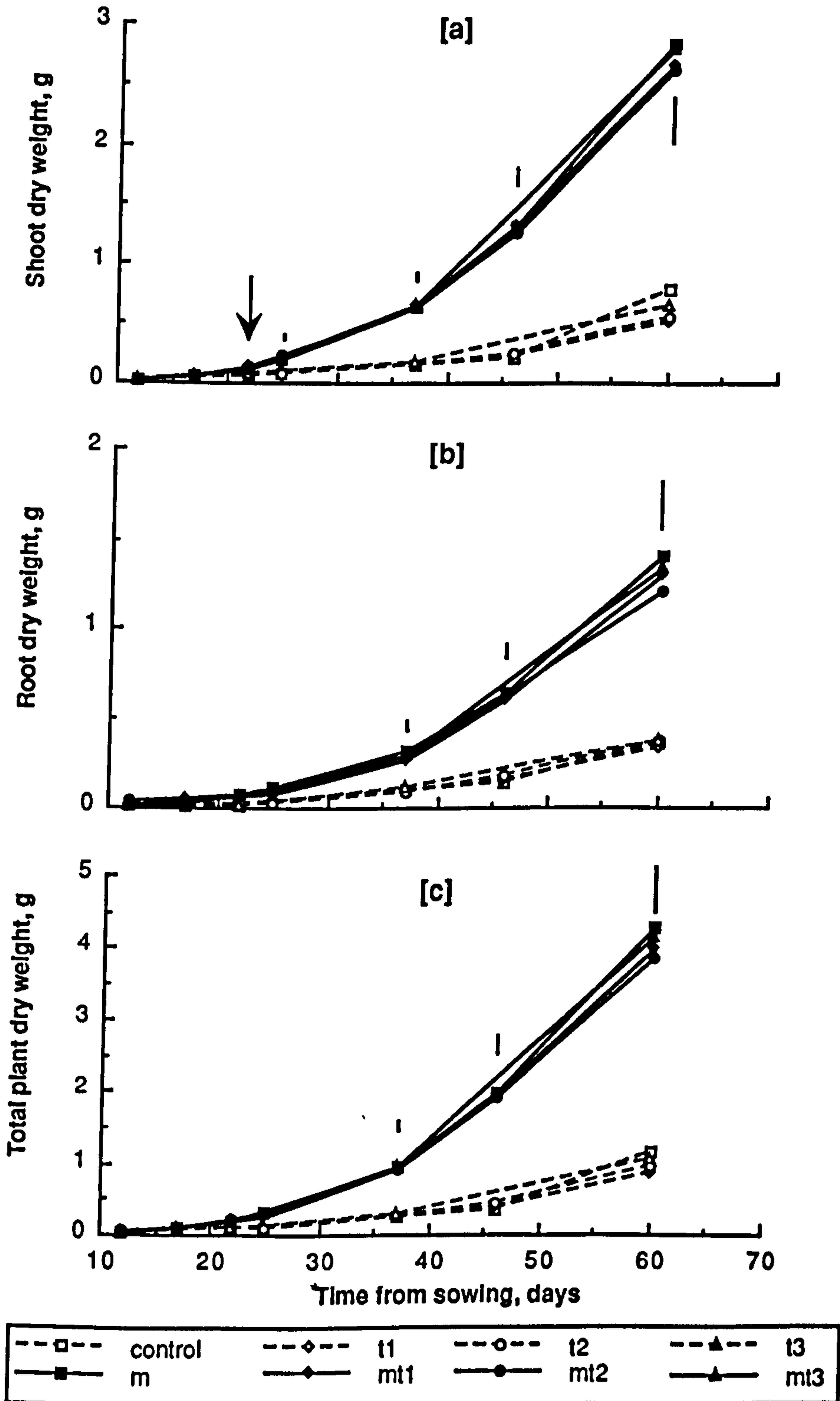


Fig. 5.1. The dry weights of shoots [a], roots [b] and total weights [c] of red clover plants as affected by *G. clarum* and *A. avenae* which was introduced to soil either 11 days prior to, at or 11 days after sowing. t1, t2 and t3: treatments with *A. avenae* alone introduced to soil 11 days prior to, at and 11 days after sowing respectively; m: treatment with *G. clarum* alone introduced to soil at sowing; mt1, mt2 and mt3: treatments with *G. clarum* introduced to soil at sowing and *A. avenae* introduced at the three times. The arrow shows the time at which a significant growth response of the plant to mycorrhizal infection was first observed. Points are means per pot and bars represent LSDs at $p=0.05$.

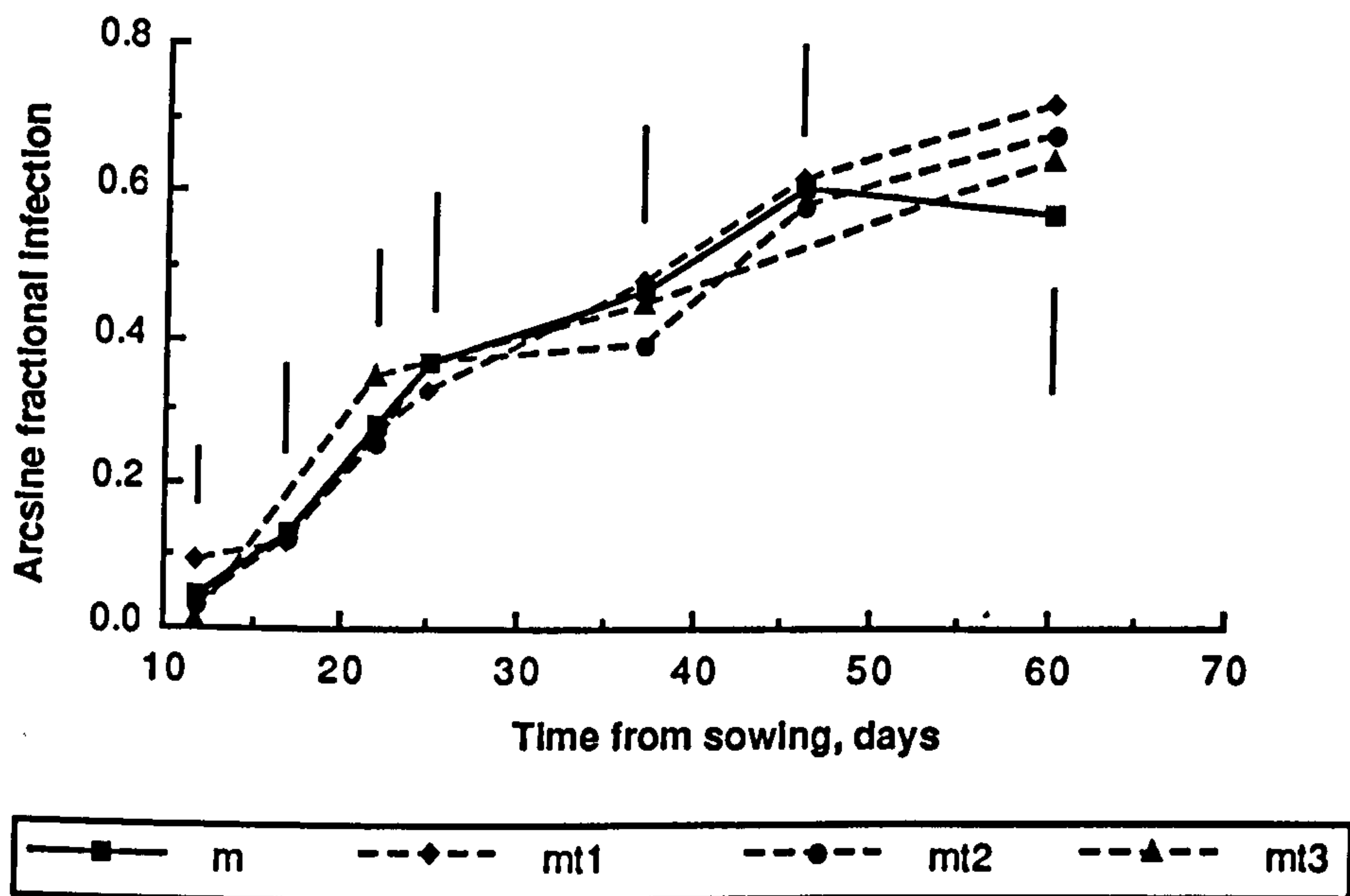


Fig. 5.2. The effect of *A. avenae* added to soil 11 days prior to, at or 11 days after sowing on infection (expressed as arcsine fractional infection) of the roots by *G. clarum*. Points are means per pot and bars represent LSDs at $p=0.05$. Treatment abbreviations as in Fig. 5.1.

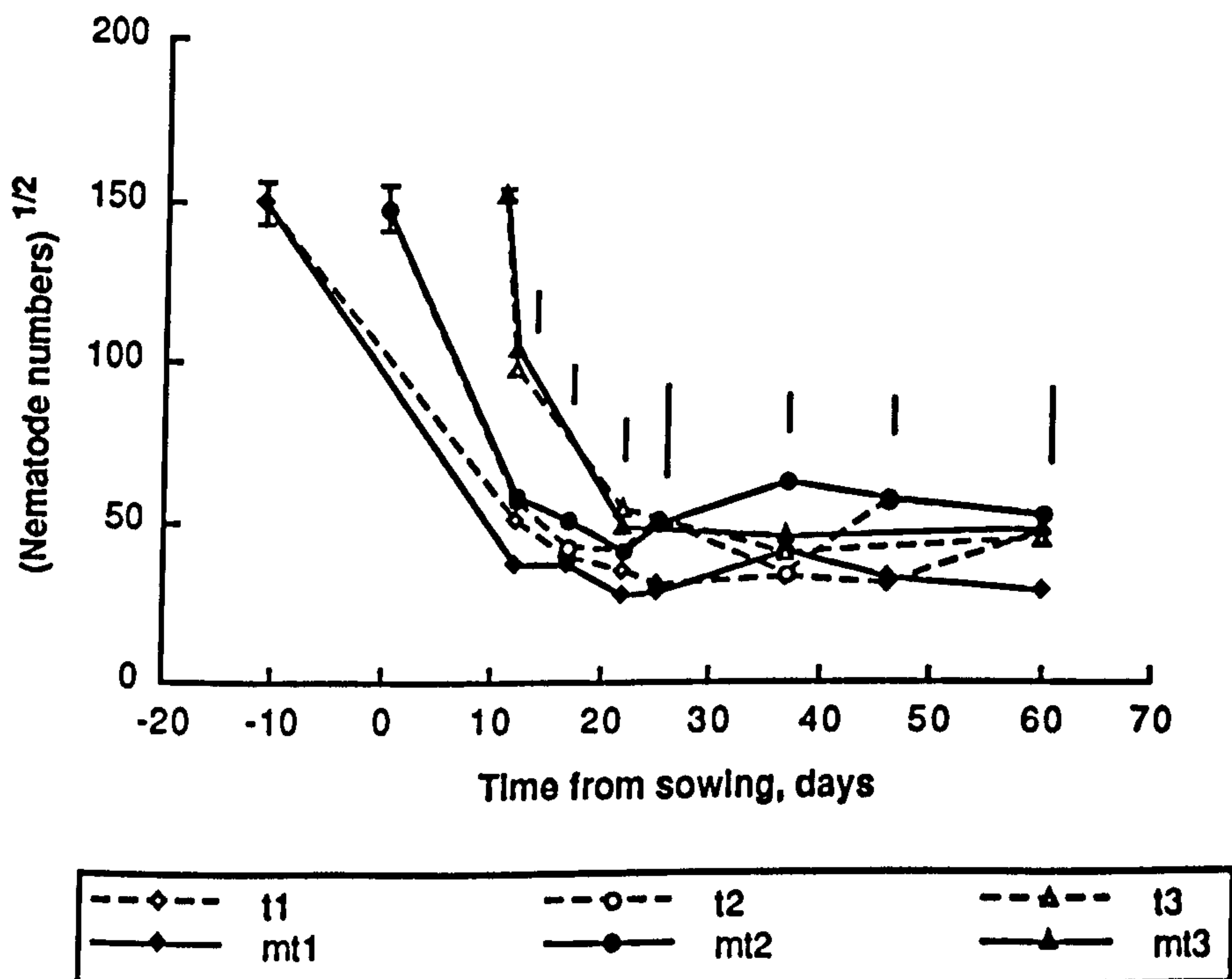


Fig. 5.3. The effect of *G. clarum* on the numbers (expressed as numbers^{1/2}) of *A. avenae* recovered from soil. Points at -11, 0 and 11 days are the means of the numbers of *A. avenae* added at the three times t1, t2 and t3. Bars on either side of these points represent ± 1 standard error. Other points are means per pot and all other bars represent LSDs at $p=0.05$. Treatment abbreviations as in Fig. 5.1.

combination of the model described by Al-Nahidh & Sanders (1987) and the model described in section 4.5.

Since root length was not measured in the present experiment, the variables 'total root length' and 'infected root length' were replaced by 'total root dry weight' (W_t) and 'infected root dry weight' (W_i) respectively. It is assumed that the weight of the root increases exponentially so that:

$$W_t = W_{t0} e^{rt} \dots \dots \dots 5.1$$

where W_{t0} is the starting total root dry weight, r the proportional growth rate of the root and t is time in days. When nematodes are present in the soil, infected root weight is assumed to consist of two categories in which the mycelium is either live (W_{il}) or dead (W_{id}). Dead mycelium is assumed to be created as a consequence of the feeding of nematodes on live mycelium.

When nematodes are absent and hence $W_{id} = 0$ the rate of increase of live infected root weight is assumed to be proportional to the already live infected root weight (W_{il}), which can be regarded as the inoculum from which new infection arises, and is proportional to the probability that regions of the root into which infection may extend are uninfected $\left[1 - \frac{W_{il}}{f_s W_t}\right]$, such that ,

$$\frac{1}{W_{il}} \frac{d(W_{il})}{dt} = \frac{d(\ln W_{il})}{dt} = s \left[1 - \frac{W_{il}}{f_s W_t}\right] \dots \dots \dots 5.2$$

where s is a parameter, equivalent to a in the previous model, termed the 'intrinsic spread rate' of infection and is the proportional growth rate of W_{il} when the fraction of infected root weight is very small. f_s is a second parameter equal to the fraction of root weight susceptible to infection (see Sanders 1986a; Sanders 1986b; Al-Nahidh & Sanders 1987).

When the VAM mycelium is being destroyed by the nematodes measurement of fractional infection does not distinguish between live and dead infection. Assuming that new infection arises only from live infected root, equation 5.2 becomes:

$$\frac{1}{W_{il}} \frac{d(W_{il})}{dt} = s \left[1 - \frac{W_{il} + W_{id}}{f_s W_t} \right] - bN \dots\dots\dots 5.3$$

where b is a constant termed the 'growth depression coefficient', which is related to the damage caused by nematodes to the mycorrhizal mycelium and is the proportional rate of death of live infection per nematode measured as $\text{gg}^{-1}\text{nematode}^{-1}\text{day}^{-1}$. N is the number of nematodes.

Assuming that the rate of death of nematodes is negligible, the rate of change of numbers of nematodes is proportional to the existing number of nematodes N and the live infected root weight available as food for the nematodes:

$$\frac{dN}{dt} = cNW_{il} \dots\dots\dots 5.4$$

where c is another constant termed the 'nematode increase coefficient' which is the proportional rate of increase in numbers of nematodes per g of infected root weight, measured in $\text{nematode}^{-1}\text{g}^{-1}\text{day}^{-1}$.

These equations were solved numerically by simulation using a computer program written in C (Fig. 5.4), given values for starting total dry root weight (W_{t0}), proportional growth rate of the root (r), starting number of nematodes (N_0), starting infected root weight (W_{i0}), intrinsic spread rate (s), maximum fraction of root susceptible to infection (f_s), growth depression coefficient (b), and the nematode increase coefficient (c). The computer program was then able to calculate iteratively daily values of total root dry weight, daily increments ΔN , ΔW_{il} and ΔW_{id} , accumulated totals of N , W_{il} and W_{id} and values of fractional infection (f_i) for up to 100 days. As with the previous model such solutions are only approximate.

The starting values of the variables and the values of all parameters except b and c were obtained from the actual data and are shown in Table 5.1. Measurements taken at the first harvest were used as the starting values W_{t0} , N_0 and W_{i0} . According to equation 5.1 the value of r is equal to the slope of the best straight line fitted to a plot of the natural logarithm of dry root weight against time and was equal to 0.08 day^{-1} . Using this value of r a curve described by equation 5.1 was fitted to the data (Fig. 5.5).

Fig. 5.4. The C program written according to the modified model of Al-Nahidh & Sanders (1987) to simulate the effects of grazing of nematodes on VAM fungi.

```

main ()
{
    double Wt, Wil, Wid, Wit, fi, n, Wt0, Wil0, Wid0, Wit0, N0, fs, s, b, c, r, dWi, dWd, dN, dt;
    int day, t;
    printf ('Starting root weight, g?\n');
    scanf ('%lf', &Wt0);
    printf ('Root rgr?\n');
    scanf ('%lf', &r);
    printf ('Starting number of nematodes?,\n');
    scanf ('%lf', &N0);
    printf ('Starting infected root weight, g?\n');
    scanf ('%lf', &Wil0);
    printf ('Intrinsic rgr of infected root weight (s)?\n');
    scanf ('%lf', &s);
    printf ('Maximum fractional infection allowed?\n');
    scanf ('%lf', &fs);
    printf ('Growth depression coefficient?\n');
    scanf ('%lf', &b);
    printf ('Nematode increase coefficient?\n');
    scanf ('%lf', &c);
    Wil = Wil0; N = N0; t = 0; Wid0 = 0; Wid = Wid0; Wit = Wil+Wid;
    for (day = 0; day < 100; day++)
    {
        1dN = c*N*Wil;
        Wt = Wt0*ert;
        dWid = b*N*Wil;
        dWil = s*Wil*(1-Wit/(fs*Wt))-dWid;
        N += dN;
        Wil += dWil;
        Wid += dWid;
        Wit += dWil+Wid;
        fi = Wit/Wt;
        t += 1;
        printf ('%3d %6.31f %6.3f %6.31f %6.31f %6.31f\n', t, Wt, Wil, Wid, fi, N);
    }
}

```

¹: d is a symbol used here instead of Δ.

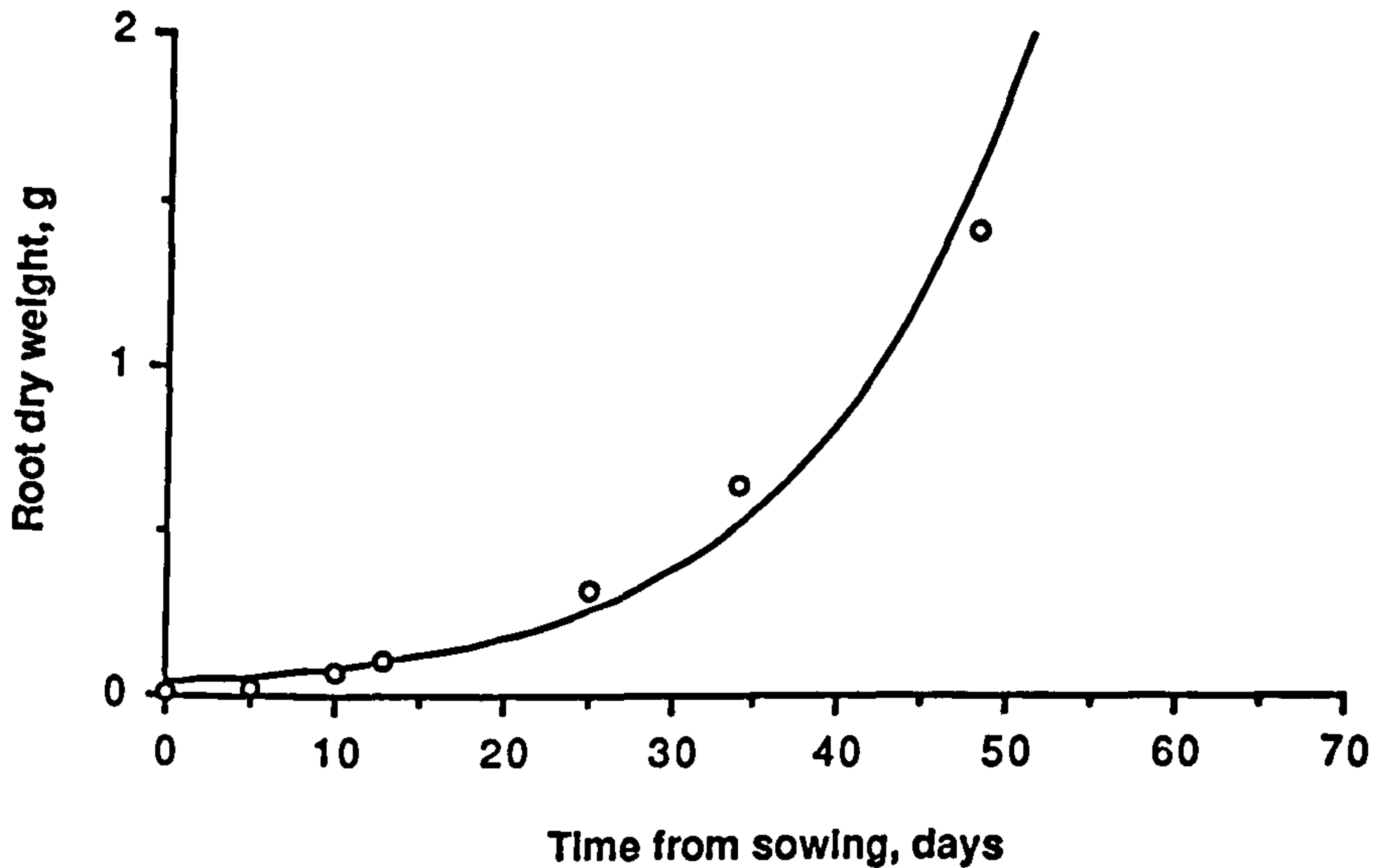


Fig. 5.5. Change in dry weights of mycorrhizal roots of red clover with time. Discrete points (O) actual data. The line is the change in dry weight predicted using equation 5.1. The values of r and W_{i0} used in the equation were those shown in Table 5.1.

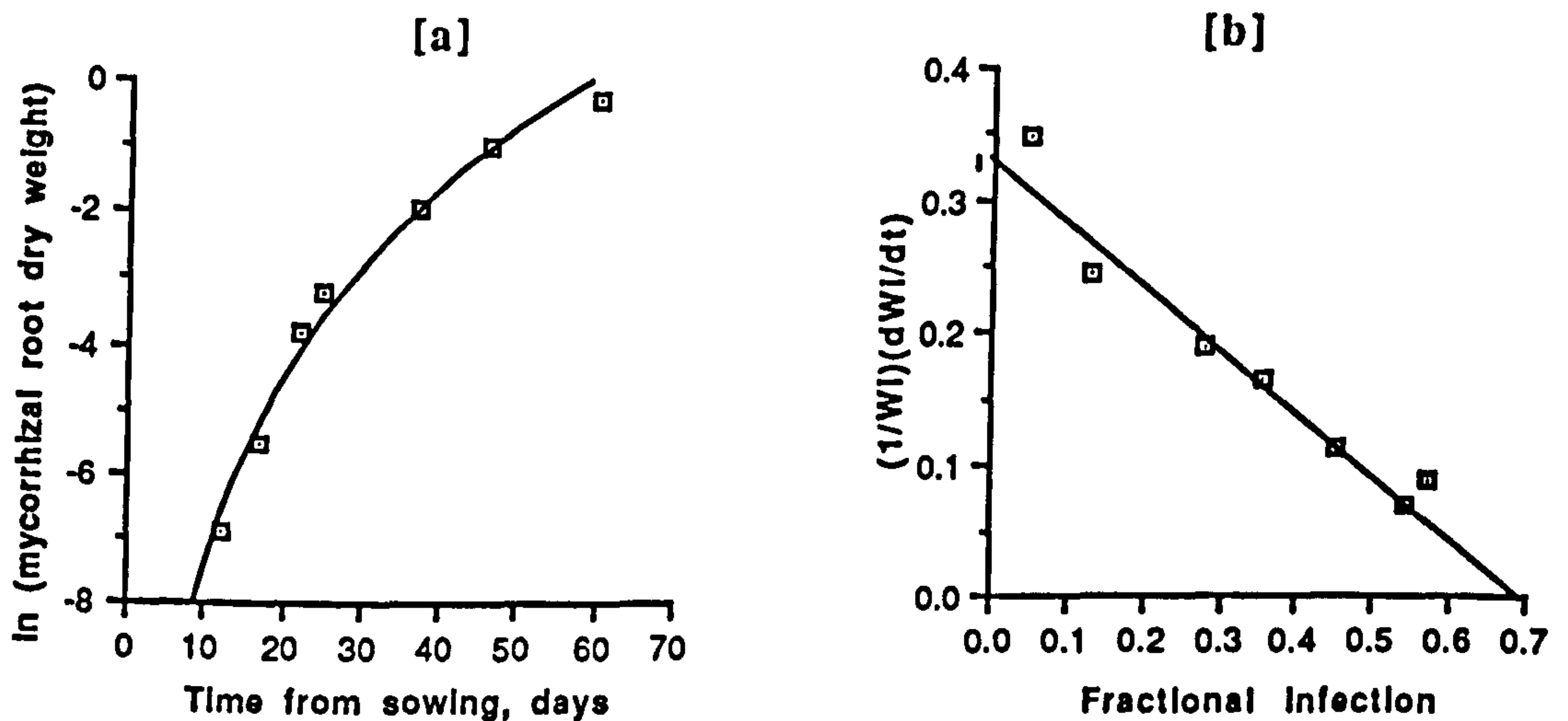


Fig. 5.6. [a] Change in $\ln(\text{dry weight of mycorrhizal root})$ with time. The curve was described by the following logarithmic equation: $\ln(\text{mycorrhizal root weight}) = 4.179 \ln t - 17.079$, $r^2 = 0.985$.

[b] Graph used to estimate parameters s and f_s of the modified model of Al-Nahidh & Sanders (1987). The differentials of the equation in [a] at each harvest time were plotted against fractional infection. The straight line which gave the best fit was described by the following equation:

$\frac{1}{W_i} \frac{dW_i}{dt} = -0.47670 f_i + 0.33651$, $r^2 = 0.947$. The intercept of the line with the y axis estimated the value of s and the intercept of the line with the x axis estimated f_s . f_i is the fractional infection.

Table 5.1. Parameters and values used in the simulation program.

Parameter	Value
Starting root weight, W_{i0}	0.037 g
Proportional root growth rate, r	0.08 day ⁻¹
Starting number of nematodes, N_0	3000 nematode
Starting infected root weight, W_{i10}	0.001 g
Intrinsic growth rate of infected root weight, s	0.4 day ⁻¹
Maximum fractional infection allowed, f_s	0.63

Table 5.2. [a] The ratios $\frac{c}{b}$ calculated by dividing the numbers of *A. avenae* by the dry weight of mycelium recovered from plates of different species of fungi.

[b] The values assigned to parameters b and c which were used in the simulation program and the corresponding $\frac{c}{b}$ ratios.

Species of fungus	[a]		[b]		
	$\frac{c}{b}$	$\frac{c}{b}^*$	b	c	$\frac{c}{b}$
ab	10 ⁶	10 ⁵	10 ⁻⁵	1	10 ⁵
cg	10 ⁵	10 ⁴	10 ⁻⁶	1	10 ⁶
d	10 ⁶	10 ⁵	10 ⁻⁴	1	10 ⁴
hc	10 ⁶	10 ⁵	10 ⁻⁵	0.1	10 ⁴
ll	10 ⁶	10 ⁵	10 ⁻⁵	10	10 ⁶
pi	10 ⁵	10 ⁴			
rr	10 ⁵	10 ⁴			
rs	10 ⁷	10 ⁶			
c	10 ³	10 ²			

$\frac{c}{b}^*$ are the values which would be appropriate to roots infected with VAM fungi. These are derived by dividing the values of $\frac{c}{b}$ by a factor of 10 to take into account that the values of c appropriate to the model of roots infected with VAM fungi will be 10 times smaller those appropriate to the agar plate model (see text).

s and f_s were estimated as described by Al-Nahidh & Sanders (1987). First the natural logarithms of the infected root dry weights from treatment m were plotted against time and a logarithmic curve was fitted (Fig. 5.6a). The equation which described the curve was differentiated with respect to time and the values of the differentials at each harvest time were plotted against fractional infection (Fig. 5.6b). If equation 5.2 describes the progress of infection correctly the points on this plot should lie on a straight line with y and x intercepts which estimate s and f_s respectively (Sanders 1986; Al-Nahidh & Sanders 1987). The estimated values of s and f_s were equal to 0.34 day^{-1} and 0.71 respectively. These were adjusted slightly to improve agreement between model predictions and data (Table 5.1).

Parameters b and c could not be calculated using experimental data and therefore possible values were derived from those used in the model for agar plates. If the nematodes were equally capable of finding and killing hyphae of VAM fungi in roots and soil as hyphae of the fungi grown on agar plates, the value of b chosen could be the same. The same values of c , could also be used but these need to be modified to take into account the fact that the mycelium available to the nematodes is represented in the present model as infected root weight and not as absolute biomass of mycelium as in the previous model (compare equation 5.3 to 4.2). The biomass of the mycelium of *G. clarum* growing inside and outside the roots was assumed to be equal to approximately 10% of the total dry biomass of the mycorrhizal root system (Bethlenfalvay *et al.* 1982). Since c has the units $(\text{g mycelium})^{-1}\text{day}^{-1}$, the values of c appropriate in the agar plate model need to be divided by 10 to take account of the fact that the mycorrhizal fungus constitutes only 10% of infected root weight.

The actual values assigned to b and c , were such as to produce a wide range of $\frac{c}{b}$ ratios within the range of ratios of $\frac{c}{b}$ * derived from the results of the plate experiment with *A. avenae* (Table 5.2a). Combinations of values given to b and c each time the program was run on the computer are shown in Table 5.2b. The corresponding predictions for fractional infection and numbers of nematodes are shown in Fig. 5.7 .

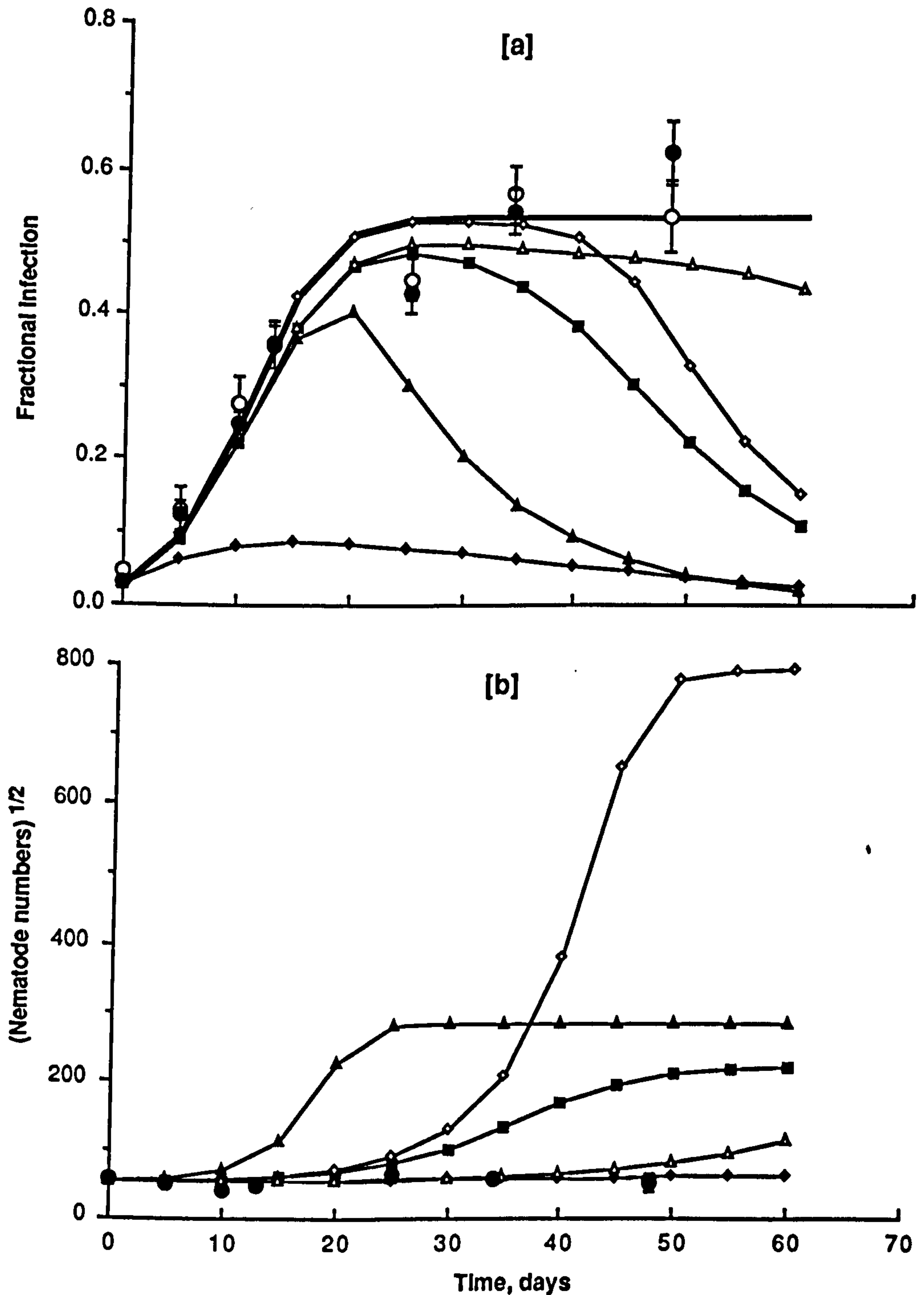


Fig. 5.7. Comparison of actual data for fractional infection [a], square roots of numbers of nematodes [b] and predictions for these variables using the program shown in Fig. 5.4. O: data from treatment m, thick line: prediction. ●: data from treatment maa; thin lines: predictions. Bars represent ± 1 standard error.

The influence of different values of b and c (see Table 5.1) on: [a] the effect of nematodes on fractional infection and [b] the increase of the numbers of nematodes due to feeding on the mycorrhizal mycelium. Predictions were calculated using the program shown in Fig. 5.4 and the combinations of values shown in Table 5.2b.

■: $b = 10^{-5}, c = 1$; ◇: $b = 10^{-6}, c = 1$; ◆: $b = 10^{-4}, c = 1$; △: $b = 10^{-5}, c = 0.1$; ▲: $b = 10^{-5}, c = 10$.

When b and c , are equal to 0, equivalent to a mycorrhizal control, fractional infection reaches a maximum of 0.54 and shows no decline (Fig. 5.7a). When b is given the value of 10^{-5} and c the value of 1, to correspond to an average ratio of $\frac{c}{b}$ in plate cultures, fractional infection reaches a lower maximum of 0.49 and has a medium rate of decline. Numbers of nematodes expressed as (nematode numbers)^{1/2} increase at a medium rate and reach a maximum of 220 (Fig. 5.7b). When the value of 1 is again given to c while b is reduced to 10^{-6} , fractional infection reaches the same maximum as in the mycorrhizal control and then declines at a later stage and rapid rate. The rate of increase in numbers of nematodes which occurs at an earlier time than before is rapid with numbers reaching a maximum of 800. When the value of c is kept the same and b given the value of 10^{-4} , fractional infection reaches a low maximum of 0.08 and shows an early and slow decline. Numbers of nematodes show practically no increase.

When b is given the value of 10^{-5} and c is reduced to 0.1, fractional infection reaches a maximum of 0.5 and has a late and slow decline (Fig. 5.7a). There is a late and slow increase in the numbers of nematodes (Fig. 5.7b). Finally, a maximum of 0.4 of fractional infection and an early and rapid decline are predicted if the value of b is kept at 10^{-5} and c increased to 10. Numbers of nematodes increase at an early stage and a rapid rate reaching a maximum of 280.

When the nematodes were assumed to be absent, equivalent to treatment m, there was good agreement between fractional infection measured experimentally and that predicted by the simulation (Fig. 5.7a). When nematodes were present, none of the combinations of b and c employed could the predictions of the model be made to agree with experimental results. The best prediction was achieved with values of b and c which were smaller than 10^{-5} nematode⁻¹day⁻¹ and 10^{-2} g⁻¹day⁻¹ respectively when no important reduction in fractional infection or increase in numbers of nematodes were predicted. Such values of b and c gave $\frac{c}{b}$ ratios smaller than 10^3 nematode g⁻¹ which was nearer the value calculated as appropriate to fungus C which in plate culture did not support the reproduction of nematodes and whose mycelium was not affected at all by *A. avenae*. It is therefore probable that *A. avenae* either did not feed on the hyphae of *G.*

clarum or its feeding had an effect on the fungal mycelium which was too small to be important.

In the experiment, numbers of nematodes in the non-mycorrhizal and mycorrhizal treatments were not significantly different. This indicates that the small population of nematodes which was maintained until the end of the experiment probably survived by feeding on volunteer saprotrophic mycelia which had recolonized the initially sterile soil. This was investigated in a subsequent experiment (see next chapter).

CHAPTER 6. THE EFFECT OF NEMATODES AND SOME OF THEIR SAPROTROPHIC HOSTS ON VAM INFECTION AND ASSOCIATED GROWTH RESPONSES OF RED CLOVER

6.1. INTRODUCTION

Nematodes may damage the mycelia of fungi without increasing in numbers (Mankau & Mankau 1963). Chin & Estey (1966) observed that *A. avenae* pierced the roots of plants with its stylet without feeding and attempts to probe other materials were also reported. It is possible then that this action could cause extensive damage to fungal mycelia if the numbers of nematodes were large.

It has been usual practice to add large numbers of nematodes to sterile soil in attempt to reduce VAM infection and hence plant growth responses (Salawu & Estey 1979; Hussey & Roncadori 1981; Chapters 3 & 5 of this thesis). The most consistent outcome of such experiments has been that only a small percentage of the number of nematodes added initially could be recovered, even after periods as short as 1 day after their introduction to the soil (see Fig. 5.3). This indicates that high rates of nematode application do not necessarily lead to high densities of nematodes in soil perhaps because it does not contain sufficient fungal biomass to sustain the introduced nematodes.

As an alternative strategy it may be possible to stimulate the reproduction of an initially small number of nematodes by introducing them into a soil containing abundant mycelium on which they can feed. For example, Mankau (1962) found that low initial numbers of indigenous *A. avenae* and *A. composticola* increased significantly when certain organic materials that encouraged the growth of saprotrophic mycelia were added to the soil (see also Mankau & Sitanath 1969).

Little is known about the effects on VAM fungi of organic material, or the saprotrophic fungi which may colonize it, and the results of experiments that have been carried-out to date are contradictory (Kruckelmann 1975; Azcon-Aguilar, Diaz-Rodriguez & Barea 1986b; Avio & Giovannetti 1988).

6.2. EXPERIMENT 9

6.2.1. INTRODUCTION

The main objective of this experiment was to study the spread of different species of saprotrophic fungi inoculated into sterile soil in the form of bran colonized with mycelium and the ability of nematodes to multiply on these food sources. The effects of such inoculation on the growth of red clover were also examined to help in the choice of a species of fungus which stimulated the reproduction of the nematode yet had no deleterious effects on the plant. *A. composticola* was chosen for this and the next experiment because it was capable of a high rate of multiplication when presented with a suitable food source (see Fig. 4.1b).

6.2.2. MATERIALS AND METHODS

Pots in the control treatment were filled with 210 g of sterile soil. Pots of the inoculated treatments also received 2 g of either sterile bran (treatment br) or bran colonized with the mycelium of *Agaricus bisporus* (treatment ab), *Rhizoctonia solani* (treatment rs) or the unidentified fungus D (treatment d). The sterile or colonized bran was placed as a layer beneath the surface of the soil. After watering, clover seed was sown on the surface of the soil and covered with alkathene beads. The quantity of beads was adjusted so that all pots weighed the same and could subsequently be watered by weight to 50% field capacity. The control and treatments were replicated eight times and pots were fully randomized in the growth room.

21 days later, 4 pots from the control and each of the inoculated treatments received 500 *A. composticola* which had been extracted from agar plate cultures on *A. bisporus*. Plants were harvested 44 days after sowing and the dry weights of shoots were recorded. Root segments from each treatment were stained with lactophenol-trypan blue to reveal fungal infection. Numbers of nematodes were measured following their extraction by method BF2. The development of saprotrophic mycelia through the soil

was examined under the microscope and photographed and the root systems from control, ab and d pots were photocopied.

6.2.3. RESULTS

Mycelium of *A. bisporus* grew out of the bran inoculum (Plate 6.1a) and through the soil producing a clearly visible complex of hyphae, rhizomorphs and primordia of fruiting bodies (Plates 6.1b & 6.2a). In pots treated with both *A. bisporus* and *A. composticola*, the mycelium appeared to be severely damaged. Mushroom primordia in the soil treated with *A. composticola* were noticeably smaller and discoloured (Plate 6.2a,b) and the mycelium was less abundant or even completely absent (Plate 6.3a,b).

The mycelia of *R. solani* or D were not readily visible under the microscope and as a result it is not certain whether these fungi were able to grow out of the bran and into the soil.

Numbers of *A. composticola* increased significantly in soil inoculated with colonized bran or bran alone but not in the soil of the control. *A. bisporus* produced significantly larger numbers of nematodes than fungus D (Fig. 6.1b). In soil inoculated with *A. bisporus*, nematodes increased in number by 100 times. In soil inoculated with bran alone, *R. solani* or D, numbers increased by factors of 60, 50 and 40 respectively. Nematodes in the control increased in number only by a factor of 2.

Inoculation of the soil with the mycelium of D, significantly reduced shoot weight (Fig. 6.1a) and root systems were severely stunted with stubby and sparse lateral roots in the region of the fungal inoculum (Plate 6.4). However, examination of root segments stained with lactophenol-trypan blue did not reveal any obvious fungal infection.

The growth of roots was stimulated by *A. bisporus* (Plate 6.4). Dry weights of shoots were not affected by inoculation with either *A. bisporus*, *R. solani* or sterile bran (Fig. 6.1a).

A. composticola did not damage the roots and had no effect on the growth of clover plants (Fig. 6.1a). There was some evidence that the nematodes counteracted the negative effect that D had on the growth of the plant since the dry weights of shoots

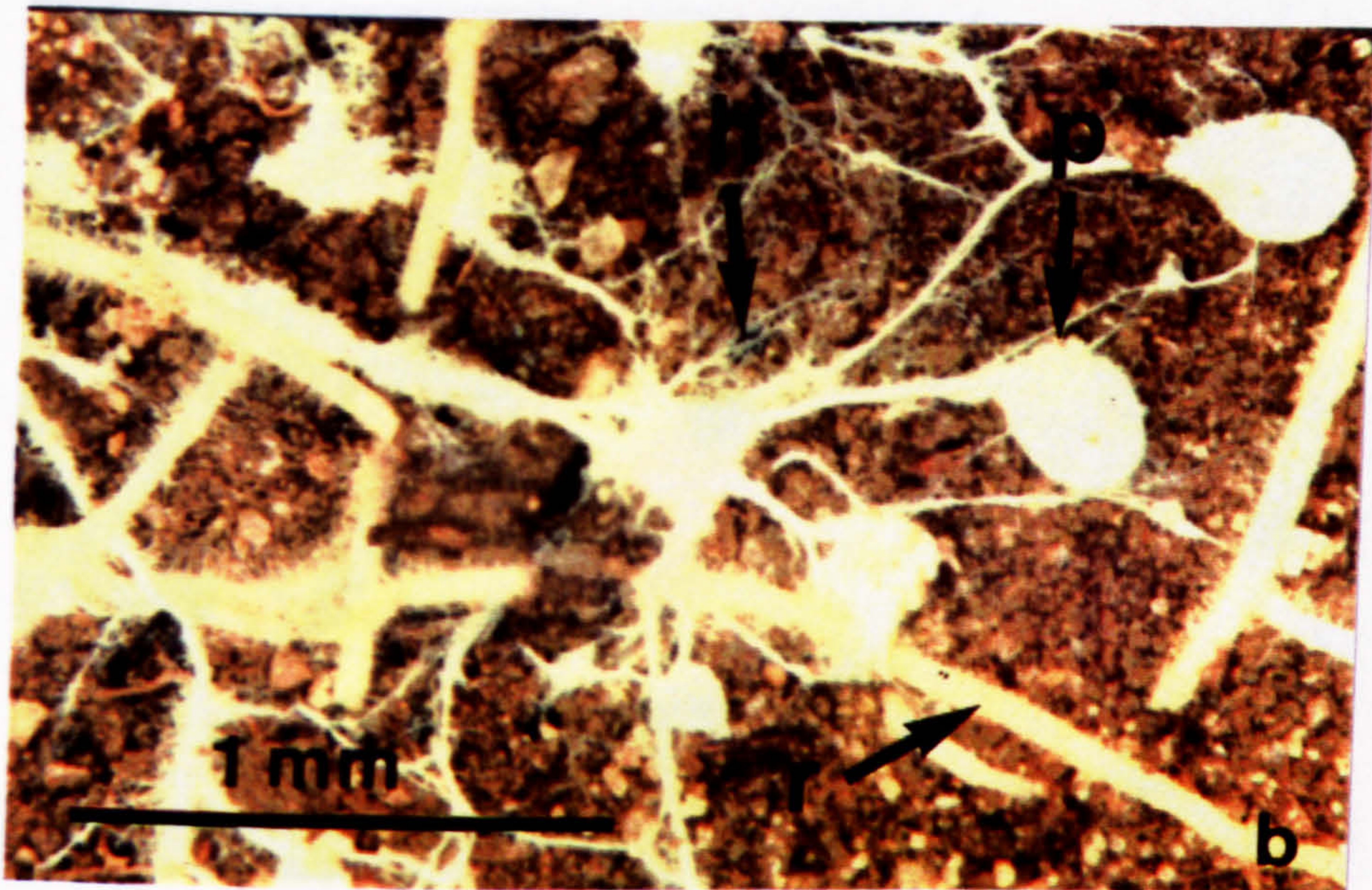
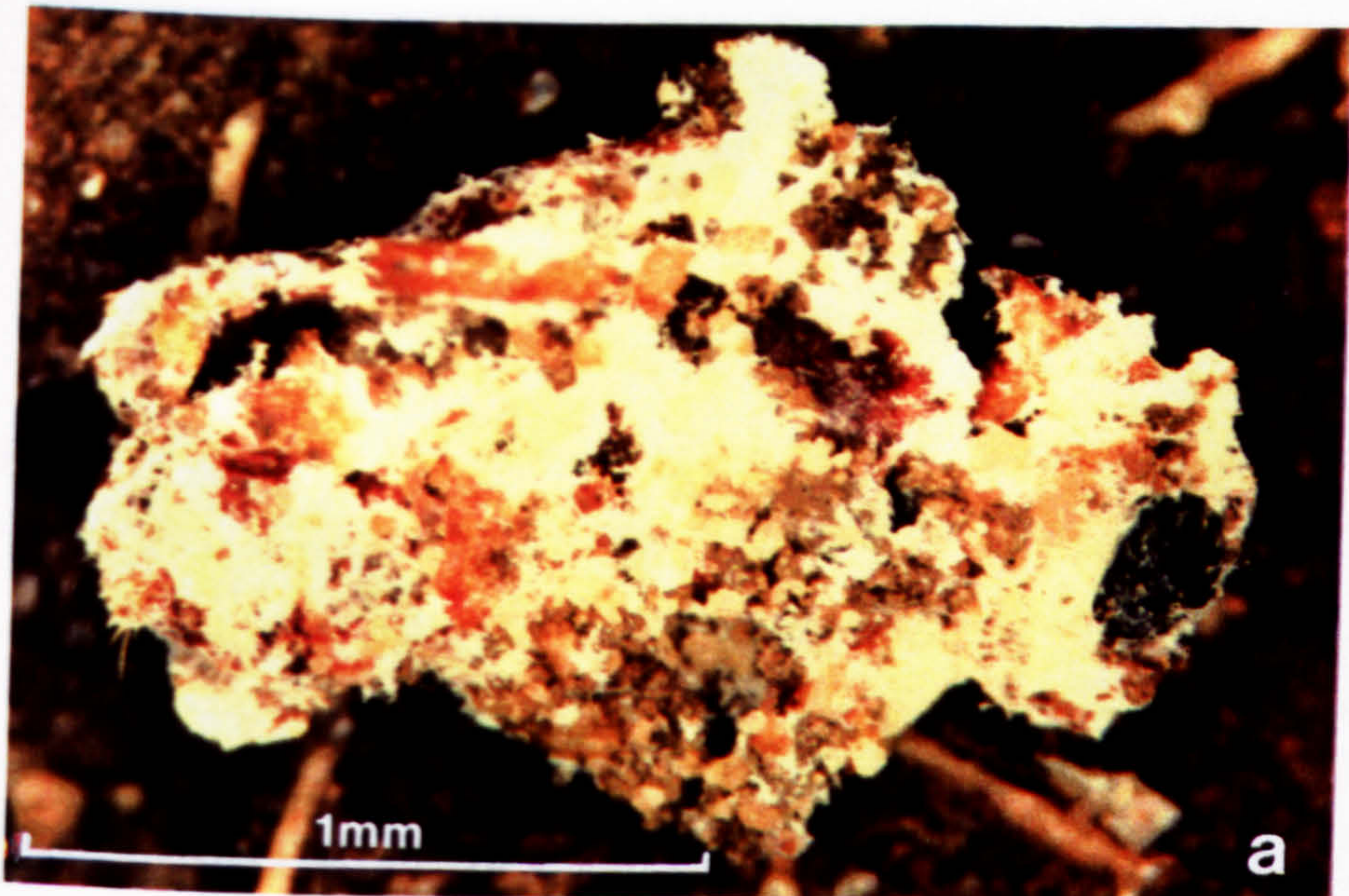


Plate 6.1. [a] Bran colonized with the mycelium of *A. bisporus*.

[b] Hyphae (h) and primordia (p) of *A. bisporus* growing in the soil amongst the roots of red clover (r).

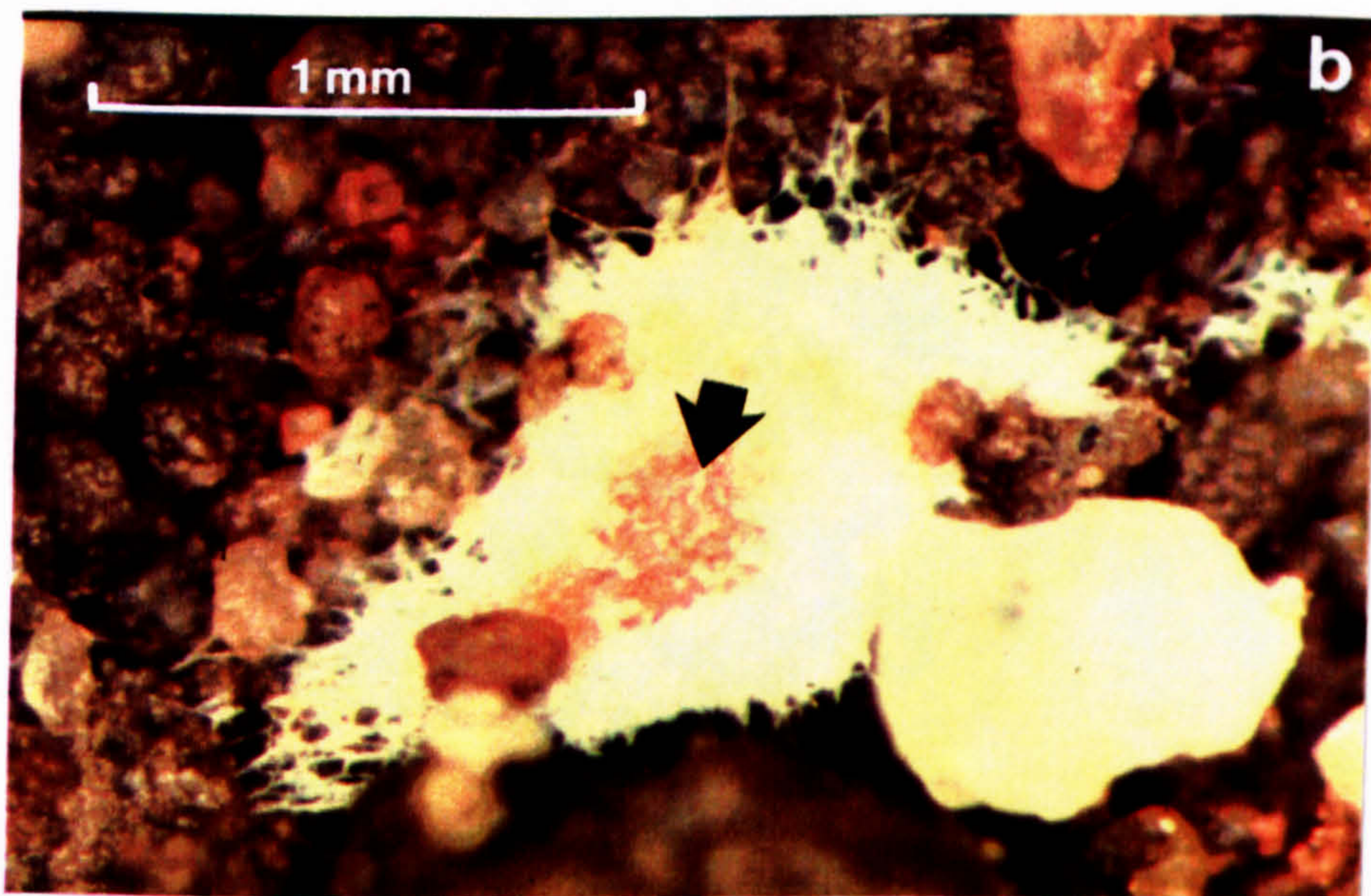
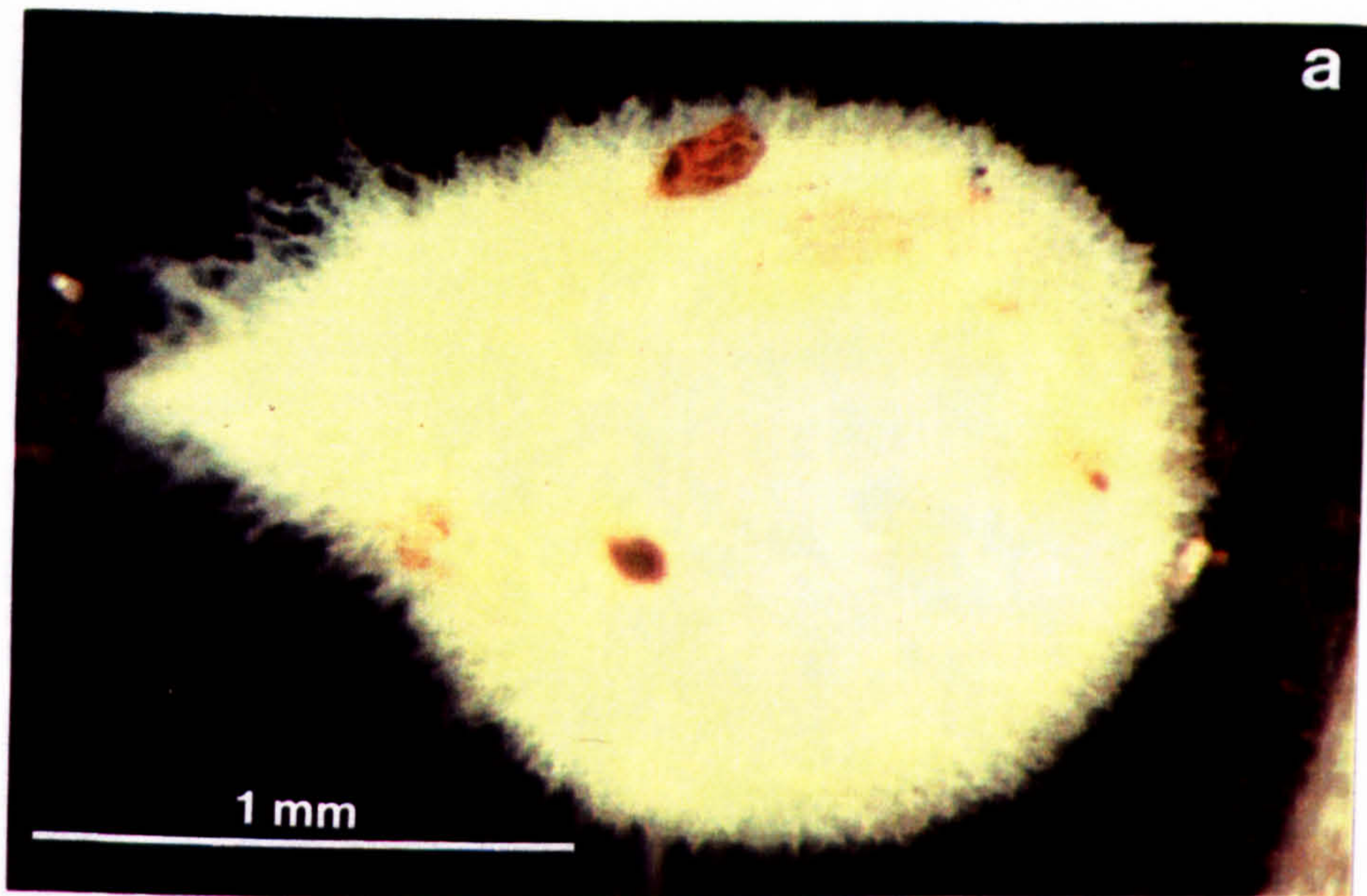


Plate 6.2. [a] A magnified view of a healthy primordium of a fruiting body (mushroom) of *A. bisporus* growing in the absence of nematodes.

[b] A magnified view of a primordium of a fruiting body of *A. bisporus* whose growth has been reduced as a result of grazing by *A. composticola*. Note the characteristic brown discolouration of the hyphae in the centre of the primordium (arrow).

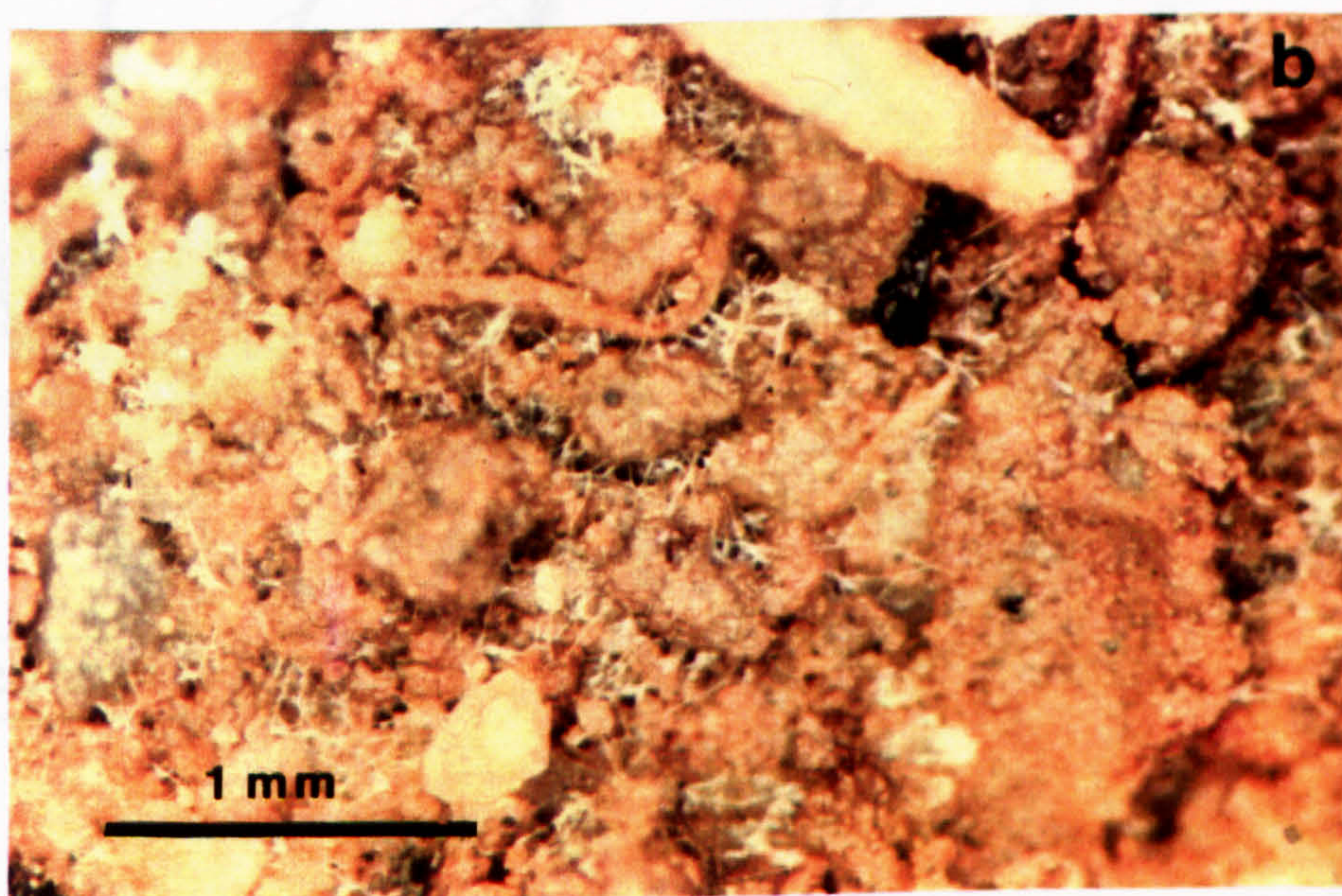
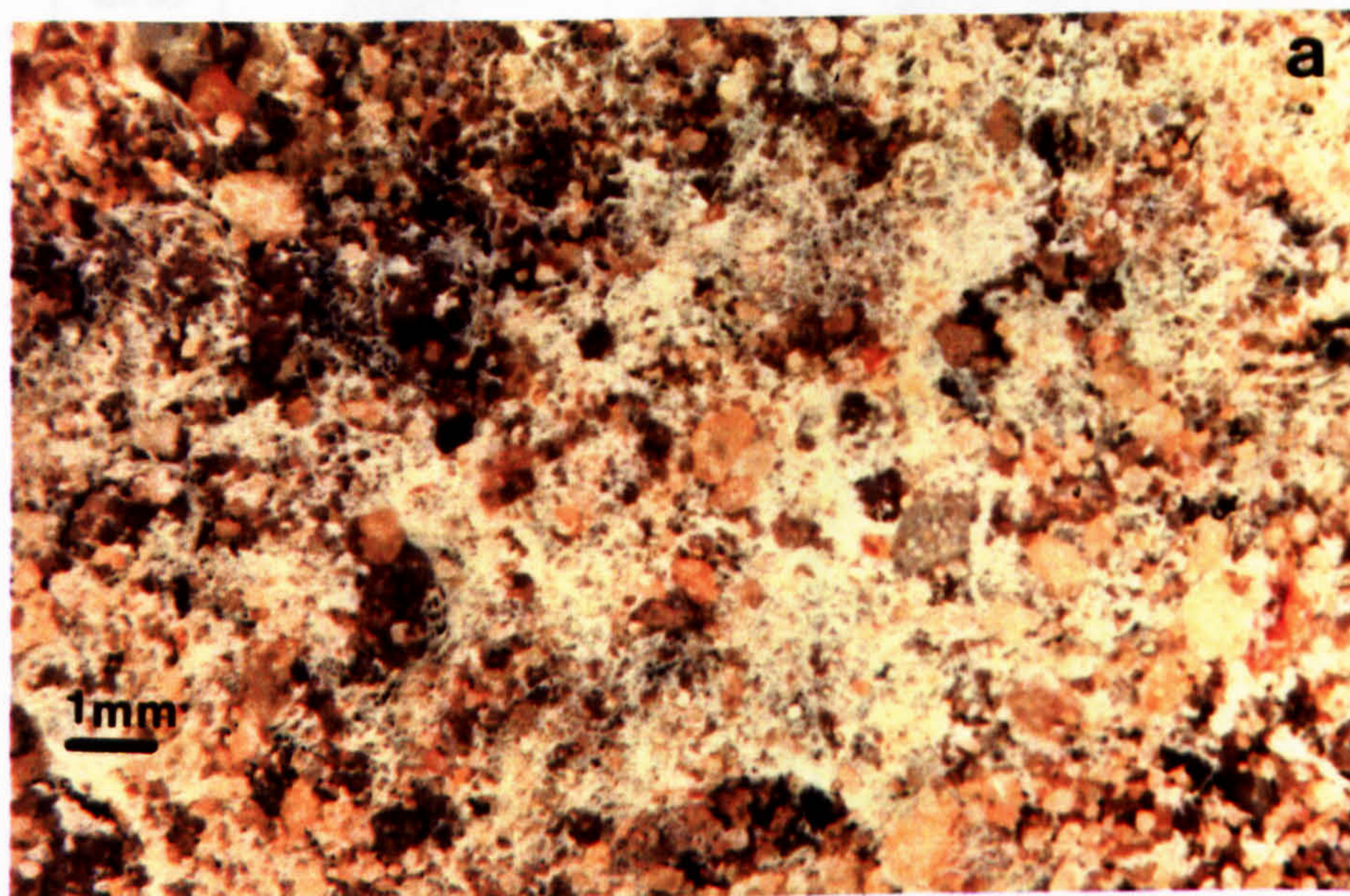


Plate 6.3. Mycelium of *A. bisporus* spreading through the soil in the absence (a) or presence of *A. composticola* whose grazing on the hyphae leads to a considerable reduction in density of mycelium in the soil (b).

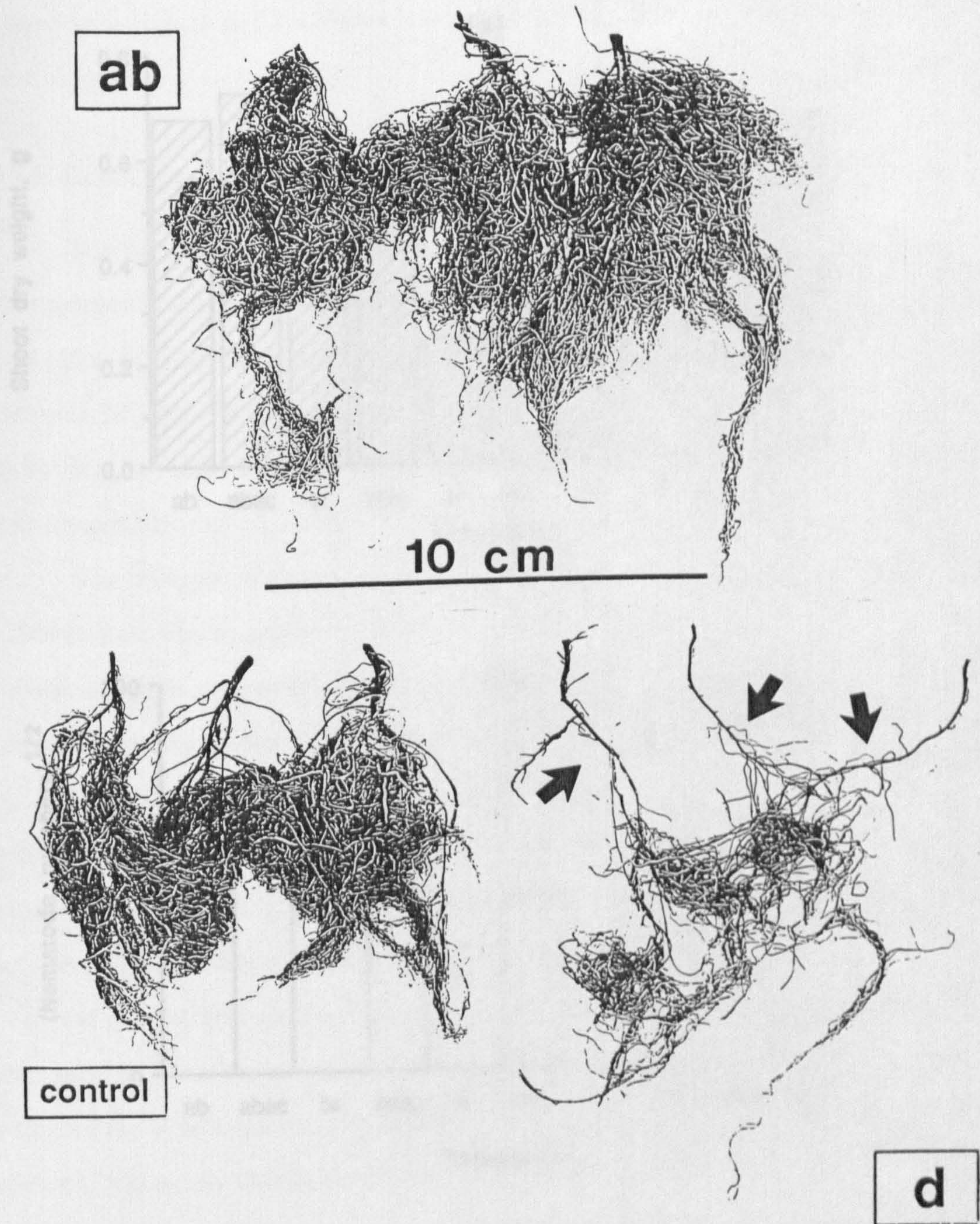


Fig. 6.1. (a) Dry weights of shoots of red clover grown in soil with previously sterilized from soil with either fungus B or *F. solani*.

(b) The effect of inoculation of dry soil with *F. solani* compared from soil.

Plate 6.4. Photocopies of root systems of red clover removed from soil which had been inoculated with either *A. bisporus* (ab), or fungus D (d), or from soil to which nothing was added (control). The presence of *A. bisporus* in soil enhanced the growth of the roots. The presence of D reduced the development of lateral roots in the region of its inoculum (arrows) and generally reduced root growth.

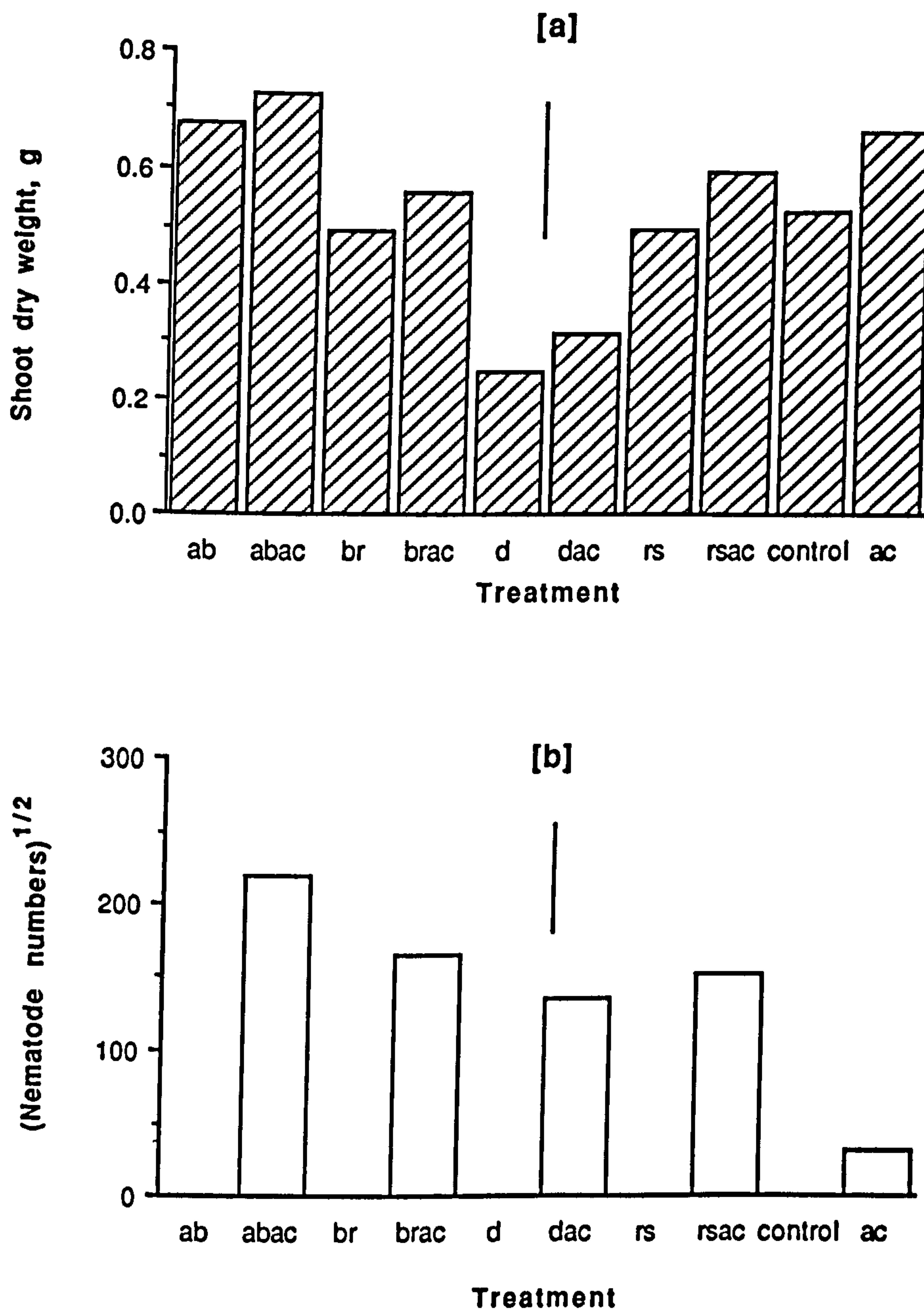


Fig. 6.1. [a] Dry weights of shoots of red clover as affected by *A. composticola* and by inoculation of the soil with previously sterilized bran either alone or colonized by either *A. bisporus*, the unidentified fungus D or *R. solani*.

[b] The effect of inoculation of the soil as in [a] on the numbers (expressed as numbers^{1/2}) of *A. composticola* recovered from soil.

ab, br, d and rs: treatments with bran colonized with *A. bisporus*, bran alone, bran colonized with D and bran colonized with *R. solani* respectively; abac, brac, dac and rsac: treatments as before but with *A. composticola* also added to the soil; ac: treatment with *A. composticola* alone. Columns are means per pot and bars represent LSDs at p=0.05.

treated with both D and *A. composticola*, were not significantly smaller than those of control plants.

6.3.3. DISCUSSION

Saprotrophic fungi contribute to the mineralization of organic nitrogen and phosphorus and thus may enhance the growth of plants growing in nutrient limiting soils. This effect may be influenced by nematodes grazing on the mycelia of the fungi (see section 1.3.5). Except in the case of the effect of *A. bisporus* on the growth of clover roots, there was no evidence of a positive response of the plant to inoculation of the soil with saprotrophic mycelia or bran and the presence of *A. composticola* had no effect.

The presence of fungus D in soil was deleterious to the growth of red clover although there was no apparent root infection. It is possible that D released a phytotoxic substance which suppressed the growth of the roots (see Lynch 1983, 1985). When grown in plate culture this fungal species stained the agar by releasing metabolites.

The experiment showed that it is possible to inoculate soil with bran colonized with a saprotrophic fungus, which then spreads in the soil. When nematodes which are capable of feeding on the fungus are added to the inoculated soil they may increase substantially in number.

It was therefore decided to use bran colonized with *A. bisporus* as a means of increasing the numbers of nematodes in the following experiment, because this fungus produced the largest number of nematodes, it had no adverse effect on the growth of the plant and was clearly visible in the soil.

6.3. EXPERIMENT 10

6.3.1. INTRODUCTION

The aim of this experiment was to investigate whether a large density of *A. composticola*, produced by allowing the nematode to feed on the mycelium of *A. bisporus* introduced into the soil on bran, was capable of reducing the response of red

clover to infection of its roots by *G. clarum*. The interactions between bran and *G. clarum* as well as the interactions between the saprotrophic and the mycorrhizal fungus were also examined.

6.3.2. MATERIALS AND METHODS

The design of the experiment is shown in Fig. 6.2. Pots of the br and ab treatments were filled with 130 g of soil (a mixture of 1 part clay loam and 1 part sand) followed by a layer of 2 g of sterile bran (br treatments) or bran colonized with ab (ab treatments) and a further layer of soil to a total of 160 g. Pots of the 0 treatments were filled with 160 g of sterile soil. Pots were placed in the growth room, watered and temporarily covered with a black plastic sheet.

A week later the soil surface in the ab treatments was covered with the white mycelium of ab whereas the soil of the br treatment was colonized by various volunteer saprotrophic fungi. At that time, approximately 1000 *A. composticola* were added to each pot of the nematode treatments (5/g soil). 7 days later nematodes from 3 replicate pots of the 0, br and ab treatments were extracted by method BF2 and counted.

5 days later, all pots were taken out of the growth room. A layer of live inoculum of *G. clarum* was added to pots selected at random (mycorrhizal treatments) and a layer of inoculum sterilized by autoclaving was added to the remaining pots (non-mycorrhizal treatments). This was done by first spreading on the surface of soil in each pot 20 g of sterile soil, on top of which the mycorrhizal inoculum was added and covered by another layer of 20 g of sterile soil. Pots were then watered, placed in a randomized design in the growth room and covered with a plastic sheet. 8 days later (day 0) pots were watered, and red clover seed was sown and covered with alkathene beads. Following germination, seedlings were gradually thinned to an eventual 3 per pot and the water content of the soil was from then on maintained at 50% of field capacity.

Five replicate pots from each treatment were harvested on three occasions (19, 43 and 63 days after sowing). Weights of shoots and roots and levels of mycorrhizal infection were measured. Nematodes were extracted by method BF2 and counted. Shoot

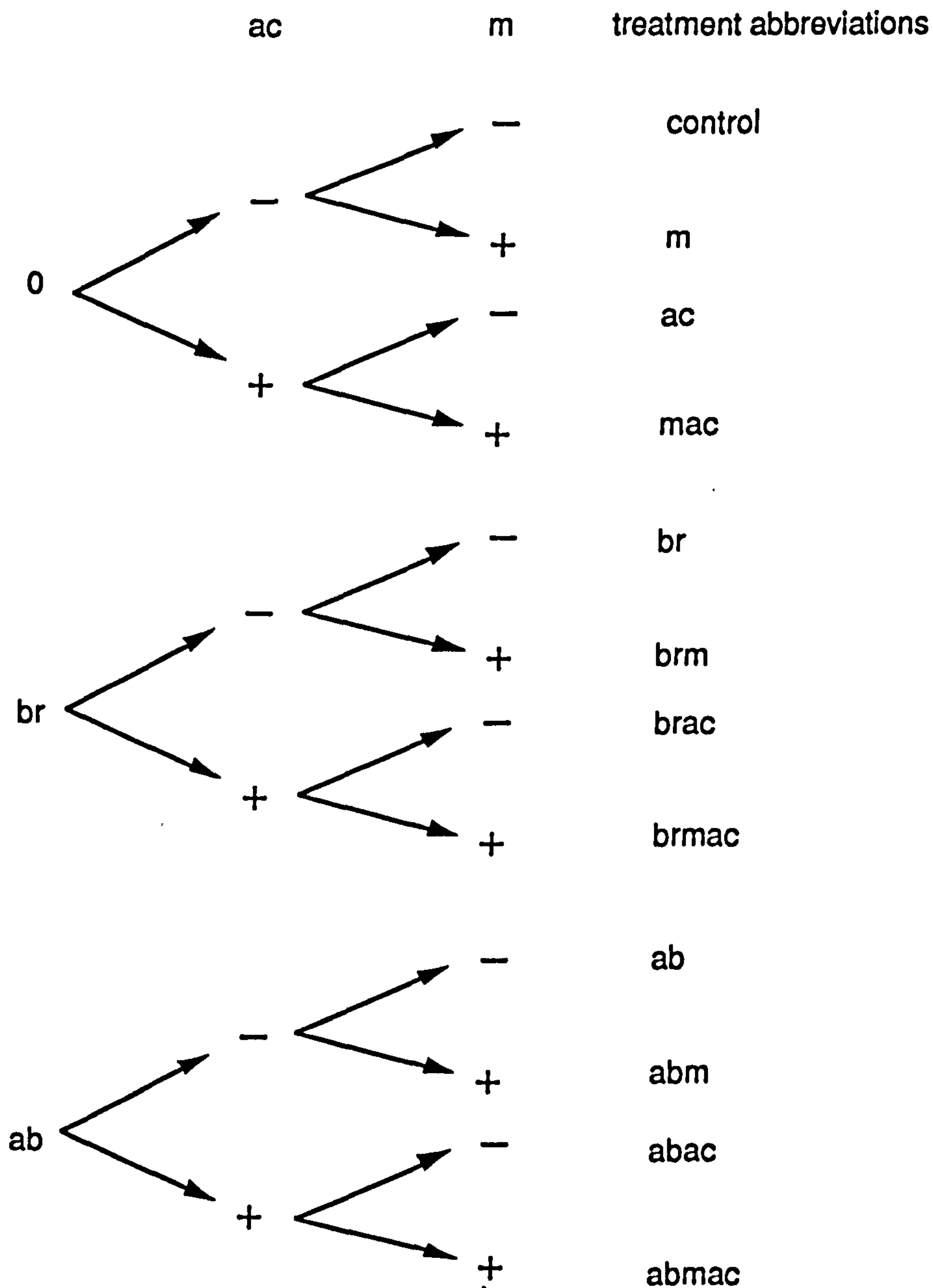


Fig. 6.2. The design of experiment 10.

Pots without added bran (0), pots with added sterile bran (br) and pots with added bran colonized with *A. bisporus* (ab) received (+) or not (-) *A. composticola* (ac) which were then inoculated (+) or not (-) with *G. clarum* (m). This resulted in 12 treatments.

concentrations and contents of phosphorus were measured by the vanado-molybdate method following dry combustion (MAFF 1986).

Phosphorus unit absorption rates (mg shoot P g⁻¹ of fresh root day⁻¹) were calculated from the following equation (see Brewster & Tinker 1972):

$$UAR = \frac{dP}{dt} \frac{1}{W_f} \dots\dots\dots 6.1$$

where $\frac{dP}{dt}$ is the instantaneous rate of phosphorus uptake and W_f the root fresh weight.

The natural logarithms of phosphorus contents ($\ln P$) were plotted against time and quadratic equations fitted (Table 6.1). Values of $\frac{dP}{dt}$ were derived by differentiating each equation in respect to time for each treatment and harvest time. $\ln W_f$ was also plotted against time and quadratic equations were fitted (Table 6.1) from which smoothed values of W_f were calculated.

Table 6.1. The equations used to estimate the weight of fresh root W_f (g) and the instantaneous rate of phosphorus uptake $\frac{dP}{dt}$ (mg/day) for the twelve treatments at different times t (days). $r^2 = 1.000$ for all equations.

Treatment	W_f (g)	$\frac{dP}{dt}$ (mg/day)
control	$\ln W_f = -2.5347 + 0.0455t - 0.00002t^2$	$\ln P = -2.5734 - 0.0493t + 0.0011t^2$
ac	$\ln W_f = -2.8487 + 0.0581t - 0.0003t^2$	$\ln P = -2.2325 - 0.0602t + 0.0012t^2$
m	$\ln W_f = -3.7724 + 0.1452t - 0.0008t^2$	$\ln P = -6.2848 + 0.2528t - 0.0023t^2$
mac	$\ln W_f = -3.7749 + 0.1415t - 0.0008t^2$	$\ln P = -6.0245 + 0.2336t - 0.0020t^2$
br	$\ln W_f = -3.4969 + 0.0701t - 0.0003t^2$	$\ln P = -2.3457 - 0.0460t + 0.0008t^2$
brac	$\ln W_f = -0.8572 - 0.0994t - 0.0019t^2$	$\ln P = -1.1921 - 0.1394t + 0.0024t^2$
brm	$\ln W_f = -4.8046 + 0.1975t - 0.0014t^2$	$\ln P = -6.9397 + 0.2617t - 0.0021t^2$
brmac	$\ln W_f = -4.6186 + 0.1731t - 0.0011t^2$	$\ln P = -6.4059 + 0.2437t - 0.0020t^2$
ab	$\ln W_f = -3.1952 + 0.0332t + 0.0005t^2$	$\ln P = -3.6799 + 0.0086t + 0.0007t^2$
abac	$\ln W_f = -4.7769 + 0.1150t - 0.0005t^2$	$\ln P = -3.2020 - 0.0008t + 0.0007t^2$
abm	$\ln W_f = -4.9257 + 0.2120t - 0.0016t^2$	$\ln P = -6.5537 + 0.2719t - 0.0024t^2$
abmac	$\ln W_f = -3.7473 + 0.1344t - 0.0007t^2$	$\ln P = -6.6151 + 0.2587t - 0.0022t^2$

6.3.3. RESULTS

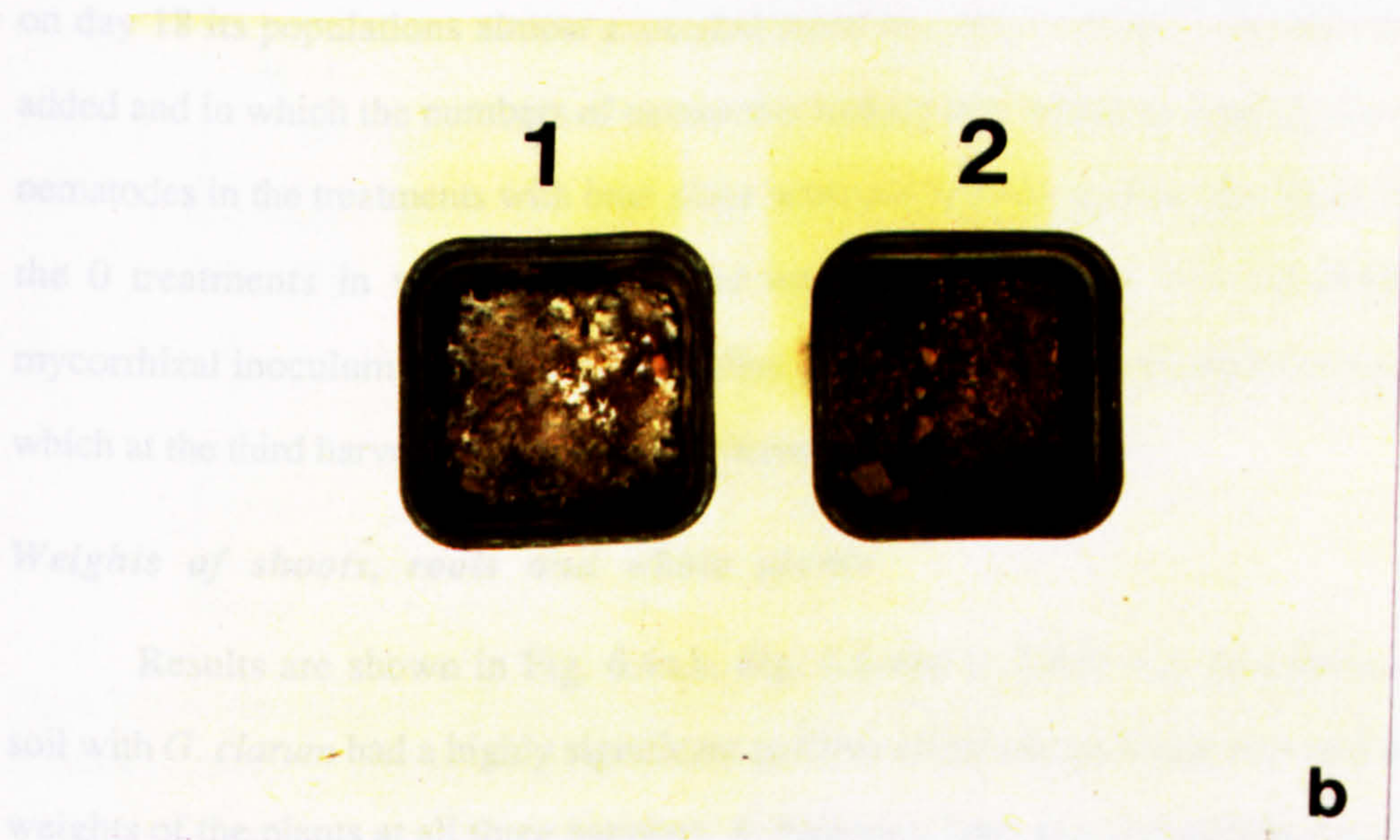
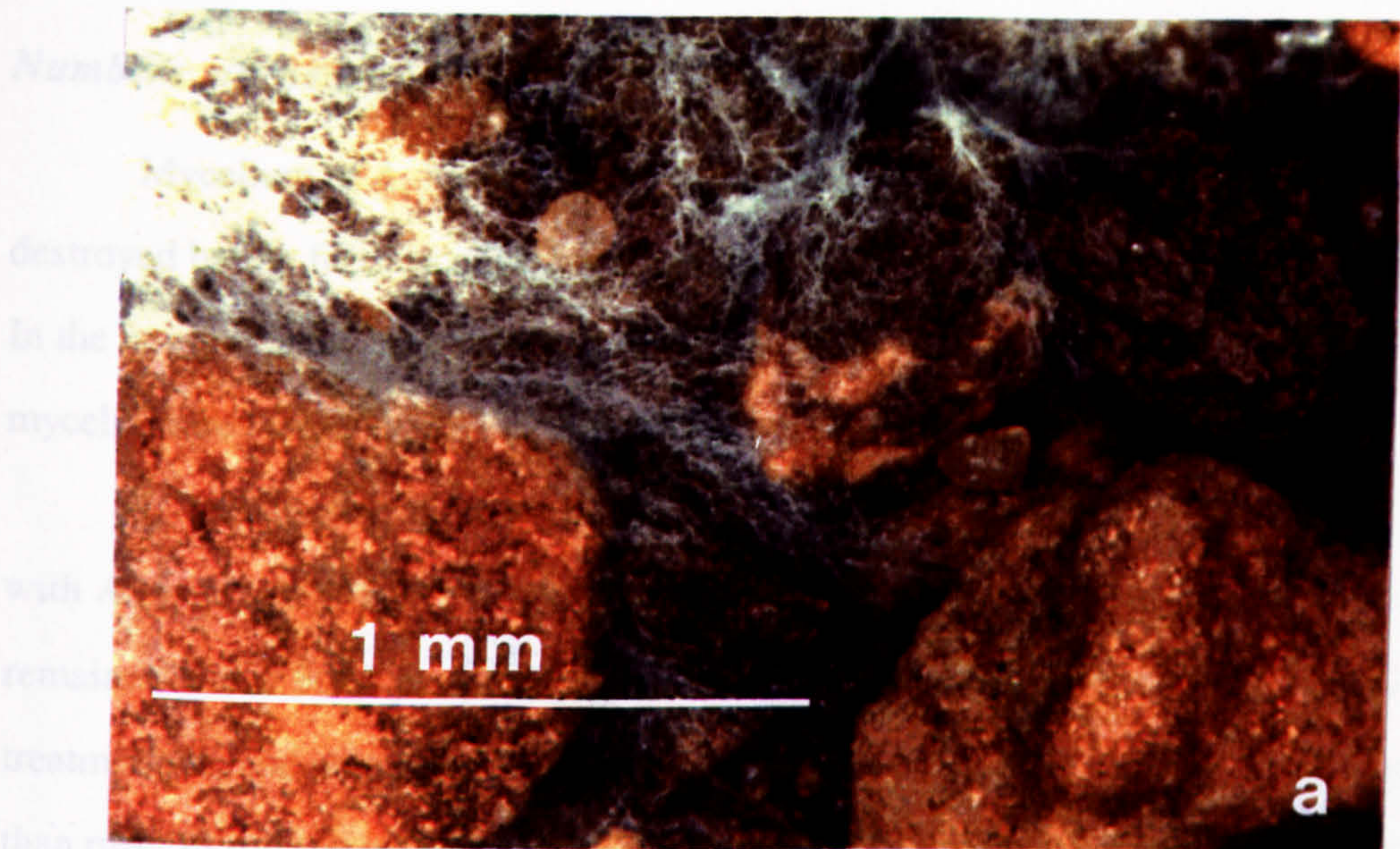


Plate 6.5. [a] Mycelium of *A. bisporus* spreading through the soil.

[b] A macroscopical view of the effect of grazing of *A. composticola* on the mycelium of *A. bisporus* growing in soil. The soil of pot 1 was inoculated with the mycelium of *A. bisporus* whereas in pot 2, 1000 *A. composticola* were added to the soil 7 days after it had been inoculated with *A. bisporus*.

6.3.3. RESULTS

Numbers of nematodes

Mycelium of *A. bisporus* rapidly colonized the soil in the ab treatments and was destroyed by the feeding of nematodes very soon after these were added (Plate 6.6a,b). In the br treatments, bran was also colonized by the volunteer saprotrophic fungi whose mycelia were broken down in a similar fashion to that of *A. bisporus*.

Within seven days the numbers of *A.composticola* increased by 40 times in soil with *A. bisporus* (Fig.6.3). After this, numbers dropped to a lower level but always remained above 9000 per pot and were generally significantly higher than numbers in treatments ac and mac except at the third harvest when numbers in abac were not greater than mac. In soil treated with bran alone, *A. composticola* multiplied at a slower rate and on day 18 its populations almost exceeded those in soil to which *A. bisporus* had been added and in which the numbers of nematodes had already started to decline. Numbers of nematodes in the treatments with bran alone were consistently greater than those in soil of the 0 treatments in which numbers did not increase greatly. In soil treated with mycorrhizal inoculum only, there was a slow gradual increase in numbers of nematodes which at the third harvest were greater in treatment mac than in ac.

Weights of shoots, roots and whole plants

Results are shown in Fig. 6.4a,b, Fig. 6.5 and in Table 6.2. Inoculation of the soil with *G. clarum* had a highly significant positive effect on the shoot, root and total dry weights of the plants at all three harvests. *A. bisporus*, bran and nematodes also affected plant growth but to a lesser extent.

At the first harvest, bran had a negative main effect on shoot, root and total weights of the plant. *A. bisporus* had a positive main effect on shoot and total plant weight. There was also a positive interaction between *A. bisporus* and *G. clarum* on the weight of the roots (Table 6.2).

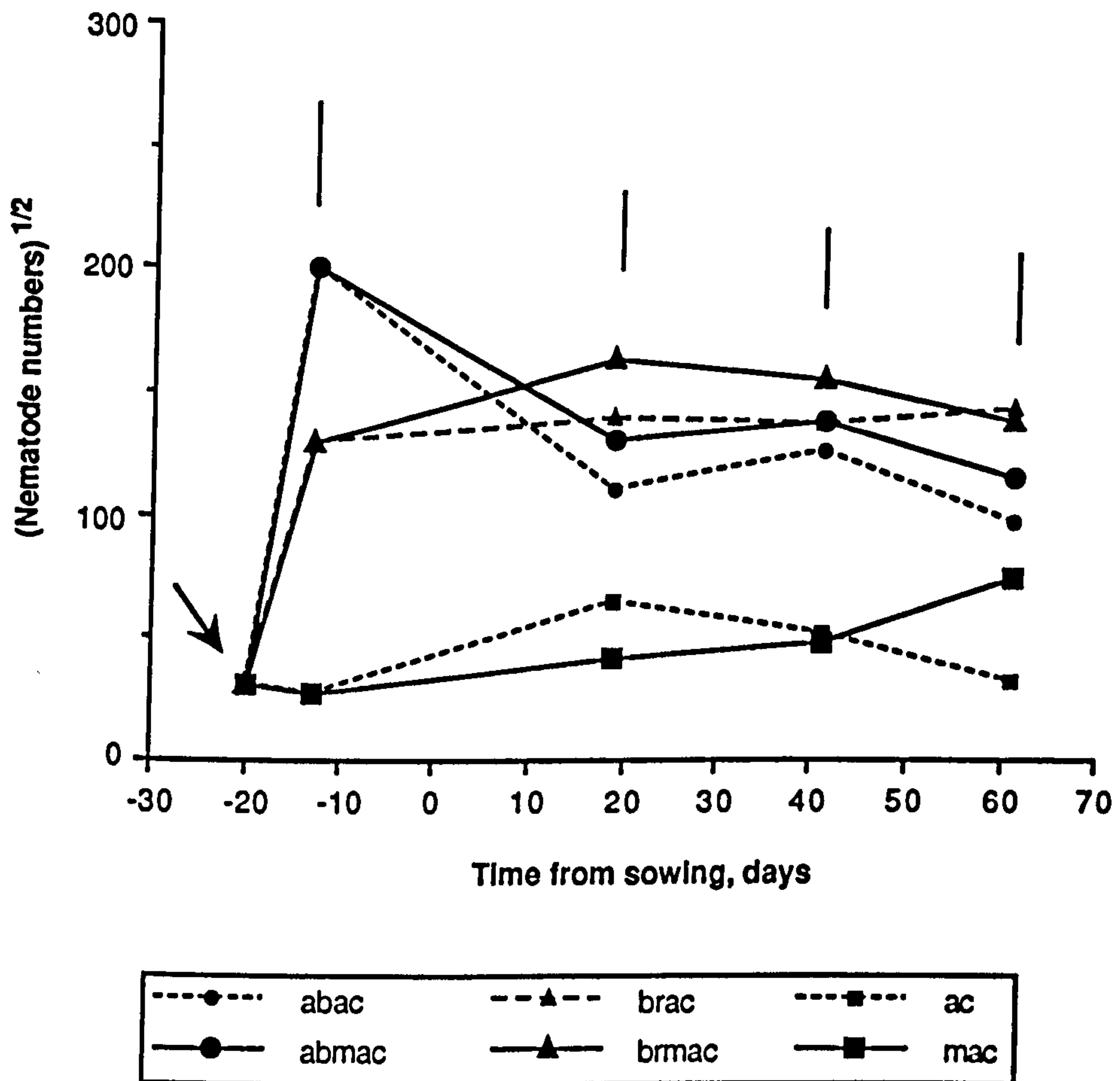


Fig. 6.3. The effect of *G. clarum* and of sterile bran, either alone or colonized with *A. bisporus* on the numbers (expressed as numbers^{1/2}) of *A. composticola* recovered from soil.

Treatment abbreviations as in Fig. 6.2. Points are means per pot and bars represent LSDs at $p=0.05$.

The arrow shows the square root of the number of nematodes added to each pot.

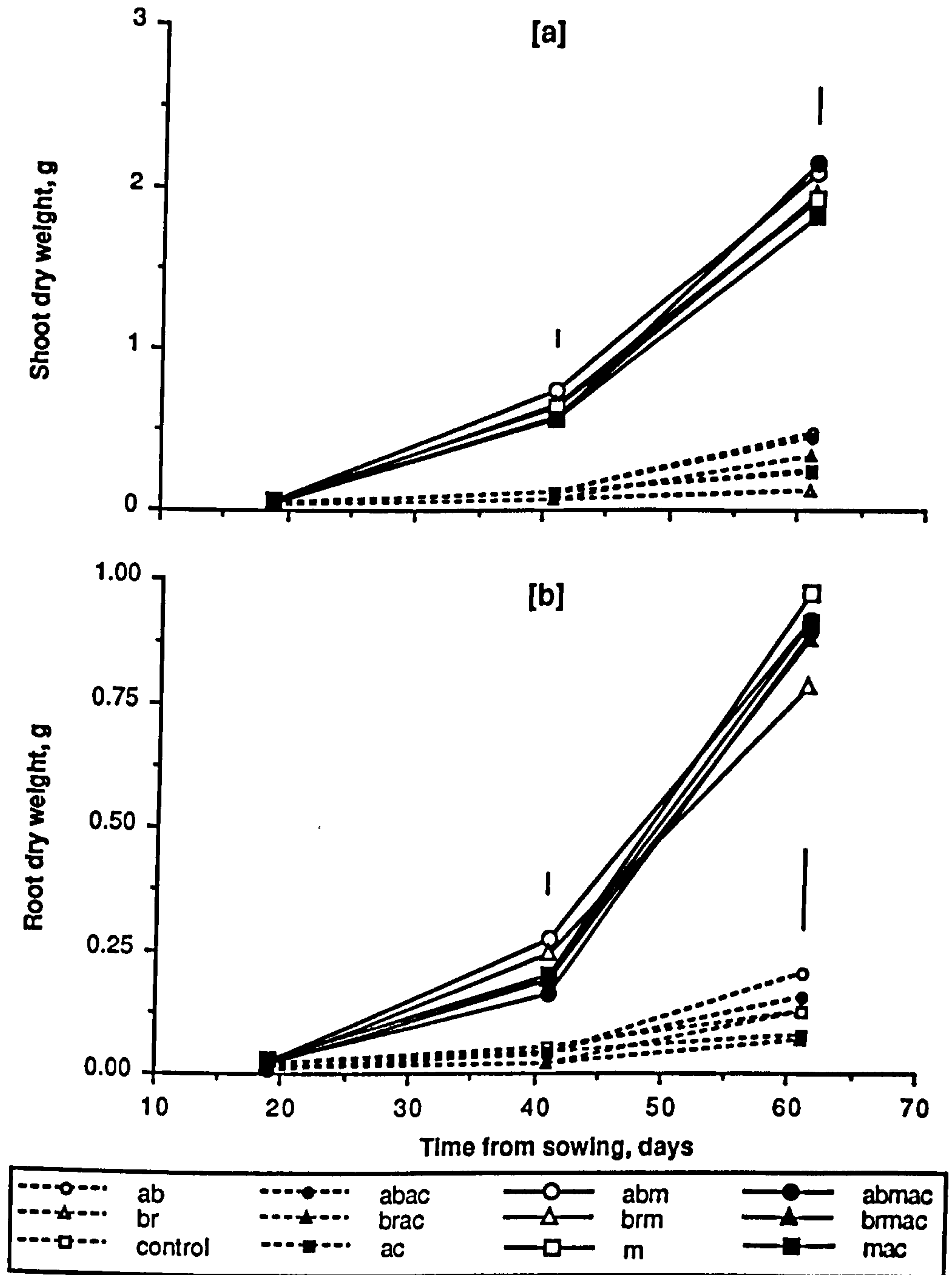


Fig. 6.4. Dry weights of shoots and roots of red clover as affected by *G. clarum*, *A. composticola* and bran either alone or colonized with *A. bisporus*.

Treatment abbreviations as in Fig. 6.2. Points are means per pot and bars represent LSDs at $p=0.05$.

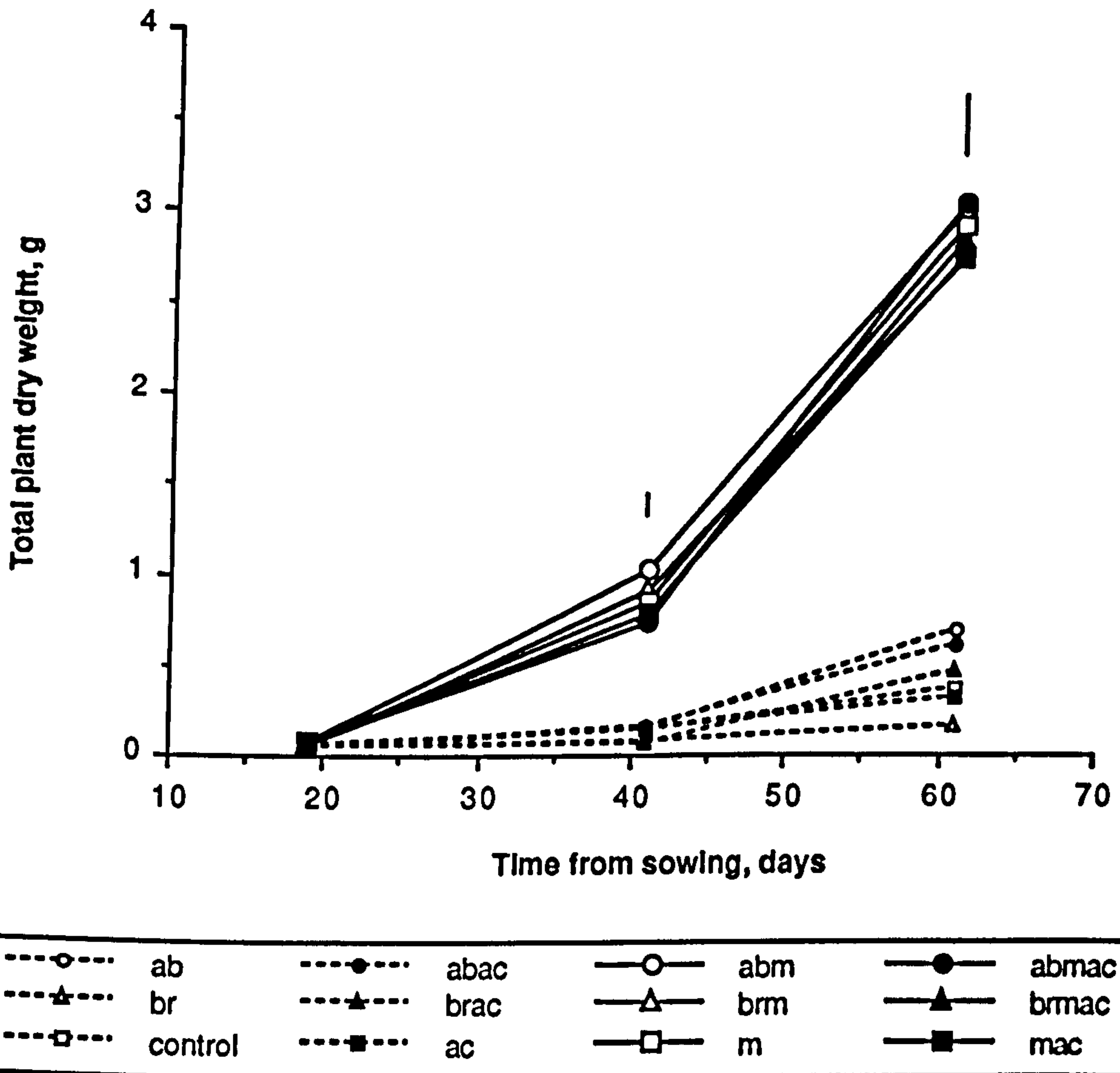


Fig. 6.5. Total dry weights of red clover as affected by *G. clarum*, *A. composticola* and bran either alone or colonized with *A. bisporus*.

Treatment abbreviations as in Fig. 6.2. Points are means per pot and bars represent LSDs at $p=0.05$.

At the second harvest, *A. bisporus* had a positive main effect on shoot weight.

A. composticola had a negative effect on shoot, root and total plant weights (Table 6.1). The shoots, roots and whole plants in treatment abm were heavier than those in all other mycorrhizal treatments (Figs 6.4a,b & 6.5). There was a positive interaction of bran and *G. clarum* on root weight and negative interactions between *G. clarum* and *A. composticola* on shoot, root and total plant weight (Table 6.2).

At the third harvest, *A. bisporus* had a positive main effect on shoot and total plant weight. Bran and *A. composticola* interacted negatively on total plant weight (Table 6.2).

Mycorrhizal infection

Mycorrhizal infection increased with time in all treatments. Fractional infection appeared to reach its plateau in the roots of m and abmac after the second harvest (Fig. 6.6). Neither bran alone, *A. bisporus* nor *A. composticola* had a consistently significant effect on fractional infection.

Concentration and content of phosphorus in the shoots

Results are shown in Fig. 6.7a,b and Table 6.2. Inoculation of the soil with *G. clarum* had a positive main effect on the concentration and content of phosphorus of the shoots at all three harvests.

At the first harvest there was a negative three way interaction between *A. bisporus*, *G. clarum* and *A. composticola* and a negative three way interaction between bran, *G. clarum* and *A. composticola* on the concentration of phosphorus in the shoots.

At the second harvest *A. bisporus* had a positive main effect on the content of phosphorus of the shoots. *A. composticola* had a negative effect on phosphorus content. There was a positive interaction between *A. bisporus* and the mycorrhizal fungus, a negative interaction between *A. bisporus* and nematodes and a negative interaction between mycorrhizal fungus and nematodes (Table 6.2). The content of phosphorus of the shoots in treatment abm was higher than in the shoots of all other mycorrhizal treatments (Fig. 6.7b). Bran had a positive main effect on the concentration of

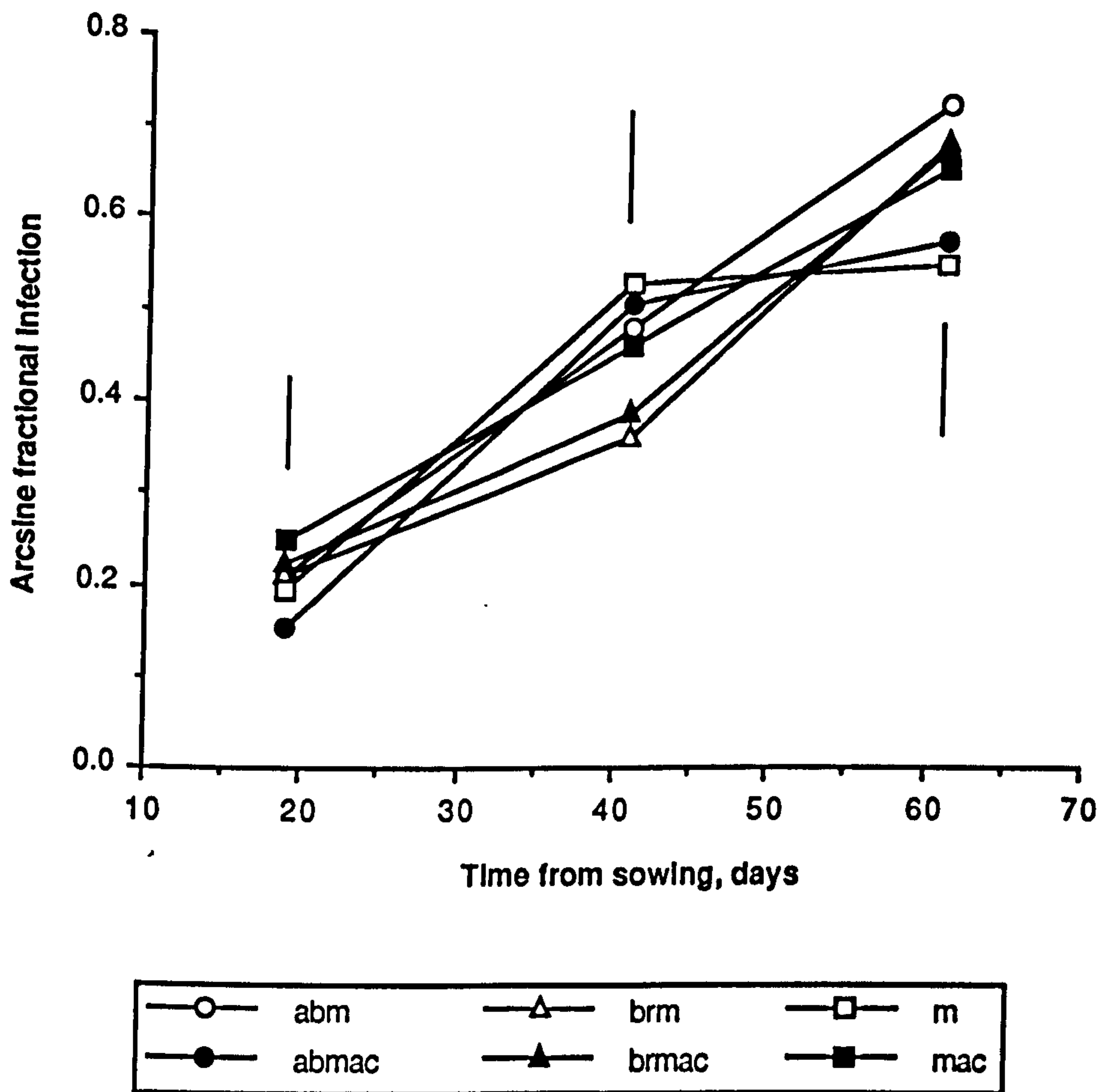


Fig. 6.6. The effect of *A. composticola*, of bran alone and of bran colonized with *A. bisporus* on the infection (expressed as arcsine fractional infection) of roots by *G. clarum*.

Treatment abbreviations as in Fig. 6.2. Points are means per pot and bars represent LSDs at $p=0.05$.

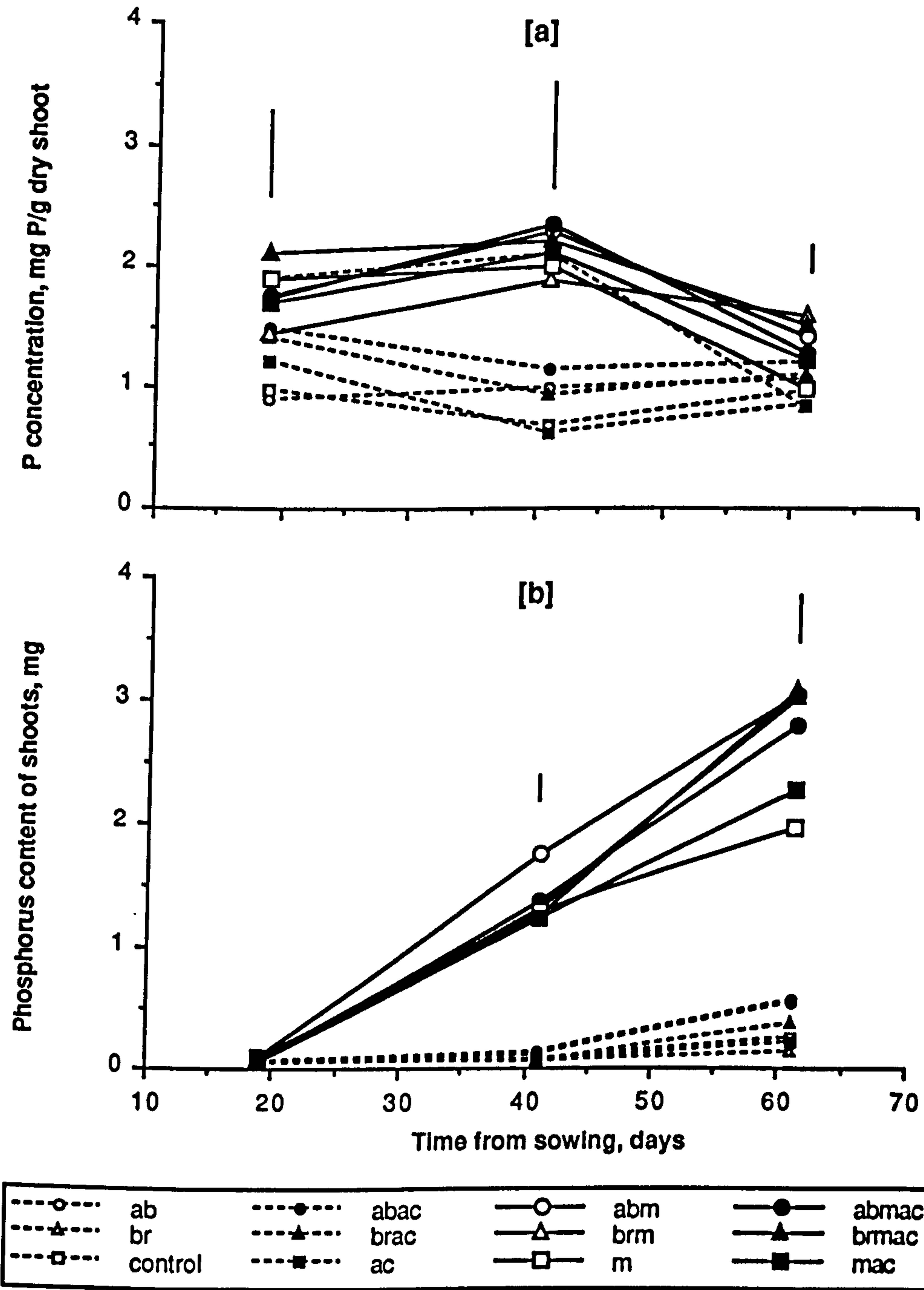


Fig. 6.7. The effect of *G. clarum*, of *A. composticola* and of bran either alone or colonized with *A. bisporus* on the concentration of phosphorus [a] or the content of phosphorus [b] in the shoots of red clover.

Treatment abbreviations as in Fig. 6.2. Points are means per pot and bars represent LSDs at $p=0.05$.

Table 6.2. The result of the analysis of variance at each harvest of experiment 10 of the data on shoot, root and total dry weights of the plants and phosphorus contents and concentrations of the shoots.

Day	Variable	Main effects												Interactions											
		br		ab		m		ac		br x m		br x ac		ab x m		ab x ac		m x ac		ab x m x ac		br x m x ac			
		F	LOS	F	LOS	F	LOS	F	LOS	F	LOS	F	LOS	F	LOS	F	LOS	F	LOS	F	LOS	F	LOS		
19	Shoot dry weight	14.6	---	9.1	++	15.7	+++	0.9	ns	0.0	ns	0.3	ns	0.8	ns	0.4	ns	0.0	ns	0.0	ns	1.1	ns		
	Root dry weight	12.0	--	0.3	ns	32.2	+++	0.4	ns	0.5	ns	0.9	ns	4.7	+	0.4	ns	0.4	ns	0.4	ns	0.1	ns		
	Total dry weight	16.7	---	4.9	+	27.4	+++	0.8	ns	0.1	ns	0.0	ns	2.5	ns	0.5	ns	0.1	ns	0.1	ns	0.7	ns		
	P content	2.1	ns	1.0	ns	46.8	+++	0.1	ns	3.5	ns	0.1	ns	2.9	ns	0.7	ns	2.5	ns	3.8	ns	1.3	ns		
	P concentration	2.4	ns	1.8	ns	13.5	+++	0.6	ns	3.0	ns	0.0	ns	1.3	ns	2.0	ns	1.2	ns	6.1	-	5.5	-		
43	Shoot dry weight	0.4	ns	4.3	+	686	+++	7.5	--	2.2	ns	0.0	ns	0.1	ns	1.1	ns	9.6	--	1.7	ns	0.0	ns		
	Root dry weight	0.4	ns	1.1	ns	252	+++	7.2	--	4.7	+	1.2	ns	1.0	ns	1.1	ns	7.2	--	2.9	ns	1.8	ns		
	Total dry weight	0.5	ns	4.0	ns	593	+++	9.3	--	3.5	ns	0.3	ns	0.5	ns	1.5	ns	9.4	--	2.1	ns	0.3	ns		
	P content	0.0	ns	14.7	+++	811	+++	5.3	-	0.1	ns	0.3	ns	5.6	+	4.8	-	6.1	-	6.1	-	6.3	-		
	P concentration	4.3	+	0.2	ns	40.6	+++	0.1	ns	4.4	-	1.2	ns	3.1	ns	1.6	ns	0.0	ns	3.8	ns	2.6	ns		
63	Shoot dry weight	0.4	ns	14.5	+++	1209	+++	0.3	ns	0.8	ns	3.7	ns	0.5	ns	1.1	ns	0.0	ns	1.7	ns	0.2	ns		
	Root dry weight	2.1	ns	3.5	ns	441	+++	1.4	ns	1.9	ns	2.7	ns	0.0	ns	1.9	ns	0.0	ns	0.0	ns	0.1	ns		
	Total dry weight	0.2	ns	11.8.	++	1069	+++	0.9	ns	0.0	ns	4.4	-	0.3	ns	2.0	ns	0.0	ns	0.7	ns	0.03	ns		
	P content	27.9	+++	1.3	ns	663	+++	0.0	ns	29.2	+++	0.0	ns	6.4	+	1.6	ns	0.2	ns	0.1	ns	4.5	-		
	P concentration	16.3	+++	0.0	ns	15.4	+++	0.3	ns	12.3	+	0.2	ns	9.7	+	0.8	ns	0.1	ns	0.1	ns	10.6	-		

br: bran, ab: *A. bisporus*, m: *G. clarum* and ac: *A. composticola*.

The value of F is generated by the procedure of analysis of variance and is equal to the ratio of the mean sum of squares of each factor to the mean sum of squares of the error.

LOS is the level of significance: +++ or -- indicate a significant response, positive or negative respectively, for $p \leq 0.001$, ++ or - - for $0.001 < p \leq 0.01$ and + or - for $0.01 < p \leq 0.05$.

phosphorus in the shoots and there was a negative interaction between bran and *G.clarum*.

At the third harvest bran had a positive main effect on both the content and concentration of phosphorus in the shoots. There were positive interactions between bran and *G. clarum* as well as *A. bisporus* and *G. clarum* on the content of phosphorus (Table 6.2). Phosphorus contents of shoots in the mycorrhizal treatments with bran or *A. bisporus* were higher than those in the two other mycorrhizal treatments m and mac (Fig. 6.7b). There was a positive interaction between bran and *G. clarum* or *A. bisporus* and *G. clarum* on the concentration of phosphorus in the shoots. Finally there was a negative three way interaction between bran, *G. clarum* and *A. composticola* on phosphorus content and phosphorus concentration (Table 6.2).

Unit absorption rate of phosphorus

The UAR of phosphorus was generally higher in mycorrhizal than non-mycorrhizal treatments and was not influenced by the presence of nematodes (Fig. 6.8). With the exception of treatment brm at the first and second harvests and treatment abmac at the first harvest, UAR was greatest in mycorrhizal treatments with bran or *A. bisporus*. The presence of *A. bisporus* in the soil caused an increase in the UAR of non-mycorrhizal plants.

6.3.4. DISCUSSION

In soil treated with *A. bisporus*, nematodes had a large biomass of mycelium available on which they could feed. As a result they reproduced at a fast rate and quickly reached a population maximum on day -13 after which they declined presumably having depleted their food supply. In soil with bran alone, nematodes multiplied at a slower rate, feeding on the volunteer saprotrophic fungi which gradually colonized the bran substrate. In this case nematodes reached a population maximum on day 20. These results confirmed the results of experiment 9 which showed that significant responses of mycophagous nematodes to food supply may occur in soil just as they do in the agar plate system. In addition, the present experiment showed that numbers of nematodes may

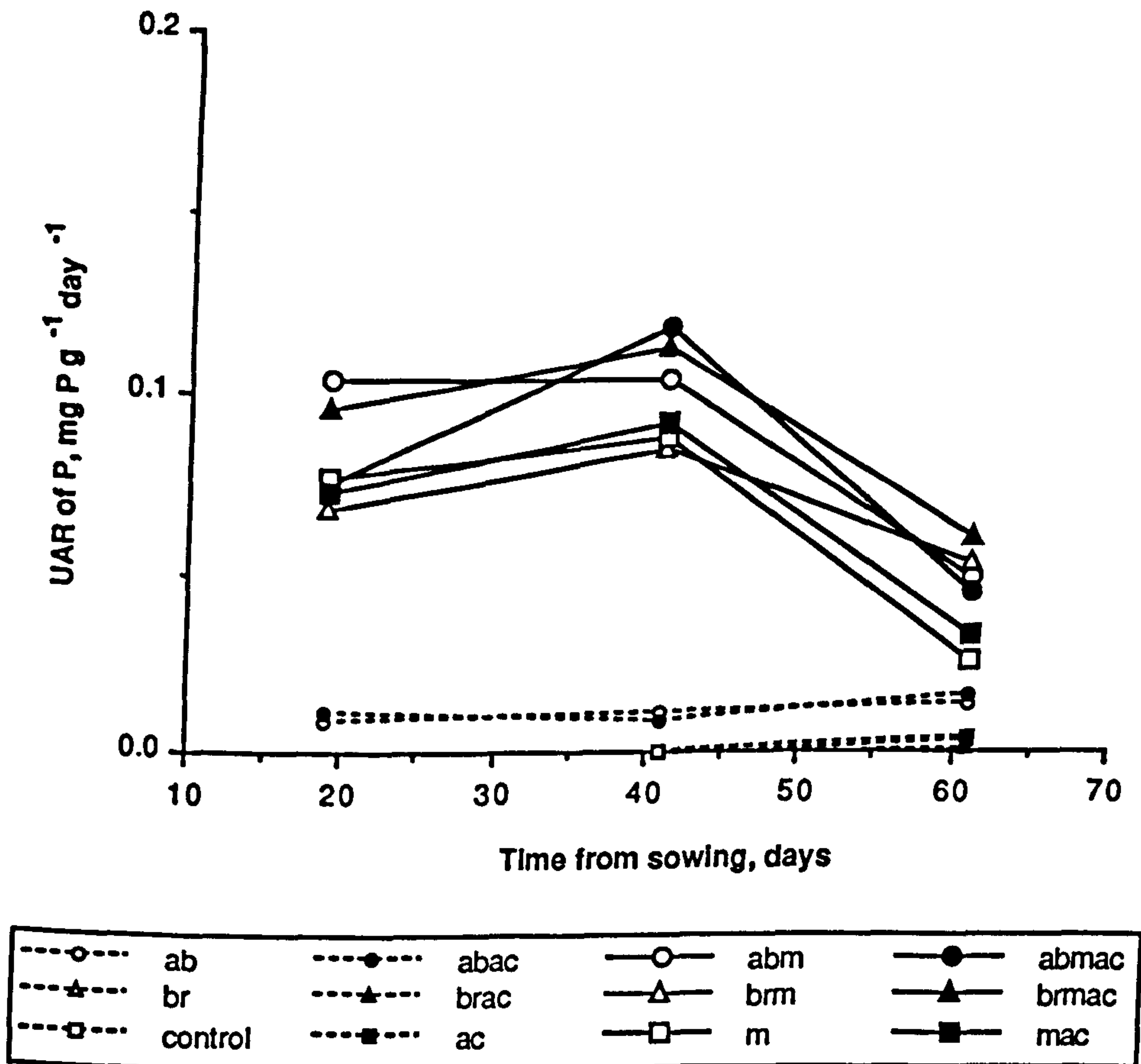


Fig. 6.8. The effect of *G. clarum*, *A. composticola*, and bran either alone or colonized with *A. bisporus* on the unit absorption rate (UAR) of phosphorus by the roots of red clover.

Treatment abbreviations as in Fig. 6.2. Points are means per pot.

increase substantially, even in the presence of VA mycorrhizas, as a result of feeding on fungal mycelium.

In contrast to experiment 8 (Chapter 5) numbers of nematodes in treatments ac and mac did not decline from their initial values. Numbers in mac were generally not different to those in treatment ac and it is unlikely that nematodes fed on the mycorrhizal mycelium itself. This is in agreement with the conclusion from experiment 8 in which *A. avenae* was also not capable of feeding on *G. clarum*. The slightly higher numbers of nematodes in treatment mac compared to treatment ac at the third harvest were probably a consequence of the nematodes feeding on fungi other than *G. clarum*. Fungi which parasitize VAM spores and other saprotrophic fungi commonly found in mycorrhizal inocula (Abbott & Robson 1984; Bagyaraj 1984) may have provided nematodes in the mac treatment with an additional supply of food. Fungi in the genera of *Fusarium*, *Penicillium*, *Trichoderma* and *Chaetomium* have been isolated from mycorrhizal inocula (Sylvia & Schenck 1983). These genera include common hosts of mycophagous nematodes (Faulkner & Darling 1961; Mankau & Mankau 1963) and may have caused the slight increase of numbers of *A. composticola* in soil inoculated with *G. clarum*.

Mycorrhizal red clover plants generally had higher concentrations, contents and UARs of phosphorus than non-mycorrhizal controls. These results were in agreement with the work of Al-Nahidh (1985) who experimented with the same species of plant and VAM fungus. Less is known about the ways in which the presence of organic material or other non-mycorrhizal soil fungi may influence phosphorus uptake and yield of mycorrhizal plants. There is a variety of possible interactions between saprotrophic and VAM fungi (see Avio & Giovannetti 1988; Hetrick 1989). In the present experiment, treatment of the soil with bran or *A. bisporus* clearly increased the rate of uptake of phosphorus by mycorrhizal roots as was indicated by the positive interactions on phosphorus uptake between bran and *G. clarum* at the third harvest and *A. bisporus* and *G. clarum* at the second and third harvest. The mechanism for this effect is not known but since it is generally agreed that mycorrhizal roots take up phosphate only from readily soluble sources of phosphorus (see Hayman 1978) it is unlikely that the mycorrhizas of

red clover were able to make use of phosphorus immobilized within the microbial population. Furthermore, neither bran nor *A. bisporus* reduced the phosphorus UAR of the non-mycorrhizal plants which would have occurred if there was a net immobilization of phosphorus. On the contrary, UAR in treatments ab and abac was consistently higher than in the control indicating net mineralization of phosphorus. Mycorrhizal roots were probably able to take up more of the phosphate mineralized by *A. bisporus* or other saprotrophs which colonized the bran by means of their external VAM mycelium.

Increased mineralization of phosphorus in soil with *A. bisporus* or bran resulting in an increased uptake of phosphorus by mycorrhizal plants in this soil was probably the reason for the increased yields of mycorrhizal plants measured at the second or third harvest. Increased uptake of other nutrients such as nitrogen as a result of their mineralization may have also contributed (Curl & Truelove 1986; Harley & Smith 1983). Dighton, Thomas & Latter (1987) found that the presence of the saprotrophic fungus *Mycena galopus* caused a more significant increase in the shoot and total weights of ectomycorrhizal pine seedlings than those of non-mycorrhizal seedlings. They suggested that the saprotrophic fungus was able to release nutrients through decomposition which would otherwise have been unavailable to the mycorrhizal roots. A similar mechanism could explain the generally higher uptake and UAR of phosphorus by mycorrhizal plants in the presence of *A. bisporus*. The weights of these mycorrhizal plants were not also generally greater indicating that the supply of phosphorus via the mycorrhizas of these plants alone was optimal.

VAM infection was not affected by any increased supply of soluble phosphate via mineralization. Judging by the content of phosphorus of the shoots of mycorrhizal plants at the third harvest (Fig. 6.7b), an average of 4 mg phosphorus/Kg soil had probably been made available to the plants in soil amended with bran colonized with *A. bisporus* as a result of mineralization. This would have increased the effective available phosphorus from 19.5 mg/Kg (see section 2.2.1) to 23.5 mg/Kg which was probably still too low to have any effect on the spread of VAM infection (Schubert & Hayman 1986).

Although it was significant, the negative effect of *A. composticola* on the dry weights of shoots and roots and the total weights of plants in soil with *A. bisporus* at harvest 2, was not important when compared to the magnitude of the effect that VAM infection had on the growth of the plant (see Table 6.2 and Figs 6.4a,b & 6.5). Since nematodes did not influence fractional infection and did not have greater numbers in mycorrhizal than in non-mycorrhizal soil, the effect of nematodes on the growth of mycorrhizal plants cannot be attributed to damage to the mycorrhizal mycelium. This is in agreement with experiment 8. In the present experiment it is more likely that the nematodes temporarily reduced the capacity of *A. bisporus* to release phosphorus or other nutrients from the bran or the soil by damaging the mycelium of this fungus on which they had apparently fed. Trofymow & Coleman (1982) found that *A. avenae* had a similar negative effect on the mineralization of organic nitrogen by *Fusarium oxysporum* but the results of Ingham *et al.* (1985b) with the same species of nematode and fungus indicated that the influence of mycophagous nematodes on the mineralization of nutrients by saprotrophic fungi is likely to be small.

6.4. CONCLUSION

Red clover responded to the infection of its roots by *G. clarum* had increased levels of phosphorus concentrations and contents in its shoots as well as increased shoot, root and total plant dry weights in all mycorrhizal treatments compared to the non-mycorrhizal controls.

A. composticola was capable of increasing its numbers in soil inoculated with one of its host fungi or with an organic substrate which is colonizable with volunteer fungi. In soil inoculated with *G. clarum* but not with bran or bran plus *A. bisporus*, the numbers of nematodes were small indicating that the available food sources in that soil were either sparse or absent. It is unlikely, therefore, that *A. composticola* was capable of feeding on *G. clarum*.

The capability of *G. clarum* to infect the roots of red clover and increase shoot, root and total plant dry weight was not affected by the presence of *A. composticola* in the

soil. This was so, even when the nematodes were present in great numbers at the time when mycorrhizal infection first became established and later throughout the whole period of its spread in the roots.

Neither mycorrhizal infection nor the associated growth responses of the plant were counteracted by the presence in soil of other saprotrophic fungi or increased quantities of organic material in the form of bran. Mycorrhizal plants appeared to benefit more than non-mycorrhizal from the mineralization of nutrients by the saprotrophic fungi.

CHAPTER 7. GENERAL DISCUSSION

Suitability of fungi as food sources for nematodes

The present work has shown that measurements of the numbers of nematodes produced after a period of feeding on fungal mycelia are not on their own a satisfactory basis for revealing food preferences. Use of this means of ranking in earlier studies (Townshend 1964; Pillai & Taylor 1967b) has almost certainly led to incorrect conclusions by ignoring the effect that nematodes have on the growth of the fungal mycelium.

Whether or not a fungus is a good host for a nematode species appears to be a consequence of two opposing processes, namely the increase in numbers of nematodes and the reduction in weight of mycelium. A model was developed based on the Lotka-Volterra equations (see Crawley 1983) in which two of the parameters determined the rates of these two processes. Computer simulations using this model gave reasonable agreement with experimental data (Hickson pers. comm.) on fungus-nematode dynamics in agar plates. The simulations showed that the ratio of numbers of nematodes to weight of mycelium recovered from a fungal culture at the time when almost all mycelium had been killed by the nematodes, was largely independent of the initial weight and growth rate of the fungus and of initial numbers of nematodes. Therefore, this ratio could be used to rank different fungi in terms of their suitability as hosts for the same species of nematode. This new way of ranking is advantageous in that it combines measurements of the effects that nematodes and fungi have on each other.

Consideration of feeding preferences of the nematodes leads to discussion of whether the fungi preferred by the nematodes have any characteristics in common. Mankau & Mankau (1963) suggested that *A. avenae* may reproduce better in cultures of root-infecting pathogenic fungi rather than saprotrophs because the latter produce antibiotics which may be toxic. In contrast, Townshend (1964) found that a fungus that

repelled *A. avenae* and retarded its reproduction was a plant-pathogenic *Sclerotium* species.

The results of the present study agree with those of Mankau & Mankau (1963) in that a strain of *R. solani*, which was potentially phytopathogenic was the favourite host of *A. avenae* and amongst the favourite hosts of *A. composticola*. However, there was no indication from the present study that root-infecting fungi are in general preferred by the nematodes since some ectomycorrhizal species which produced coloured metabolites or had pigmented mycelia were the least preferred hosts of both *A. avenae* and *A. composticola*. Exudation of metabolites or pigmentation of the hyphae may therefore be a characteristic of fungi which is important in determining whether or not a particular fungus is a good host. Sutherland & Fortin (1968) concluded that a toxic substance produced by the ectomycorrhizal *R. roseolus* was the cause for the recovery of small numbers of *A. avenae* from plate cultures of this fungus. Mankau (1969) found that oxalic acid produced by *Aspergillus niger* was toxic to *A. avenae*. Less is known about whether pigmented mycelia are themselves toxic to nematodes. Fungal metabolites are potentially harmful to fungal-feeding insects (Kukor & Martin 1987). Flavonoids in the leaves of plants are examples of pigments which by being toxic may act as feeding deterrents for insects (Harborne 1976). Pigments produced by ectomycorrhizal fungi may act in a similar way to deter nematode grazing of their mycelia.

The fact that nematodes still managed to feed on mycelia of ectomycorrhizal fungi in spite of their production of pigments and metabolites could mean that a mechanism other than toxicity of these substances may partly explain their potential to deter nematode feeding. It is possible that substances produced by some ectomycorrhizal fungi may interfere with reproductive development of nematodes. Besl & Blumreisinger (1984) showed that the fungi *Paxillus atrotomentosus* and *Dermocybe* spp. retarded larval development of the insect *Drosophila melanogaster* in laboratory cultures and were rarely infected by its larvae in the field. This was apparently caused by the production of substances by the fungi which were inhibitory to the larvae. *Dermocybe* spp. in particular produced anthraquinone pigments which were shown to be involved in this inhibition.

Quantitative or qualitative differences in the nutritional status of hyphae of different species (see Foster 1949) may also account in part for the dietary preferences of nematodes. For example, variation in the levels of phosphorus and potassium supplied to a fungus grown in plate culture influenced numbers of nematodes produced (Smart & Darling 1963). Unfortunately the authors did not measure the growth of the fungus in the absence of nematodes and therefore, it is not known whether the effect of different levels of nutrients in the medium on the numbers of nematodes was caused by differences in the contents of nutrients of the hyphae or by differences in the quantity of mycelium produced.

In the present study one species of fungus (C) which was capable of rapid extension growth and produced large numbers of spores but little vegetative mycelium was not affected by the nematodes and presumably the nematodes were not able to feed on its spores. Little is known about whether mycophagous nematodes are in general capable of feeding on fungal spores or rather prefer to feed on hyphae as suggested by Yeates (1971). Ingestion of spores appears to be a feature common with non stylet-bearing nematodes (Wood 1973) and it is unlikely that this type of feeding could occur in the Tylenchida which include the majority of mycophagous species of nematodes (Goodey 1963) and whose stylets are too narrow to allow spores to pass. The spores of *Verticillium alboatrum* were attacked by *Aphelenchoides besseyi* (Nickle & McIntosh 1968) and *D. myceliophagus* has been observed to feed on the conidia of *Botrytis cinerea* (Doncaster 1966). In contrast, Riffle (1971) reported that *Aphelenchoides cibolensis* fed on the hyphae but not on the spores of a *Leptographium* species and suggested that this allowed the fungus to remain viable in spite of having been severely damaged by the feeding of the nematode. Further experiments are needed to examine the possibility that the production of large numbers of spores but little vegetative mycelium is a strategy which makes such fungi less suitable as food sources for nematodes.

Interactions between mycophagous nematodes and VAM fungi

Pure culture of VAM fungi is one of the important objectives of research on mycorrhizas (Hall 1988). If successful, it may become possible in the future to observe

directly the behaviour of nematodes in plate cultures of VAM fungi. Until then one has to rely on pot experiments with VAM fungi grown in association with host plants in the soil. Results of several experiments in the present study showed that none of the various species of mycophagous nematodes had any effect on VAM infection by *G. clarum* or on the mycorrhizal responses of red clover. Thus previous beliefs that nematodes may be capable of counteracting the beneficial effects of VAM infection on the growth of plants by grazing on the mycorrhizal mycelium (Salawu & Estey 1979; Ingham *et al.* 1986b) cannot be confirmed.

Inconsistencies in the results of the experiments of Hussey & Roncadori (1981) on the interactions between *A. avenae* and VAM fungi were attributed by the authors to differences in the numbers of nematodes introduced to the soil – higher numbers only were associated with reductions in mycorrhizal plant yields. However, in the light of the results of the present study it is unlikely that the application rate of nematodes to soil is the factor which mainly influences the outcome of nematode-VAM fungus interactions because, even when high numbers of nematodes were added, there was no effect on the association between the VAM fungus and the plant.

Some progress towards finding out why nematodes did not affect the development of the VAM fungus has been made by modelling the spread of mycorrhizal infection in the roots and the change of numbers of nematodes in the soil. This modelling was done using the equations of Al-Nahidh & Sanders (1987) in combination with the modified Lotka-Volterra equations used previously to describe fungus-nematode interactions in agar plates. To simulate the actual experimental results, it was necessary to assume that nematodes were not capable of feeding on the fungus.

Production of toxic substances by the VAM fungi themselves has not yet been demonstrated but there is evidence that mycorrhizal roots contain increased concentrations of isoflavonoids (Morandi, Bailey & Gianinazzi-Pearson 1984; Morandi & Gianinazzi-Pearson 1986). Accumulation of these metabolites in the roots are considered to be involved in the mechanism by which the formation of mycorrhizal roots has increased plant resistance to damage by plant-parasitic nematodes. For example, coumestrol which

was found in high concentrations inside the roots of Lima beans infected by *Pratylenchus scribneri*, inhibited the motility of the nematode *in vitro* at concentrations smaller than those found in the roots (Rich, Keen & Thomason 1977). As suggested by Hetrick (1989) such compounds could also affect the behaviour of non-pathogenic organisms in the rhizosphere of mycorrhizal plants if they were exuded from the roots or released from dead root cells. Therefore, in the present study, the release of such substances from the mycorrhizal roots of red clover may have protected *G. clarum* from nematode grazing. Successful observation of nematodes feeding on hyphae of a VAM fungus *in vitro* may not necessarily mean that the nematodes would feed on the fungus when it was in association with the roots of its host.

The composition of the mycelium of *G. clarum* itself may have made it unpalatable to the nematodes but nothing is known about whether the hyphae of VAM fungi contain toxic or inhibitory substances or whether they are an unsuitable food source for mycophagous nematodes. These possibilities require further investigation.

Whatever the mechanism, the above evidence indicates that VAM infection caused by *G. clarum* may be immune to attack by mycophagous nematodes but it is not known whether this is the case with all VAM species. Experiments on the interactions between VAM fungi and plant-parasitic nematodes have shown that their outcome may in fact depend on the species of VAM fungus involved (Thomas *et al.* 1989; Strobel, Hussey & Roncadori 1982). Further experiments using different species of VAM are necessary before general conclusions can be reached. *G. clarum* has some of the characteristics of an r-strategist because it can colonize the roots of its host rapidly and sporulate profusely (Daft & Hogarth 1983). Experiments with slow growing species of VAM could show that the effect of nematodes is more detrimental on these species.

The result of experiment 4 on the interactions between red clover, *G. clarum* and *D. triformis*, *D. myceliophagus*, *A. avenae* or *A. composticola* in which non-mycorrhizal plants in the presence of either one of these four species of nematodes were significantly smaller than control plants and had severely damaged roots was not repeated but is noteworthy. Controversy has existed at various times as to whether so called

mycophagous nematodes may damage plant roots. The work of Chin & Estey (1966) showed that *A. avenae* may feed on root hairs and was also found inside the roots of tomato plants. In further tests with tomato plants inoculated with both *Verticillium albo-atrum* and *A. avenae*, they found that percentage of wilting plants was considerably higher and the plant weights lower than in the case of plants inoculated with either organism alone. The results of experiment 4 support these findings since root damage was probably the result of a similar type of interaction between the nematodes and an unidentified root pathogen. The appearance of the roots indicated the possibility that the nematodes may have wounded them by probing the root cells with their stylets thus facilitating the invasion of the pathogen. Taken together, this evidence provides some basis for the opinion that the classification of nematodes into mycophagous or plant-parasitic is rather misleading. More specifically, the role of mycophagous nematodes in the soil may not be limited to the influence that these may have on fungal biomass. Further studies need to be done to validate the hypothesis that mycophagous nematodes may directly affect plant yield.

The way in which the presence of *G. clarum* compensated for root damage in this same experiment is not known but is in agreement with a large number of studies on the interactions between VAM fungi and root pathogens which have led to the conclusion that mycorrhizas are capable of increasing the tolerance or the resistance of plants to infections by pathogens (see Hussey & Roncadori 1982; Garcia-Garrido & Ocampo 1988; Smith 1988).

Methods for the recovery of nematodes from soil

Quantitative studies of interactions between nematodes and VAM fungi depend heavily on accurate methods for the recovery and measurement of numbers of nematodes in soil. Unfortunately, no method exists which is reliable for the quantitative recovery of different nematodes from the soil under different sets of conditions. The size and behaviour of the individual species of nematodes, as well as the extent to which the reliability of a given method is dependent on various other factors (e.g. ambient

temperature) need to be carefully considered before a method is chosen (see also sections 1.2.2 & 2.2.3).

Sieving of soil suspensions containing nematodes and the subsampling of soil before extraction begins were two major sources of error in estimates of numbers of nematodes extracted. The errors associated with two standard methods in which sieving or subsampling were essential steps made it difficult to reach any firm conclusions from the results of all preliminary experiments. The implication of this is that many of the estimates of numbers of nematodes reported in the literature may be inaccurate and conclusions invalid. Extreme care should be taken when using any one of the so called standard techniques and extraction efficiencies should always be determined and reported. The method developed in the present study was based on the principle that nematodes are able to swim out from a soil saturated with water. It was shown to produce relatively consistent recoveries but was limited in its use by the small quantity of soil that could be processed each time and by the need for nematodes to be active. Further research is necessary to find methods which may be used for the quantitative recovery of nematodes under a variety of conditions. The behavioural reaction of the nematodes to external stimuli such as temperature, electrical fields or chemical substances (Nicholas 1984) could possibly be utilized in the design of new extraction techniques. For example, it has been suggested that predacious nematode-trapping fungi possess or release substances that attract nematodes into their traps (Balan *et al.* 1976; Jansson & Nordbring-Hertz 1980; Jansson 1982; Balan 1985). Such substances could possibly be employed in the construction of artificial traps for the capture and recovery of nematodes from soil.

The effect of organic material and saprotrophic fungi on VAM infection

The results of the present study showed that the addition of organic material alone or organic material colonized by saprotrophic fungi to partially-sterilized soil did not interfere with the establishment of VA mycorrhizas of *G. clarum*. This is important in the context of the use of additions of organic matter or saprotrophic organisms to soil as means of biological control of root pathogens (Mangenot & Diem 1979). The precise

mechanisms of such biological control are largely unknown but it appears that amendments with organic matter may induce competition for niches between pathogens and antagonists or result in the release of toxic substances through decomposition of the organic matter. Introduced antagonists may act by producing antibiotics, competing for space with or parasitizing the pathogens.

Clearly the practice of biological control may be potentially hazardous towards VAM fungi. Several fungi capable of parasitizing the VAM spores and hyphae and others, mainly saprotrophic, which produce antimicrobial metabolites are believed to be responsible for reduced germination of spores and lower levels of VAM infection and may affect the physiological functions of mycorrhizas (Ross & Ruttencutter 1977; Sylvia & Schenck 1983). VAM infection by three species of *Glomus* was also reduced in the presence of organic material colonizable by saprotrophs but the mycorrhizal responses of the plant were not always significantly affected (Avio & Giovannetti 1988). The results of the present study are in contrast with the above reports, indicating that the establishment of VA mycorrhizas may not be affected by the practice of biological control in the field. Further work is needed before it is possible to make general statements on the interactions between saprotrophic and VAM fungi. Substantial variation may exist between different VAM species in terms of their sensitivity towards antibiotics (Sylvia & Schenck 1983) and therefore different species of VAM fungi should be tested. Species which are antibiotic producers (e.g. *Trichoderma* spp. and *Penicillium* spp.) could also be employed to examine the effects on VAM infection of such substances which are known to reduce root infection by pathogens (Mangenot & Diem 1979).

Conclusion

It appears that mycophagous nematodes have dietary preferences which are not related to the common nutritional groups of fungi (i.e. mycorrhizal, saprotrophic or phytopathogenic) but instead are determined by characteristics related to each individual fungal species such as production of toxic metabolites, production of pigments or differences in the nutritional status of hyphae. The fact that these nematodes can significantly reduce the growth of several fungi, including one root pathogen, in plate

culture supports the hypothesis that the nematodes have the potential to benefit yields of plants by reducing the ability of phytopathogenic fungi to infect plant roots. Experiments that have been performed under greenhouse conditions lend support to this view (Roessner & Umland 1983; Hong & Estey 1985). In contrast, damage by the nematodes to mycelia of ectomycorrhizal fungi which was observed in plate culture indicates that the nematodes may also be detrimental for growth of ectomycorrhizal plants by reducing ectomycorrhizal infection of their roots. Although VAM infection by *G. clarum* was not affected by the nematodes it would not be surprising to find that other VAM fungi are not immune to nematode attack because of the non-specific feeding habits of these nematodes. This would preclude choosing such species of VAM fungi for inoculation of crops in possible future exploitation of VAM fungi as biological fertilizers, if this can ever be achieved. Further research on the interactions between mycophagous nematodes and fungi is essential in order to determine whether these nematodes are potentially harmful or beneficial to plant growth.

REFERENCES

- Abbott, L.K. & Robson, A.D. (1977). Growth stimulation of subterranean clover with vesicular arbuscular mycorrhizas. *Australian Journal of Agricultural Research*, 28, 639-649.
- Abbott, L.K. & Robson, A.D. (1984). The effect of VA mycorrhizae on plant growth. *VA Mycorrhiza* (Ed. by C.L. Powell & D.J. Bagyaraj), pp. 113-130. CRC Press, Boca Raton.
- Abbott, L.K. & Robson, A.D. (1985). Formation of external hyphae in soil by species of vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, 99, 245-255.
- Agnihotri, V.P. (1970). Solubilization of insoluble phosphates by some soil fungi isolated from nursery seedbeds. *Canadian Journal of Microbiology*, 16, 877-880.
- Agrios, G.N. (1978). *Plant Pathology*. Academic Press, New York.
- Alexander, M. (1977). *Introduction to soil microbiology*. John Wiley & Sons, New York.
- Al-Nahidh, S. (1985). *Host-endophyte compatibility in mycorrhizal crop plants*. Ph.D. thesis, University of Leeds.
- Al-Nahidh, S. & Sanders, F.E. (1987). Comparisons between vesicular-arbuscular mycorrhizal fungi with respect to the development of infection and consequent effects on plant growth. *Fungal infection of plants* (Ed. by G.F. Pegg & P.G. Ayers), pp. 239-252. Press Syndicate of the University of Cambridge, Cambridge.
- Amijee, F., Tinker, P.B. & Stribley, D.P. (1989). The development of endomycorrhizal root systems. VII. A detailed study of effects of soil phosphorus on colonization. *New Phytologist*, 111, 435-446.
- Anderson, R.V., Gould, W.D., Ingham, R.E. & Coleman, D.C. (1979). A staining method for nematodes: determination of nematode resistant stages and direct counts from soil. *Transactions of the American Microscopical Society*, 98, 213-218.

- Arrold, N.P. & Blake, C.D. (1966). Some effects of *Ditylenchus myceliophagus* and *Aphelenchoides composticola* on the growth on agar plates of the cultivated mushroom, *Agaricus bisporus*. *Nematologica*, 12, 501–510.
- Arrold, N.P. & Blake, C.D. (1968). Some effects of the nematodes *Ditylenchus myceliophagus* and *Aphelenchoides composticola* on the yield of the cultivated mushroom. *Annals of Applied Biology*, 61, 161–166.
- Atilano, R.A., Menge J.A. & Van Gundy, S.D. (1981). Interaction between *Meloidogyne arenaria* and *Glomus fasciculatus* in grape. *Journal of Nematology*, 13, 52–57.
- Avio, L. & Giovannetti, M. (1988). Vesicular–arbuscular mycorrhizal infection of lucerne roots in a cellulose–amended soil. *Plant and Soil*, 112, 99–104.
- Azcon, R., Barea, J.M. & Hayman, D.S. (1976). Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phosphate–solubilizing bacteria. *Soil Biology and Biochemistry*, 8, 135–138.
- Azcon, R., Azcon–G De Aguilar, C., Barea, J.M. (1978). Effects of plant hormones present in bacterial cultures on the formation of responses to VA endomycorrhiza. *New Phytologist*, 80, 359–364.
- Azcon–Aguilar, C. & Barea, J.M. (1985). Effect of soil micro–organisms on formation of vesicular–arbuscular mycorrhizas. *Transactions of the British Mycological Society*, 84, 536–537.
- Azcon–Aguilar, C., Diaz–Rodriguez, R.M. & Barea J.M. (1986a). Effect of soil micro–organisms on spore germination and growth of the vesicular–arbuscular mycorrhizal fungus *Glomus mosseae*. *Transactions of the British Mycological Society*, 86, 337–340.
- Azcon–Aguilar, C., Diaz–Rodriguez, R.M. & Barea, J.M. (1986b). Effect of free–living fungi on the germination of *G. mosseae* on soil extract. *Physiological and genetical aspects of mycorrhizae* (Ed. by V. Gianinazzi–Pearson & S. Gianinazzi), pp. 515–519. INRA, Paris.
- Baath, E., Lohm, U., Lundgren, B., Rosswall T., Soderstrom, B. & Sohlenius, B. (1981). Impact of microbial–feeding animals on total soil activity and nitrogen dynamics: a soil microcosm experiment. *Oikos*, 37, 257–264.

- Baermann, G. (1917). Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdroben. *Geneeskundig Tijdschrift voor Nederlandsch-Indie*, 57, 131–137.
- Bagyaraj, D.J. (1984). Biological interactions with VA mycorrhizal fungi. *VA Mycorrhiza* (Ed. by C.L. Powell & D.J. Bagyaraj), pp. 131–153. CRC Press, Boca Raton.
- Bailey, N.T.J. (1983). *Statistical methods in biology*. Hodder & Stoughton, London.
- Balan, J., Krizkova, L., Nemeč, P. & Kolozsvary, A. (1976). A qualitative method for detection of nematode attracting substances and proof of production of three different attractants by the fungus *Monocrosporium rutgeriense*. *Nematologica*, 22, 306–311.
- Balan, J. (1985). Measuring minimal concentrations of attractants detected by the nematode, *Panagrellus redivivus*. *Biological Abstracts*, 79(11), Ref. No. 96082.
- Barea, J.M., Azcon, R. & Hayman, D.S. (1975). Possible synergistic interactions between Endogone and phosphate-solubilizing bacteria in low-phosphate soils. *Endomycorrhizas* (Ed. by F.E. Sanders, B. Mosse & P.B. Tinker), pp. 409–417. Academic Press, London.
- Barker, K.R. (1964). On the disease reduction and reproduction of the nematode *Aphelenchus avenae* on isolates of *Rhizoctonia solani*. *Plant Disease Reporter*, 48, 428–432.
- Barker, K.R. & Darling H.M. (1965). Reproduction of *Aphelenchus avenae* on plant tissues in culture. *Nematologica*, 11, 162–166.
- Barker, K.R., Nusbaum, C.J. & Nelson, L.A. (1969a). Seasonal population dynamics of selected plant-parasitic nematodes as measured by three extraction procedures. *Journal of Nematology*, 1, 232–239.
- Barker, K.R., Nusbaum, C.J. & Nelson, L.A. (1969b). Effects of storage temperature and extraction procedure on recovery of plant-parasitic nematodes from field soils. *Journal of Nematology*, 1, 240–247.
- Barker, K.R. & Campbell, C.L. (1981). Sampling nematode populations. *Plant parasitic nematodes*, Vol. III (Ed. by B.M. Zuckerman & R.A. Rohde), pp. 451–474. Academic Press, New York.

- Barnes, G.L., Russell, C.C. & Foster, W.D. (1981a). *Aphelenchus avenae*, a potential biological control agent for root rot fungi. *Plant Disease*, 65, 423–424.
- Barnes, G.L., Russell, C.C. & Foster, W.D. (1981b). Evidence for non-pathogenicity of the mycophagous nematode *Aphelenchus avenae*. *Phytopathology*, 71, 858.
- Besl, H. & Blumreisinger, M. (1984). *Drosophila melanogaster* suitable for testing the susceptibility of higher fungi to larval feeding. *Biological Abstracts*, 78(1), Ref. No. 2000.
- Bethlenfalvay, G.W., Pakovsky, R.W., Brown, M.S. & Fuller, G. (1982). Mycotrophic growth and mutualistic development of host plant and fungal endophyte in an endomycorrhizal symbiosis. *Plant and Soil*, 68, 43–54.
- Bevege, D.I. & Bowen, G.D. (1975). *Endogone* strain and host plant differences in development of vesicular–arbuscular mycorrhizas. *Endomycorrhizas* (Ed. by F.E. Sanders, B. Mosse & P.B. Tinker), pp. 77–86. Academic Press, London.
- Bird, G.W., Rich, J.R. & Glover, S.U. (1974). Increased endomycorrhizae of cotton roots in soil treated with nematicides. *Phytopathology*, 64, 48–51.
- Bostrom, S. & Sohlenius, B. (1986). Short-term dynamics of nematode communities in arable soil. Influence of a perennial and an annual cropping system. *Pedobiologia*, 29, 345–357.
- Brewster, J.L. & Tinker, P.B.H. (1972). Nutrient flow rates into roots. *Soils and Fertilizers*, 35, 355–359.
- Bridge, J. & Hunt, D.J. (1985). *Aphelenchoides arachidis*. C.I.H. Descriptions of Plant-parasitic Nematodes, Set 8, No. 116.
- Brown, R.H. & Kerry, B.R. (Eds) (1987). *Principles and practice of nematode control in crops*. Academic Press, Marrickville Australia.
- Byrd, D.W., Barker, K.R., Ferris, H., Nusbaum, C.J., Griffin, W.E., Small, R.H. & Stone, C.A. (1976). Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. *Journal of Nematology*, 8, 206–212.

- Chi, C.C., Childers, W.R. & Hanson, E.W. (1964). Penetration and subsequent development of three *Fusarium* species in alfalfa and red clover. *Phytopathology*, 54, 434–437.
- Chin, D.A. & Estey, R.H. (1966). Studies on the pathogenicity of *Aphelenchus avenae* Bastian, 1865. *Phytoprotection*, 47, 66–72.
- Christie, J.R. (1953). Ectoparasitic nematodes of plants. *Phytopathology*, 43, 295–297.
- Clark, W.C. (1964). Fungal-feeding nematodes as possible plant pathogens. *New Zealand Journal of Agricultural Research*, 7, 441–443.
- Cobb, N.A. (1918). Estimating the nema population of the soil. *U.S. Department of Agriculture, Bureau of Plant Industry, Circular No. 1*.
- Cole, C.V., Elliott, E.T., Hunt, H.W. & Coleman, D.C. (1978). Trophic interactions in soils as they affect energy and nutrient dynamics. V. Phosphorus transformations. *Microbial Ecology*, 4, 381–387.
- Coleman, D.C., Cole, C.V., Anderson, R.V., Blaha, M., Champion, M.K., Clarholm, M., Elliott, E.T., Hunt, H.W., Shaefer, B. & Sinclair, J. (1977). An analysis of rhizosphere-saprophage interactions in terrestrial ecosystems. *Soil organisms as components of ecosystems* (Ed. by U. Lohm & T. Persson), pp. 299–309. Ecological Bulletins, Swedish Natural Science research Council, Stockholm.
- Cooke, R.C. & Pramer, D. (1968). Interactions of *Aphelenchus avenae* and some nematode-trapping fungi in dual culture. *Phytopathology*, 58, 659–661.
- Cooke, R.C. & Rayner, A.D.M. (1984). *Ecology of saprotrophic fungi*. Longman, London.
- Cooper, K.M. & Grandison, G.S. (1986). Interaction of vesicular-arbuscular mycorrhizal fungi and root-knot nematode on cultivars of tomato and white clover susceptible to *Meloidogyne hapla*. *Annals of Applied Biology*, 108, 555–565.
- Cooper, K.M. & Grandison, G.S. (1987). Effects of vesicular-arbuscular mycorrhizal fungi on infection of tamarillo (*Cyphomandra betacea*) by *Meloidogyne incognita* in fumigated soil. *Plant Disease*, 71, 1101–1106.

- Crawley, M.J. (1983). *Herbivory: The dynamics of animal-plant interactions*. Blackwell Scientific, Oxford.
- Crompton, A. & Matthews, B. (1970). *Soils of the Leeds district*. Sheet 70, pp. 81-82 & 144. *Memoirs of the Soil Survey of Great Britain (England and Wales)*, Harpenden.
- Curl, E.A. (1982). The rhizosphere: Relation to pathogen behavior and root disease. *Plant Disease*, 66, 624-630.
- Curl, E.A. & Truelove, B. (1986). *The rhizosphere*. Springer-Verlag, Berlin.
- Curl, E.A. (1988). The role of soil microfauna in plant-disease suppression. *CRC Critical Reviews in Plant Sciences*, 7, 175-196.
- Daft, M.J. & Hogarth, B.G. (1983). Competitive interactions amongst four species of *Glomus* on maize and onion. *Transactions of the British Mycological Society*, 80, 339-345.
- Dighton, J., Thomas E.D. & Latter, P.M. (1987). Interactions between tree roots, mycorrhizas, a saprotrophic fungus and the decomposition of organic substrates in a microcosm. *Biology and Fertility of Soils*, 4, 145-150.
- Doncaster, C.C. (1966). Nematode feeding mechanisms. 2. Observations on *Ditylenchus destructor* and *D. myceliophagus* feeding on *Botrytis cinerea*. *Nematologica*, 12, 417-427.
- Elliott, J. M. (1971). *Some methods for the statistical analysis of samples of benthic invertebrates*. Freshwater Biological Association, Scientific Publications no. 25, Ambleside.
- Elmiligy, I.A. & de Grisse, A. (1970). Effect of extraction technique and adding fixative to soil before storing on recovery of plant-parasitic nematodes. *Nematologica*, 16, 353-358.
- Evans, A.A.F. & Fisher, J.M. (1969). Development and structure of populations of *Ditylenchus myceliophagus* as affected by temperature. *Nematologica*, 15, 395-402.
- Evans, A.A.F. & Fisher, J.M. (1970). Some factors affecting the number and size of nematodes in populations of *Aphelenchus avenae*. *Nematologica*, 16, 295-304.

- Faulkner, L.R. & Darling, H.M. (1961). Pathological histology, hosts, and culture of the potato rot nematode. *Phytopathology*, 51, 778–786.
- Finlay, R.D. (1985). Interactions between soil micro-arthropods and endomycorrhizal associations of higher plants. *Ecological interactions in soil: plants, microbes and animals* (Ed. by A.H. Fitter), pp.319–331. Blackwell Scientific Publications, Oxford.
- Fisher, J.M. & Evans, A.A.F. (1967). Penetration and feeding by *Aphelenchus avenae*. *Nematologica*, 13, 425–428.
- Fitter, A.H. (Ed.) (1985). *Ecological interactions in soil: plants, microbes and animals*. Blackwell Scientific Publications, Oxford.
- Flegg, J.J. (1967). Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Annals of Applied Biology*, 60, 429–437.
- Foster, J. W. (1949). *Chemical activities of fungi*. Academic Press, New York.
- Fox, J.A. & Spasoff, L. (1972). Interaction of *Heterodera solanacearum* and *Endogone gigantea* on tobacco. *Journal of Nematology*, 4, 224–225.
- Freckman, D.W. (Ed.) (1982a). *Nematodes in soil ecosystems*. University of Texas Press, Austin.
- Freckman, D.W. (1982b). Parameters of the nematode contribution to ecosystems. *Nematodes in soil ecosystems* (Ed. by D.W. Freckman), pp. 81–97. University of Texas Press, Austin.
- Freckman, D.W., Cromack Jr., K. & Wallwork, J.A. (1986). Recent advances in quantitative soil biology. *Microfloral and faunal interactions in natural and agro-ecosystems* (Ed. by M.J. Mitchell & J.P. Nakas), pp. 399–442. Martinus Nijhoff, Dordrecht.
- Fulton, N.D. & Hanson, E.W. (1960). Studies on root rots of red clover in Wisconsin. *Phytopathology*, 50, 541–550.
- Garcia-Garrido J.M. & Ocampo, J.A. (1988). Interaction between *Glomus mosseae* and *Erwinia carotovora* and its effects on the growth of tomato plants. *New Phytologist*, 110, 551–555.

- Gerdemann, J.W. (1964). The effect of mycorrhiza on the growth of maize. *Mycologia*, 56, 342–349.
- Germani, G., Ollivier, B. & Diem, H.G. (1981). Interaction of *Scutellonema cavenessi* and *Glomus mosseae* on growth and N₂ fixation of soybean. *Revue de Nematologie*, 4, 277–280.
- Giannakis, N. & Sanders, F.E. (1990). Interactions between mycophagous nematodes, mycorrhizal and other soil fungi. *Agriculture, Ecosystems and the Environment*, 29, 163–167.
- Giovannetti, M. & Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, 84, 489–500.
- Golden, J.K. & Van Gundy, S.D. (1975). A disease complex of okra and tomato involving the nematode, *Meloidogyne incognita*, and the soil-inhabiting fungus, *Rhizoctonia solani*. *Phytopathology*, 65, 265–273.
- Goodell, P.B. (1982). Soil sampling and processing for detection and quantification of nematode populations for ecological studies. *Nematodes in soil ecosystems* (Ed. by D.W. Freckman), pp. 178–198. University of Texas Press, Austin.
- Goodey, J.B. (1960). Observations on the effects of the parasitic nematodes *Ditylenchus myceliophagus*, *Aphelenchoides composticola* and *Paraphelenchus myceliophthorus* on the growth and cropping of mushrooms. *Annals of Applied Biology*, 48, 655–664.
- Goodey, J.B. (1963). *Soil and freshwater nematodes*. Methuen & Co., London.
- Graham, J.H., Linderman, R.G. & Menge, J.A. (1982). Development of external hyphae by different isolates of mycorrhizal *Glomus* spp. in relation to root colonization and growth of troyer citrange. *New Phytologist*, 91, 183–189.
- Griffin, G.D. & Thyr, B.D. (1988). Interaction of *Meloidogyne hapla* and *Fusarium oxysporum* f. sp. *medicaginis* on alfalfa. *Phytopathology*, 78, 421–425.
- Gupta, M.C. (1986). Biological control of *Fusarium moniliforme* Sheldon and *Pythium butleri* Subramaniam by *Aphelenchus avenae* Bastian in chitin and cellulose-amended soils. *Soil Biology and Biochemistry*, 18, 327–329.

- Hale, K.A. & Sanders, F.E. (1982). Effects of benomyl on vesicular-arbuscular mycorrhizal infection of red clover (*Trifolium pratense* L.) and consequences for phosphorus inflow. *Journal of Plant Nutrition*, 5, 1355-1367.
- Hall, I.R. (1988). Potential for exploiting vesicular-arbuscular mycorrhizas in agriculture. *Biotechnology in agriculture* (Ed. by A. Mizrahi), pp. 141-174. A.R. Liss, New York.
- Harborne, J.B. (1976). Functions of flavonoids in plants. *Chemistry and biochemistry of plant pigments* Vol. I (Ed. by T.W. Goodwin), pp. 736-778. Academic Press, London
- Harley, J.L. & Smith, S.E. (1983). *Mycorrhizal symbiosis*. Academic Press, London.
- Harrison, J.M. & Green, C.D. (1976). Comparison of centrifugal and other methods for standardization of extraction of nematodes from soil. *Annals of Applied Biology*, 82, 299-308.
- Hayman, D.S. (1978). Endomycorrhizae. *Interactions between non-pathogenic soil-organisms and plants* (Y.R. Dommergues & S.V. Krupa), pp. 401-442. Elsevier Scientific Publications, Amsterdam.
- Heald, C.M., Bruton, B.D. & Davis, R.M. (1989). Influence of *Glomus intraradices* and soil phosphorus on *Meloidogyne incognita* infecting *Cucumis melo*. *Journal of Nematology*, 21, 69-73.
- Henderson, V.E. & Katznelson, H. (1961). The effect of plant roots on the nematode population of the soil. *Canadian Journal of Microbiology*, 7, 163-167.
- Hepper, C.M. (1981). Techniques for studying the infection of plants by vesicular-arbuscular mycorrhizal fungi under axenic conditions. *New Phytologist*, 88, 641-647.
- Hepper, C.M. (1983). Limited independent growth of a vesicular-arbuscular mycorrhizal fungus *in vitro*. *New Phytologist*, 93, 537-542.
- Hepper, C.M. & Warner, A. (1983). Role of organic matter in growth of a vesicular-arbuscular mycorrhizal fungus in soil. *Transactions of the British Mycological Society*, 81, 155-156.

- Hesling, J.J. (1974). *Ditylenchus myceliophagus*. C.I.H. Descriptions of Plant-parasitic Nematodes, Set 3, No. 36.
- Hesling, J.J. (1977). *Aphelenchoides composticola*. C.I.H. Descriptions of Plant-parasitic Nematodes, Set 7, No. 92.
- Hetrick, B.A.D., Kitt, D.G. & Wilson, G.T. (1986). The influence of phosphorus fertilization, drought, fungal species, and nonsterile soil on mycorrhizal growth response in tall grass prairie plants. *Canadian Journal of Botany*, 64, 1199–1203.
- Hetrick, B.A.D., Wilson, G.T. & Kitt, D.G. (1988). Effects of soil microorganisms on mycorrhizal contribution to growth of big bluestem grass in non-sterile soil. *Soil Biology and Biochemistry*, 20, 501–507.
- Hetrick, B.A.D. (1989). Acquisition of phosphorus by VA mycorrhizal fungi and the growth responses of their host plants. *Nitrogen, phosphorus and sulphur utilization by fungi* (Ed. by L. Boddy, R. Marchant & D.J. Read), pp. 205–226. Cambridge University Press, Cambridge.
- Hirschmann, H. (1962). The life cycle of *Ditylenchus triformis* (Nematoda: Tylenchida) with emphasis on post-embryonic development. *Proceedings of the Helminthological Society of Washington*, 29, 30–43.
- Hong, C.P. & Estey, R.H. (1985). Control of the damping-off disease of pea by *Aphelenchus avenae*. *Indian Journal of Nematology*, 15, 1–4.
- Hooper, D.J. (1973). *Ditylenchus destructor*. C.I.H. Descriptions of Plant-parasitic Nematodes, Set 2, No. 21.
- Hooper, D.J. (1974). *Aphelenchus avenae*. C.I.H. Descriptions of Plant-parasitic Nematodes, Set 4, No. 50.
- Hooper, D.J. (1975). *Aphelenchoides blastophthorus*. C.I.H. Descriptions of Plant-parasitic Nematodes, Set 5, No. 73.
- Hooper, D.J. (1986). Extraction of free-living stages from soil. *Laboratory methods for work with plant and soil nematodes*. Reference book 402 (Ed. by J.F. Southey), pp. 5–30. HMSO, London.

- Hussey, R.S. & McGuire, J.M. (1987). Interactions with other organisms. *Principles and practice of nematode control in crops* (Ed. by R.H. Brown & B.R. Kerry), pp. 293–328. Academic Press, Marrickville Australia.
- Hussey, R.S. & Roncadori, R.W. (1978). Interaction of *Pratylenchus brachyurus* and *Gigaspora margarita* on cotton. *Journal of Nematology*, 10, 16–20.
- Hussey, R.S. & Roncadori, R.W. (1981). Influence of *Aphelenchus avenae* on vesicular–arbuscular endomycorrhizal growth response in cotton. *Journal of Nematology*, 13, 48–52.
- Hussey, R.S. & Roncadori, R.W. (1982). Vesicular–arbuscular mycorrhizae may limit nematode activity and improve plant growth. *Plant Disease*, 66, 9–14.
- Incoll, L.D., Long, S.P. & Ashmore, M.R. (1977). SI units in publications in plant science. Commentary in Plant Sciences No. 28. *Current Advances in Plant Science*. 331–343.
- Ingham, E.R., Trofymow, J.A., Ames, R.N., Hunt, H.W., Morley, C.R., Moore, J.C. & Coleman, D.C. (1986a). Trophic interactions and nitrogen cycling in a semi–arid grassland soil. I. Seasonal dynamics of the natural populations, their interactions and effects on nitrogen cycling. *Journal of Applied Ecology*, 23, 597–614.
- Ingham, E.R., Trofymow, J.A., Ames, R.N., Hunt, H.W., Morley, C.R., Moore, J.C. & Coleman, D.C. (1986b). Trophic interactions and nitrogen cycling in a semi–arid grassland soil. II. System responses to removal of different groups of soil microbes or fauna. *Journal of Applied Ecology*, 23, 615–630.
- Ingham, R.E., Anderson, R.V., Gould W.D. & Coleman D.C. (1985a). Vertical distribution of nematodes in a short grass prairie. *Pedobiologia*, 28, 155–160.
- Ingham, R.E., Trofymow, J.A., Ingham, E.R. & Coleman, D.C. (1985b). Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. *Ecological Monographs*, 55, 119–140.
- Ingham, R.E. (1988). Interactions between nematodes and vesicular–arbuscular mycorrhizae. *Agriculture, Ecosystems and Environment*, 24, 169–182.

- Inserra, R.N., Di Vito, M., Vovlas, N. & Seinhorst, J.W. (1983). Relationship between *Paratrichodorus* sp. density, and growth of wheat in pots. *Journal of Nematology*, 15, 79–87.
- Jakobsen, I. & Andersen, A.J. (1982). Vesicular–arbuscular mycorrhiza and growth in barley: effects of irradiation and heating of soil. *Soil Biology and Biochemistry*, 14, 171–178.
- Jansson, H–B. & Nordbring–Hertz, B. (1980). Interactions between nematophagous fungi and plant–parasitic nematodes: attraction, induction of trap formation and capture. *Nematologica*, 26, 383–389.
- Jansson, H–B. (1982). Attraction of nematodes to endoparasitic nematophagous fungi. *Transactions of the British Mycological Society*, 79, 25–29.
- Jensen, V., Kjoller, A. & Sorensen, L.H. (Eds) (1986). *Microbial communities in soil*. Elsevier Applied Science Publishers, Barking Essex.
- Kerr, A. & Vythilingam, M.K. (1966). Factors influencing the extraction of nematodes from soil. *Nematologica*, 12, 511–517.
- Kimpinski, J. & Welch, H.E. (1971a). The ecology of nematodes in Manitoba soils. *Nematologica*, 17, 308–318.
- Kimpinski, J. & Welch, H.E. (1971b). Comparison of Baermann funnel and sugar flotation extraction from compacted and non–compacted soils. *Nematologica*, 17, 319–324.
- Klekowski, R.Z., Wasilewska, L., & Paplinska, E. (1972). Oxygen consumption by soil–inhabiting nematodes. *Nematologica*, 18, 391–403.
- Klink, J.W. & Barker, K.R. (1968). Effect of *Aphelenchus avenae* on the survival and pathogenic activity of root–rotting fungi. *Phytopathology*, 58, 228–232.
- Kondrollochis, M. (1977). The effect of age of fungus on the development of populations of *Aphelenchus avenae* and *Ditylenchus myceliophagus*. *Nematologica*, 23, 260–272.
- Kotcon, J.B., Bird, G.W., Rose, L.M. & Dimoff, K. (1985). Influence of *Glomus fasciculatum* and *Meloidogyne hapla* on *Allium cepa* in organic soils. *Journal of Nematology*, 17, 55–60.

- Kruckelmann, H.W. (1975). Effects of fertilizers, soils, soil tillage, and plant species on the frequency of *Endogone* chlamydospores and mycorrhizal infection in arable soils. *Endomycorrhizas* (Ed. by F.E. Sanders, B. Mosse & P.B. Tinker), pp. 511–525. Academic Press, London.
- Krupa, Y.R. & Dommergues, Y.R. (1979). *Ecology of root pathogens*. Elsevier Scientific Publishing Company, Amsterdam.
- Kucey, R.M.N. & Paul, E.A. (1982). Biomass of mycorrhizal fungi associated with bean roots. *Soil Biology and Biochemistry*, 14, 413–414.
- Kukor, J.J. & Martin, M.M. (1987). Nutritional ecology of fungus-feeding arthropods. *Nutritional ecology of insects, mites, spiders and related invertebrates* (Ed. by F. Slansky Jr. & J.G. Rodriguez), pp. 791–814. John Wiley & Sons, New York.
- Lynch, J.M. (1983). *Soil biotechnology, microbiological factors in crop productivity*. Blackwell Scientific Publications, Oxford.
- Lynch, J.M. (1985). Microbial saprophytic activity on straw and other residues: consequences for the plant. *Ecological interactions in soil: Plants, Microbes and Animals* (Ed. by A.H. Fitter), pp. 181–191. Blackwell Scientific Publications, Oxford.
- MAFF (1986). *The analysis of agricultural materials*, Reference book 427. HMSO, London.
- Magnusson, C. (1983a). Abundance and trophic structure of pine forest nematodes in relation to soil layers and ground cover. *Holarctic Ecology*, 6, 175–182.
- Magnusson, C. (1983b). Abundance, distribution and feeding relations of root fungal feeding nematodes in a Scotch pine forest *Pinus sylvestris*. *Holarctic Ecology*, 6, 183–193.
- Mangenot, F. & Diem H.G. (1979). Fundamentals of biological control. *Ecology of root pathogens* (Ed. by S.V. Krupa & Y.R. Dommergues), pp. 207–265. Elsevier Scientific Publishing Company, Amsterdam.
- Manjunath, A., Mohan, R. & Bagyaraj, D.J. (1981). Interaction between *Beijerinckia mobilis*, *Aspegillus niger* and their effects on growth of onion. *New Phytologist*, 87, 723–727.

- Mankau, R. (1962). The effect of some organic additives upon a soil nematode population and associated natural enemies. *Nematologica*, 7, 65–73.
- Mankau, R. & Mankau, S.K. (1963). The role of mycophagous nematodes in the soil. I. The relationships of *Aphelenchus avenae* to phytopathogenic soil fungi. *Soil Organisms* (Ed. by J. Doeksen J. and J. Van der Drift), pp. 271–280. North Holland Publishing Company, Amsterdam.
- Mankau, R. (1969). Toxicity of culture filtrates of *Aspergillus niger* to the mycophagous nematode, *Aphelenchus avenae*. *Phytopathology*, 59, 13.
- Mankau, R. & Sitanath, D. (1969). The influence of chitin amendments on *Meloidogyne incognita*. *Journal of Nematology*, 1, 15–16.
- Marsh, B.a'B. (1971). Measurement of length in random arrangements of lines. *Journal of Applied Ecology*, 8, 265–267.
- McSorley, R. & Parrado, J.L. (1981). Effect of sieve size on nematode extraction efficiency. *Nematropica*, 11, 165–174.
- McSorley, R. (1987). Extraction of nematodes and sampling methods. *Principles and Practice of Nematode Control in Crops* (Ed. by R.H. Brown & B.R. Kerry), pp.13–47. Academic Press, Marrickville Australia.
- Menge, J.A., Johnson, E.L.V. & Minassian, V. (1979). Effect of heat treatment and three pesticides upon the growth and reproduction of the mycorrhizal fungus *Glomus fasciculatus*. *New Phytologist*, 82, 473–480.
- Minagawa, N. (1979). Efficiencies of two methods for extracting nematodes from soil. *Applied Entomology and Zoology*, 14, 469–477.
- Minz, G. (1957). Free-living plant-parasitic and possible plant-parasitic nematodes in Israel. *Plant Disease Reporter*, 41, 92–94.
- Mitchell, M.J. & Nakas, J.P.(eds) (1986) *Microfloral and faunal interactions in natural and agro-ecosystems*. Martinus Nijhoff, Dordrecht.
- Moore, J.C., St.John, T.V. & Coleman, D.C. (1985). Ingestion of vesicular-arbuscular mycorrhizal hyphae and spores by soil microarthropods. *Ecology*, 66, 1979–1981.

- Moore, P.D. (1987). Distribution of mycorrhiza throughout the British flora. *Nature*, **327**, 100.
- Morandi, D., Bailey, J.A. & Gianinazzi-Pearson, V. (1984). Isoflavonoid accumulation in soybean roots infected with vesicular-arbuscular mycorrhizal fungi. *Physiological Plant Pathology*, **24**, 357-364.
- Morandi, D. & Gianinazzi-Pearson, V. (1986). Influence of mycorrhizal infection and phosphate nutrition on secondary metabolite contents of soybean roots. *Physiological and genetical aspects of mycorrhizae* (Ed. by V. Gianinazzi-Pearson & S. Gianinazzi), pp. 787-791. INRA, Paris.
- Mosse, B. (1959). The regular germination of resting spores and some observations on the growth requirements of an *Endogone* sp. causing vesicular-arbuscular mycorrhiza. *Transactions of the British Mycological Society*, **42**, 273-286.
- Mosse, B. (1973). Plant growth responses to vesicular-arbuscular mycorrhiza. IV. In soil given additional phosphate. *New Phytologist*, **72**, 127-136.
- Mosse, B. (1975). Specificity in VA mycorrhizas. *Endomycorrhizas* (Ed. by F.E. Sanders, B. Mosse & P.B. Tinker), pp. 469-484. Academic Press, London.
- Newman, E.I. (1966). A method of estimating the total length of root in a sample. *Journal of Applied Ecology*, **3**, 139-145.
- Nicholas, W.L. (1984). *The biology of free-living nematodes*. Clarendon Press, Oxford.
- Nickle, W.R. & McIntosh, P. (1968). Studies on the feeding and reproduction of seven mycophagous nematodes on *Rhizoctonia*, *Fusarium*, and *Verticillium*. *Nematologica*, **14**, 11-12.
- Nickle, W.R., (Ed.) (1984). *Plant and insect nematodes*. Marcel Dekker Inc., New York.
- Nicolson, T.H. (1959). Mycorrhiza in the Gramineae. I. Vesicular-arbuscular endophytes, with special reference to external phase. *Transactions of the British Mycological Society*, **42**, 421-438.
- O'Bannon, J.H., Inserra, R.N., Nemecek, S. & Vovlas, N. (1979). The influence of *Glomus mosseae* on *Tylenchulus semipenetrans* -infected and uninfected *Citrus limon* seedlings. *Journal of Nematology*, **11**, 247-250.

- O'Bannon, J.H. & Nemeč, S. (1979). The response of *Citrus limon* seedlings to a symbiont, *Glomus etunicatus*, and a pathogen, *Radopholus similis*. *Journal of Nematology*, 11, 270–275.
- Ocampo, J.A. & Hayman, D.S. (1980). Effects of pesticides on mycorrhiza in field-grown barley, maize and potatoes. *Transactions of the British Mycological Society*, 74, 413–416.
- Ocampo, J.A. & Hayman, D.S. (1981). Influence of plant interactions on vesicular–arbuscular mycorrhizal infections. II. Crop rotations and residual effects of non–host plants. *New Phytologist*, 87, 333–343.
- Oostenbrink, M. (1960). Estimating nematode populations by some selected methods. *Nematology* (Ed. by J.N. Sasser & W.R. Jenkins), pp. 85–102. Chapel Hill, University of North Carolina Press.
- Oostenbrink, M. (1971). Comparison of techniques for population estimation of soil and plant nematodes. *Methods of study in quantitative soil ecology: population, production and energy flow* (Ed. by J. Phillipson), pp. 72–82. Blackwell Scientific Publications, Oxford.
- Peters, B.G. (1952). Toxicity tests with vinegar eelworm. I. *Journal of Helminthology*, 26, 97–110.
- Phillips, J.M. & Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55, 158–160.
- Pillai, J.K. & Taylor, D.P. (1967a). Effect of temperature on the time required for hatching and duration of life cycle of five mycophagous nematodes. *Nematologica*, 13, 512–516.
- Pillai, J.K. & Taylor, D.P. (1967b). Influence of fungi on host preference, host suitability, and morphometrics of five mycophagous nematodes. *Nematologica*, 13, 529–540.
- Pitcher, R.S. (1978). Interactions of nematodes with other pathogens. *Plant nematology* (Ed. by J.F. Southey), pp. 63–77. HMSO, London.
- Powell, N.T. (1971). Interactions between nematodes and fungi in disease complexes. *Annual Review of Phytopathology*, 9, 253–274.

- Procter, D.L.C. (1977). Nematode densities and production on Truelove Lowland. *Truelove Lowland, Devon Island Canada, a high arctic ecosystem* (Ed. by L.C. Bliss), pp. 347–361. University of Alberta Press, Edmonton.
- Raj, J., Bagyaraj, D.J. & Manjunath, A. (1981). Influence of soil inoculation with vesicular–arbuscular mycorrhiza and a phosphate–dissolving bacterium on plant growth and ^{32}P –uptake. *Soil Biology and Biochemistry*, **13**, 105–108.
- Rhoades, H. L. & Linford, M. B. (1959). Control of *Pythium* root rot by the nematode *Aphelenchus avenae*. *Plant Disease Reporter*, **43**, 323–328.
- Rich, J.R. & Bird, G.W. (1974). Association of early–season vesicular–arbuscular mycorrhizae with increased growth and development of cotton. *Phytopathology*, **64**, 1421–1425.
- Rich, J.R., Keen, N.T. & Thomason, I.J. (1977). Association of coumestans with the hypersensitivity of Lima bean roots to *Pratylenchus scribneri*. *Physiological Plant Pathology*, **10**, 105–116.
- Riffle, J.W. (1971). Effect of nematodes on root–inhabiting fungi. *Mycorrhizae* (Ed. by E. Hacskeylo), pp. 97–113. U.S Department. of Agriculture, Washington D.C.
- Riffle, J.W. (1973). Effect of two mycophagous nematodes on *Armillaria mellea* root rot of *Pinus ponderosa* seedlings. *Plant Disease Reporter*, **57**, 355–357
- Riffle, J.W. (1975). Two *Aphelenchoides* species suppress formation of *Suillus granulatus* ectomycorrhizae with *Pinus ponderosa* seedlings. *Plant Disease Reporter*, **59**, 951–955.
- Roessner, J. & Urland, K. (1983). Mycophagous nematodes of the genus *Aphelenchoides* on the stem base of cereals and their effect against foot rot organisms. *Nematologica*, **29**, 454–462.
- Roncadori, R.W. & Hussey, R.S. (1977). Interaction of the endomycorrhizal fungus *Gigaspora margarita* and root–knot nematode on cotton. *Phytopathology*, **67**, 1507–1511.
- Ross, J.P. & Ruttencutter, R. (1977). Population dynamics of two vesicular–arbuscular endomycorrhizal fungi and the role of hyperparasitic fungi. *Phytopathology*, **67**, 490–496.

- Ross, J.P. (1980). Effect of nontreated field soil on sporulation of vesicular-arbuscular mycorrhizal fungi associated with soybean. *Phytopathology*, 70, 1200–1205.
- Rovira, A.D. & Bowen, G.D. (1966). The effects of micro-organisms upon plant growth. II. Detoxication of heat-sterilized soils by fungi and bacteria. *Plant and Soil*, 25, 129–142.
- Roy, A.K. (1973). Effects of *Aphelenchus avenae* on tomato plants infected with *Rhizoctonia solani* and *Colletotrichum coccodes*. *Zeitschrift für Pflanzenkrankheiten Pflanzenpathologie und Pflanzenschutz*, 80, 23–33.
- Ruehle, J.L. (1973). Nematodes and forest trees – types of damage to tree roots. *Annual Review of Phytopathology*, 11, 99–118.
- Rufelt, S. (1987). A threshold value for cutting intensity in relation to *Fusarium* root rot of red clover (*Trifolium pratense* L.). *Journal of Phytopathology*, 118, 301–305.
- Salawu, E.O. & Estey, R.H. (1979). Observations on the relationships between a vesicular-arbuscular fungus, a fungivorous nematode, and the growth of soybeans. *Phytoprotection*, 60, 99–102.
- Saleh, H. & Sikora, R.A. (1984). Relationship between *Glomus fasciculatum* root colonization of cotton and its effect on *Meloidogyne incognita*. *Nematologica*, 30, 230–237.
- Sanders, F.E. & Tinker, P.B. (1973). Phosphate flow into mycorrhizal roots. *Pesticide Science*, 4, 385–395.
- Sanders, F.E. (1975). The effect of foliar-applied phosphate on the mycorrhizal infections of onion roots. *Endomycorrhizas* (Ed. by F.E. Sanders, B. Mosse & P.B.Tinker), pp. 261–276. Academic Press, London.
- Sanders, F.E., Tinker, P.B., Black, R.L.B. & Palmerley S.M. (1977). The development of endomycorrhizal root systems: I. Spread of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte. *New Phytologist*, 78, 257–268.
- Sanders, F.E. & Sheikh, N.A. (1983). The development of vesicular-arbuscular mycorrhizal infection in plant root systems. *Plant and Soil*, 71, 223–246.

- Sanders, F.E. (1986a). The role of mycorrhiza in plant nutrient uptake from soil. *Microbial communities in soil* (Ed. by V. Jensen, A. Kjoller & L.H. Sorensen), pp. 61–72. Elsevier Applied Science Publishers, Barking Essex.
- Sanders, F.E. (1986b). Quantitative approaches to the analysis of the development of mycorrhizal root systems. *Physiological and genetical aspects of mycorrhizae* (Ed. by V. Gianinazzi-Pearson & S. Gianinazzi), pp. 209–216. INRA, Paris.
- Schenck, N.C. & Kinloch, R.A. (1974). Pathogenic fungi, parasitic nematodes, and endomycorrhizal fungi associated with soybean roots in Florida. *Plant Disease Reporter*, 58, 169–173.
- Schenck, N.C., Kinloch, R.A. & Dickson, D.W. (1975). Interaction of endomycorrhizal fungi and root-knot nematode on soybean. *Endomycorrhizas* (Ed. by F.E. Sanders, B. Mosse & P.B. Tinker), pp. 607–617. Academic Press, London.
- Schenck N.C. (1981). Can mycorrhizae control root disease? *Plant disease*, 65, 230–234.
- Schubert, A. & Hayman, D.S. (1986). Plant growth responses to vesicular-arbuscular mycorrhiza. XVI. Effectiveness of different endophytes at different levels of soil phosphate. *New Phytologist*, 103, 79–90.
- Seinhorst J.W. (1956). The quantitative extraction of nematodes from soil. *Nematologica*, 1, 249–267.
- Seinhorst, J.W. (1962). Modifications of the elutriation method for extracting nematodes from soil. *Nematologica*, 8, 117–128.
- Shafer, S.R. Rhodes, L.H. & Riedel, R.M. (1981). *In-vitro* parasitism of endomycorrhizal fungi of ericaceous plants by the mycophagous nematode *Aphelenchoides bicaudatus*. *Mycologia*, 73, 141–149.
- Sheikh, N.A. (1984). *The dynamics of spread of vesicular-arbuscular mycorrhizal infection and temperature responses*. Ph.D. Thesis, University of Leeds.
- Sikora, R.A. & Schonbeck, F. (1975). Effect of vesicular-arbuscular mycorrhiza (*Endogone mosseae*) on the population dynamics of the root-knot nematodes (*Meloidogyne incognita*) and (*Meloidogyne hapla*). *Proceedings of the VIII International Plant Protection Congress*. Section V, pp. 158–166, Moscow.

- Sikora R.A. (1978). Effect of the endotrophic mycorrhizal fungus, *Glomus mosseae*, on the host-parasite relationship of *Meloidogyne incognita* in tomato. *Journal of Plant Diseases and Protection*, 85, 197-202.
- Sikora, R.A. (1979). Predisposition to *Meloidogyne* by the endotrophic mycorrhizal fungus *Glomus mosseae*. *Root-knot nematodes (Meloidogyne species). Systematics, biology and control* (Ed. by F. Lamberti & C.E. Taylor), pp. 399-404. Academic Press, London.
- Sitaramaiah, K. & Sikora, R.A. (1981). Influence of the endomycorrhizal fungus *Glomus mosseae* on *Rotylenchulus reniformis* penetration and development on bush bean, cucumber and muskmelon. *Mededelingen van de Faculteit Lanbouwwetenschappen, Rijksuniversiteit Gent*, 46, 695-702.
- Smart Jr., G.C., & Darling, H.M. (1963). Pathogenic variation and nutritional requirements of *Ditylenchus destructor*. *Phytopathology*, 53, 374-381.
- Smith, G.S., Roncadori, R.W. & Hussey, R.S. (1986). Interaction of endomycorrhizal fungi, superphosphate, and *Meloidogyne incognita* on cotton in microplot and field studies. *Journal of Nematology*, 18, 208-216.
- Smith, G.S., Hussey, R.S. & Roncadori, R.W. (1986). Penetration and postinfection development of *Meloidogyne incognita* on cotton as affected by *Glomus intraradices* and phosphorus. *Journal of Nematology*, 18, 429-435.
- Smith, G.S. (1988). The role of phosphorus nutrition in interactions of vesicular-arbuscular mycorrhizal fungi with soilborn nematodes and fungi. *Phytopathology*, 78, 371-374.
- Smith, G.S. & Kaplan, D.T. (1988). Influence of mycorrhizal fungus, phosphorus, and burrowing nematode interactions on growth of rough lemon citrus seedlings. *Journal of Nematology*, 20, 539-544.
- Smith, S.E., Nicholas, D.J.D. & Smith, F.A. (1979). Effect of early mycorrhizal infection on nodulation and nitrogen fixation in *Trifolium subterraneum* L.. *Australian Journal of Plant Physiology*, 6, 305-316.
- Sohlenius, B. (1980). Abundance, biomass and contribution to energy flow by soil nematodes in terrestrial ecosystems. *Oikos*, 34, 186-194.

- Sohlenius, B. & Bostrom, S. (1986). Short-term dynamics of nematode communities in arable soil—Influence of nitrogen fertilization in barley crops. *Pedobiologia*, 29, 183–191.
- Southey, J.F. (Ed.) (1978). *Plant nematology*. HMSO, London.
- Steiner, G. (1936). The status of the nematode *Aphelenchus avenae* Bastian, 1865, as a plant parasite. *Phytopathology*, 26, 294–295.
- Stinner, B.R. & Crossley Jr., D.A. (1982). Nematodes in no-tillage agroecosystems. *Nematodes in soil ecosystems* (Ed. by D.W. Freckman), pp. 14–28. University of Texas Press, Austin.
- St. John, T.V. & Coleman, D.C. (1983). The role of mycorrhizae in plant ecology. *Canadian Journal of Botany*, 61, 1005–1014.
- Strobel, N.E., Hussey R.S. & Roncadori, R.W. (1982). Interactions of vesicular–arbuscular mycorrhizae, *Meloidogyne incognita*, and soil fertility on peach. *Phytopathology*, 72, 690–694.
- Sutherland, J.R. & Fortin, J.A. (1968). Effect of the nematode *Aphelenchus avenae* on some ectotrophic, mycorrhizal fungi and on a red pine mycorrhizal relationship. *Phytopathology*, 58, 519–523.
- Sutcliffe, J.F. & Baker, D.A. (1981). *Plants and mineral salts*. Studies in Biology No. 48. Edward Arnold, London.
- Sylvia, D.M. & Schenck, N.C. (1983). Germination of chlamydospores of three *Glomus* species as affected by soil matric potential and fungal contamination. *Mycologia*, 75, 30–35.
- Sylvia, D.M. (1988). Activity of external hyphae of vesicular–arbuscular mycorrhizal fungi. *Soil Biology Biochemistry*, 20, 39–43.
- Tennant, D. (1975). Test of a modified line intersect method of estimating root length. *Journal of Ecology*, 63, 995–1001.
- Thomas, G.V., Sundararaju, P., Ali, S.S. & Ghai, S.K. (1989). Individual and interactive effects of VA mycorrhizal fungi and root-knot nematode, *Meloidogyne incognita*, on cardamom. *Tropical Agriculture*, 66, 21–24.

- Thomson Cason, K.M., Hussey, R.S. & Roncadori, R.W. (1983). Interaction of vesicular-arbuscular mycorrhizal fungi and phosphorus with *Meloidogyne incognita* on tomato. *Journal of Nematology*, 15, 410–417.
- Thorp, A. (1987). Final year project, Department of Plant Sciences, University of Leeds.
- Tinker, P.B. (1984). The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. *Plant and Soil*, 76, 77–91.
- Townshend, J.L. (1963). A modification and evaluation of the apparatus for the Oostenbrink direct cottonwool filter extraction method. *Nematologica*, 9, 106–110.
- Townshend, J.L. (1964). Fungus hosts of *Aphelenchus avenae* Bastian, 1865 and *Bursaphelenchus fungivorus* Franklin & Hooper, 1962 and their attractiveness to these nematode species. *Canadian Journal of Microbiology*, 10, 727–737.
- Trofymow, J.A. & Coleman, D.C. (1982). The role of bacterivorous and fungivorous nematodes in cellulose and chitin decomposition in the context of root/rhizosphere/soil conceptual model. *Nematodes in soil ecosystems* (Ed. by D.W. Freckman), pp. 117–138. University of Texas Press, Austin.
- Trofymow, J.A., Morley, C.R., Coleman, D.C. & Anderson, R.V. (1983). Mineralization of cellulose in the presence of chitin assemblages of microflora and fauna in soil. *Oecologia*, 60, 103–110.
- Viglierchio, D.R. & Schmitt, R.V. (1983a). On the methodology of nematode extraction from field samples: Baermann funnel modifications. *Journal of Nematology*, 15, 438–444.
- Viglierchio, D.R. & Schmitt, R.V. (1983b). On the methodology of nematode extraction from field samples: comparison of methods for soil extraction. *Journal of Nematology*, 15, 450–454.
- Walker, G.E. (1984a). Ecology of mycophagous nematode *Aphelenchus avenae* in wheat-field and pine-forest soils. *Plant and Soil*, 78, 417–428.
- Walker, G.E. (1984b). Feeding trials of *Aphelenchus avenae* on soil bacteria and actinomycetes. *Plant and Soil*, 78, 431–432.
- Warcup, J.H. (1957). Chemical and biological aspects of soil sterilization. *Soils and Fertilizers*, 20, 1–5.

- Warner, A. & Mosse, B. (1980). Independent spread of vesicular-arbuscular mycorrhizal fungi in soil. *Transactions of the British Mycological Society*, **74**, 407-446.
- Warner, A. (1984). Colonization of organic matter by vesicular-arbuscular mycorrhizal fungi. *Transactions of the British Mycological Society*, **82**, 352-354.
- Warnock, A.J., Fitter, A.H. & Usher, M.B. (1982). The influence of a springtail *Folsomia candida* (Insecta, Collembola) on the mycorrhizal association of leek *Allium porrum* and the vesicular-arbuscular mycorrhizal endophyte *Glomus fasciculatus*. *New Phytologist*, **90**, 285-292.
- Wasilewska, L., Jakubczyk, H. & Paplinska, E. (1975). Production of *Aphelenchus avenae* Bastian (Nematoda) and reduction of mycelium of saprophytic fungi by them. *Polish Ecological Studies*, **1**, 61-73.
- Wasilewska, L., Paplinska E. & Zielinski J. (1981). The role of nematodes in decomposition of plant material in a rye field. *Pedobiologia*, **21**, 182-191.
- Wasilewska, L. & Bienkowski, P. (1985). Experimental study on the occurrence and activity of soil nematodes in decomposition of plant material. *Pedobiologia*, **28**, 41-57.
- Watrud, L.S. (1982). Spore germination and axenic culture of endomycorrhizae. A. Spore germination of vesicular-arbuscular mycorrhizal fungi. *Methods and principles of mycorrhizal research* (Ed. by N.C. Schenck), pp. 81-83. The American Phytopathological Society, St. Paul Minnesota.
- Whitehead, A.G. & Hemming, J.R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, **55**, 25-38.
- Willard, J.R. (1972). *Soil invertebrates: 1. Methods of sampling and extraction*. Matador Project Technical Report 7, Saskatchewan.
- Willard, J.R. & Petrovich, M.S. (1972). A direct centrifugal-flotation method for extraction of nematodes from clay soils. *Plant Disease Reporter*, **56**, 808-810.
- Wilson, G.W.T., Hetrick, B.A.D. & Kitt, D.G. (1988). Suppression of mycorrhizal growth response of big bluestem by non-sterile soil. *Mycologia*, **80**, 338-343.

- Wilson, G.W.T., Hetrick, B.A.D. & Kitt, D.G. (1989). Suppression of vesicular-arbuscular mycorrhizal fungus spore germination by nonsterile soil. *Canadian Journal of Botany*, **67**, 18–23.
- Wood, F.H. (1973). Nematode feeding relationships. Feeding relationships of soil-dwelling nematodes. *Soil Biology and Biochemistry*, **5**, 593–601.
- Wyss, U. (1981). Ectoparasitic nematodes: feeding behaviour and plant cell responses. *Plant parasitic nematodes*, Vol. III (Ed. by B.M. Zuckerman & R.A. Rohde), pp. 325–351. Academic Press, New York.
- Yeates, G.W. (1971). Feeding types and feeding groups in plant and soil nematodes. *Pedobiologia*, **11**, 173–179.
- Yeates, G.W. (1972). Nematoda of a Danish beech forest. *Oikos*, **23**, 178–189.