Title Aspects of the population biology of the cyst nematode parasites of oilseed rape

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1988

A thesis submitted to the Council for National Academic Awards in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Luton College of Higher Education
(sponsoring establishment)

Rothamsted Experimental Station
(Collaborating establishment)

CONTENTS

ACKNOWLEDGEMENTS

For the duration of this project I have been indebted to many people without whose help the work would have been considerably more difficult. I would like to take this opportunity to record my gratitude for their assistance.

Dr G W Storey and Dr K Evans for their supervision, encouragement and constructive criticism throughout the course of the experimental work and in the preparation of the thesis.

Dr R N Perry and J Beane (R.E.S.) for their assistance with interference microscopy techniques and helpful discussions.

J N Perry (R.E.S.) for providing a copy and operating details of the nematode population model.

Many colleagues, too numerous to mention individually at L.C.H.E. Science Dept., R.E.S. Nematology Dept. and E.S.C.A. Crop Protection Dept. for their support and helpful discussions.

Thanks also go to the following establishments who donated seed samples N.I.A.B. (Cambridge), P.B.I. (Cambridge), Agriculture Canada (Saskatoon) and the Nickerson Research Centre.

In addition I would like to thank A Gibson (E.S.C.A.) for his advice and assistance in computing, Sandra Williams (L.C.H.E.) and Fiona Chrystal (E.S.C.A.) for typing the manuscript.

Appreciation is extended to Bedfordshire County Council for their financial support.

DECLARATION

While registered as a candidate for the degree of Doctor of Philosophy for the C.N.A.A., the author has not been a registered candidate for any other award of the C.N.A.A., or of any university or other establishment. No material contained in this thesis has been used in any other submission for an academic award

Signed .....(Candidate)

Signed .....(Director of Studies)

ABSTRACT

S A Bowen PhD Thesis 1988

ASPECTS OF THE POPULATION BIOLOGY OF THE CYST-NEMATODE PARASITES OF OILSEED RAPE

Investigation of the host-parasite relationship between oilseed rape, Heterodera cruciferae and H.schachtii has shown that the rate of hatching, development and reproduction is strongly influenced by temperature, two possible generations occurring on an autumn-sown crop. H.schachtii preferred warmer temperatures, hatched and reproduced more than H.cruciferae. Comparisons between newly-formed eggs in cysts and egg sacs showed that their different hatching responses were related to their physiology; cyst-bound eggs hatched poorly whereas juveniles hatched readily from egg sacs and facilitated the early establishment of a second generation.

Multiplication of both species varied greatly between cultivars and differences in hatching and multiplication were attributed to the effects of plant growth and intrinsic differences between cultivars. Plant age influenced the hatching activity of root diffusates and nematode development.

Multiplication rates of single and mixed species populations declined with increasing initial population density indicating that intraspecific competition and root damage limited population growth. Nematodes multiplied synergistically in concomitant infestations suggesting that interspecific competition was less important.

In a damage assessment test, root and shoot growth of nematode-infested plants was reduced and the increased accumulation of calcium in their shoots indicated that they used water less efficiently than uninfested plants. These effects were density-dependent and H.schachtii was more damaging than H.cruciferae. Tolerance to nematode attack was attributed to good root establishment.

The rate of decline of H.cruciferae populations varied with time, soil depth and between populations; low soil moisture and temperature favouring nematode survival. The role of weeds as 'maintainer hosts' of H.cruciferae was assessed but considered negligible.

Nematode population dynamics were simulated using a computer model. Population densities fluctuated considerably under typical crop rotations but large populations had generally declined to less damaging levels before a host was cropped again. It was indicated that a long run of non-hosts or nematicide use would achieve better control of H.schachtii than H.cruciferae.

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GENERAL INTRODUCTION

(i) History and agronomy of oilseed rape in Britain

Oilseed rape belongs to the genus Brassica, a genus which has both evolved and been developed into a great variety of plants including important crops such as cabbage (Brassica oleracea), cauliflower (B.oleracea var.) and turnip (B.campestris). Oilseed rape (B.napus) differs fundamentally from most of its related crop species in that it is not grown primarily for its root or leaf (as are turnip and cabbage), but for its seed. Another brassica which shares this distinction is black mustard (B.nigra). The seeds of oilseed rape plants are crushed and the oil extracted and refined for use in edible food products such as margarine and cooking oils. Additionally, the residue material from the extraction process is a rich source of protein and can be included in compound feed for livestock. Oilseed rape does not have a long history as a traditionally grown crop in Britain like many other brassica crops although it has been grown as a crop in Europe since the middle ages. Oilseed rape is recorded as being grown in parts of England in the early seventeenth and eighteenth century, in which period oilseed rape or coleseed was especially attractive as a pioneer crop in early land reclamation schemes in East Anglia. The oil was burnt in lamps and the residual "oil cake" used as feed for livestock (Bunting, 1984). By the nineteenth century oilseed rape is recorded as being grown as an autumn forage crop, being lightly grazed over winter and then left to yield an oilcrop the following summer (McNaughton, 1976). Towards the middle of this century farmers were beginning to grow oilseed rape as a break crop, especially on chalk land farms in intensive cereal production. During 1968 between 4000 and 6000 ha of oilseed rape were grown in England and Wales and the crop was worth approximately £50 per tonne. The majority of the crops were spring sown, usually grown one year in four or five in a spring-sown cereal rotation (Bunting, 1984). By 1973 the area of oilseed rape cultivation had expanded. Breeding had helped to increase yields and the merits of growing a commercial break crop were recognised by many farmers. However, the entry of Britain into the EEC at this time had the greatest effect on oilseed rape cultivation. A need to encourage the production of oilcrops was recognised in order to compete with the large amounts of imported high grade vegetable oil, mainly from American and South American soybeans. Oilseed rape was considered the most suitable crop and a price-support system was introduced to encourage farmers to grow it. This involved a gradual increase in the value of oilseed rape from

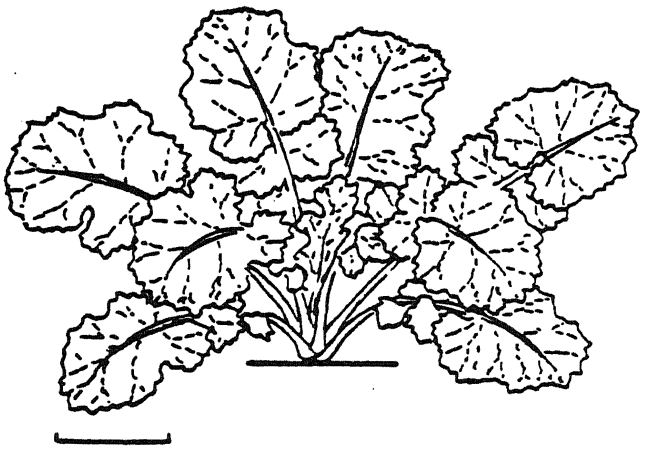
approximately £70 per tonne in 1973 to more than £250 per tonne in 1984. The success of the scheme is reflected in the rapid increase in the amount of oilseed rape grown in England and Wales. In 1973, 14,000 ha were grown; in 1978 64,000 ha were grown and in 1983 217,000 ha were grown (Bunting, 1984). The area of oilseed rape is expected to reach 300,000 ha by 1988 (Evans, 1984). Oilseed rape is now one of the most extensively grown non-cereal crops in Britain. Although there remains a need to import oil, oilseed rape has captured a large proportion of the high grade vegetable oil market and with further potential increases in the protein content of the residue for livestock feed oilseed rape is set to take a greater proportion of the market. The East Midlands and South East England are major areas of oilseed rape cultivation with a large amount of oilseed rape also grown in Humberside and East Anglia. The initial rapid expansion of oilseed rape cultivation involved spring-sown crops in preference to winter-sown crops. Winter rape crops were often late in being sown due to delays in the harvest of the previous cereal crop and later sowings resulted in winter crops returning smaller yields than spring-sown crops. The poor establishment of a late sown winter crop combined with severe pest damage has been found to depress yields (Mendham et al., 1981; Bunting, 1984). However, from about 1975 onwards many farmers began to grow winter barley which overcame the earlier rotational problems of getting rape crops established. Spring-sown rape crop yields were also being found to be very drought-sensitive especially in the dry summer of 1975. The trend towards winter oilseed rape reflected the trend towards winter cereals and currently in most areas the majority of the oilseed rape crop is autumn-sown. In some areas where there is a risk of damage over winter due to pests and diseases, oilseed rape is spring-sown. As well as providing a profitable crop for farmers, oilseed rape is also attractive as a break crop, especially on intensive cereal producing land. As the genetic base of oilseed rape is different from cereals it suffers from many different pests and diseases, the break from cereals thus preventing further increases in cereal pest and diseases. Oilseed rape provides a good entry for winter cereals due to its early harvest and in combination with a herbicide can reduce weed problems in the subsequently-sown cereal crop. Including rape in the rotation has been shown to increase yields of following cereal crops (Anon, 1985). However, land which has been cropped with brassicas in the preceding four years should not be sown with rape because of the danger of pests and disease build-up (Anon, 1983).

The rapid expansion in oilseed rape cultivation has increased the interest in breeding new cultivars of oilseed rape. Initial difficulties concerned the health implications of certain constituents of the seed (erucic acid), for both humans and animals. Fortunately, plant breeders were able to develop cultivars with a low erucic acid content in the seed and this was an important step influencing the initial expansion of the crop (Kimber, 1984). Cultivars are selected for seed yield, oil content and quality and early maturity as well as resistance to disease, lodging and pod shattering. Many of these characteristics must conform to stringent standards set by the EEC before intervention prices are paid and this helps to ensure the production of good quality edible rape oil that can successfully compete with imported oil. All the currently recommended cultivars are low in erucic acid (Anon, 1986) whilst plant breeders are now developing cultivars with a low glucosinolate content as well. In 1984, the most extensively grown cultivar was the French bred Jet Neuf although the cultivar Bienvenu has occupied a greater acreage since.

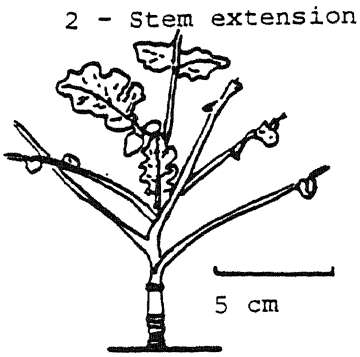
An autumn-sown crop of oilseed rape is usually sown between mid-August and the first week of September. Early sowings are desirable as the plants have more time to establish a good root system before the low winter soil temperatures halt growth. The initial stage of growth involves the production of leaves and the development of an extensive root system. In spring, plants begin to grow rapidly when the soil temperatures increase passing through the "stem elongation" phase of growth. A spring application of fertiliser ensures good growth at this stage and increases later yields. Plants begin to produce their characteristic yellow flowers in May and June after which, pods containing the seed are formed. The crop is harvested in late July or early August. A growth stage key has been proposed for oilseed rape (Sylvester-Bradley and Makepeace, 1984), the stages of which are shown in Figure 1.

The most prevalent diseases of oilseed rape are Alternaria leaf and pod spot (mainly Alternaria brassicae) and canker (Leptosphaeria maculans). Light leaf spot (Pyrenopeziza brassicae) and two soil-borne diseases, stem-rot (Sclerotinia sclerotiorum) and clubroot (Plasmodiophora brassicae) occasionally cause significant yield losses. The most widespread insect pests of oilseed rape are pollen (blossom) beetle (Meligethes spp.), seed weevil (Ceutorhynchus

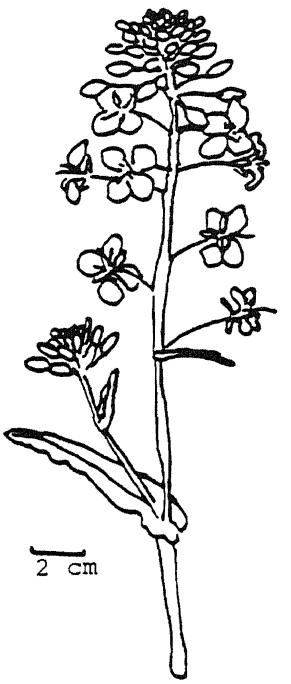
Figure 1. Principal growth stages of oilseed rape (Brassica napus L.)
(After Sylvester-Bradley & Makepeace, 1984)



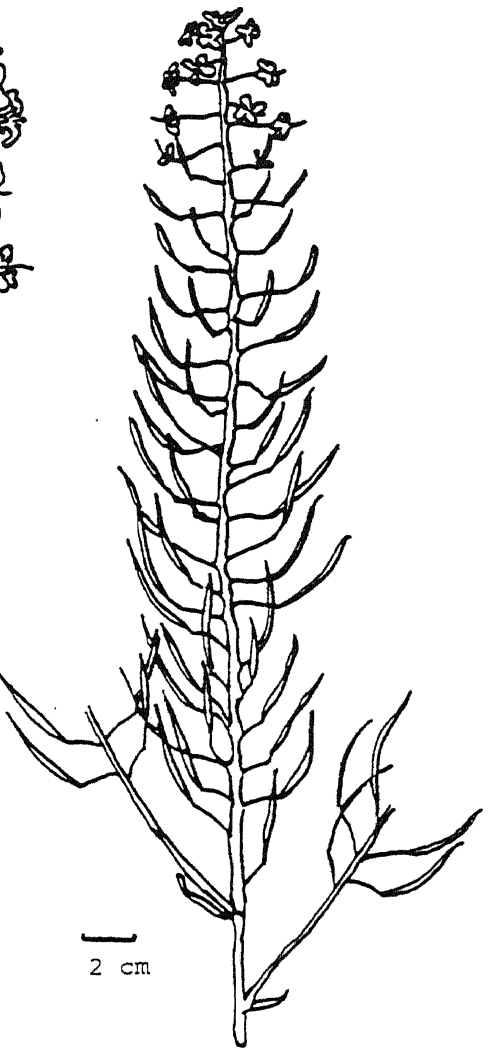
1 - Leaf production



2 - Stem extension



4 - Flowering



5 - Pod development

Principal Stages and Codes

0 - Germination and emergence
1 - Leaf production
2 - Stem extension
3 - Flower bud development
4 - Flowering
5 - Pod development
6 - Seed development

assimilis) and pod midge (Dasineura brassicae). The cabbage stem flea beetle (Psylliodes chrysocephala) is a locally important pest.

(ii) Nematode pests of oilseed rape

The cyst-forming nematodes of the genera Heterodera and Globodera are primarily root parasites and a distinguishing feature of these nematodes is the enlargement of the female body to assume a characteristic lemon or spherical shape. The males are free-living whilst the females are sedentary in or attached to the root. After the death of the female the body wall tans to form a tough protective cyst enclosing a large number of eggs in which infective second stage juveniles may remain dormant for many years.

Most crops grown in Britain can be parasitised by at least one species of cyst nematode. The potato cyst nematodes Globodera rostochiensis (Wollenweber), and G.pallida (Stone) are the most economically important (Evans, 1984). The sugar beet cyst nematode Heterodera schachtii (Schmidt) and the cereal cyst nematode Heterodera avenae (Wollenweber) are also important pests but damage by the former has been reduced by enforced rotational restrictions on the growing of host crops (Whiteway et al., 1982) and by the latter by natural control fungal control agents in the soil which parasitise the developing female (Kerry & Crump, 1977).

An examination of the host ranges of cyst nematodes (Jones, 1950; Winslow, 1954) has identified the potential cyst nematode parasites of oilseed rape in Britain and these species have been listed by Scotto la Massese (1983), Evans (1984) and Southey (1984). In Britain two species of cyst nematode are capable of parasitising oilseed rape: the brassica cyst nematode, Heterodera cruciferae (Franklin), and the beet cyst nematode H.schachtii . In the Netherlands oilseed rape is also a host for a third species of cyst nematode, a host race of the clover cyst nematode H.trifolii (Goffart), (Maas & Heijbroek, 1982). There are no reports of this host race yet in Britain (Southey, 1984) and therefore H.cruciferae and H.schachtii are the subjects of the present study. The distribution and potential threat posed by these cyst nematodes to oilseed rape are discussed in a subsequent section.

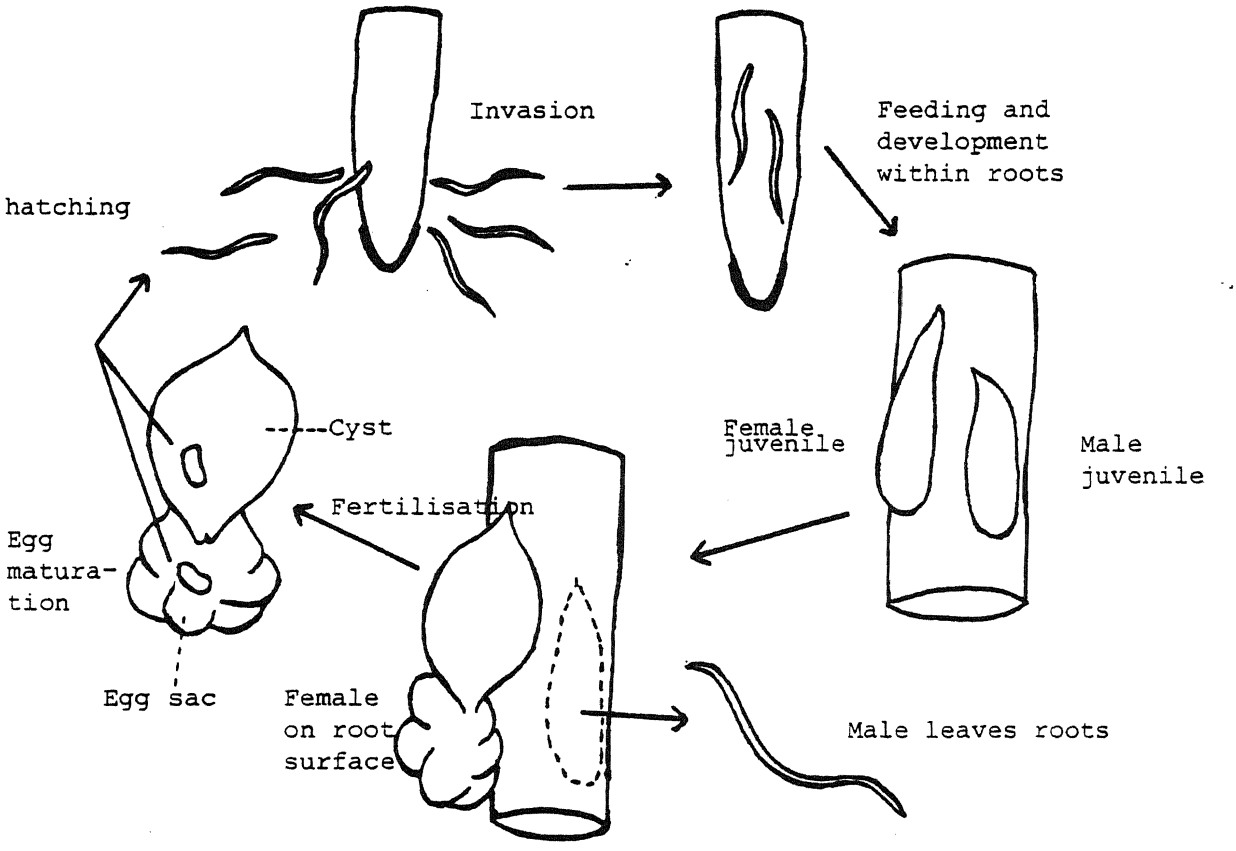
Other nematode parasites of oilseed rape have been listed by Scotto la Massese (1983) and Southey (1984). The root-knot nematode, Meloidogyne artiellia has been recorded on oilseed rape in France and Britain but little is known of the distribution and host-parasite relations of this species. The stem nematode, Ditylenchus dipsaci can also parasitise oilseed rape but little multiplication occurs on the crop and there have been few reports of damage due to infestations of this species (Southey, 1984). Two genera of the stubby-root nematodes (Trichodorus and Paratrichodorus) are often damaging to many types of crops on light soils and are often important virus vectors. Reports of damage to oilseed rape by these nematodes are usually associated with large numbers of nematodes in areas where the soil is light (Southey, 1984). A yield loss of 0.13 tonne/hectare may occur for every 1000 trichodorids/litre of soil (Evans & Spaull, 1984). Species of root-lesion nematodes (Pratylenchus spp.) occur commonly in cultivated land and are considered the most important of the plant parasitic nematodes of oilseed rape in France (Scotto la Massese, 1983). In Britain these nematode species are not considered as important pests of oilseed rape and damage is only likely to occur at extremely large population densities (Southey, 1984).

(iii) Cyst nematode life history

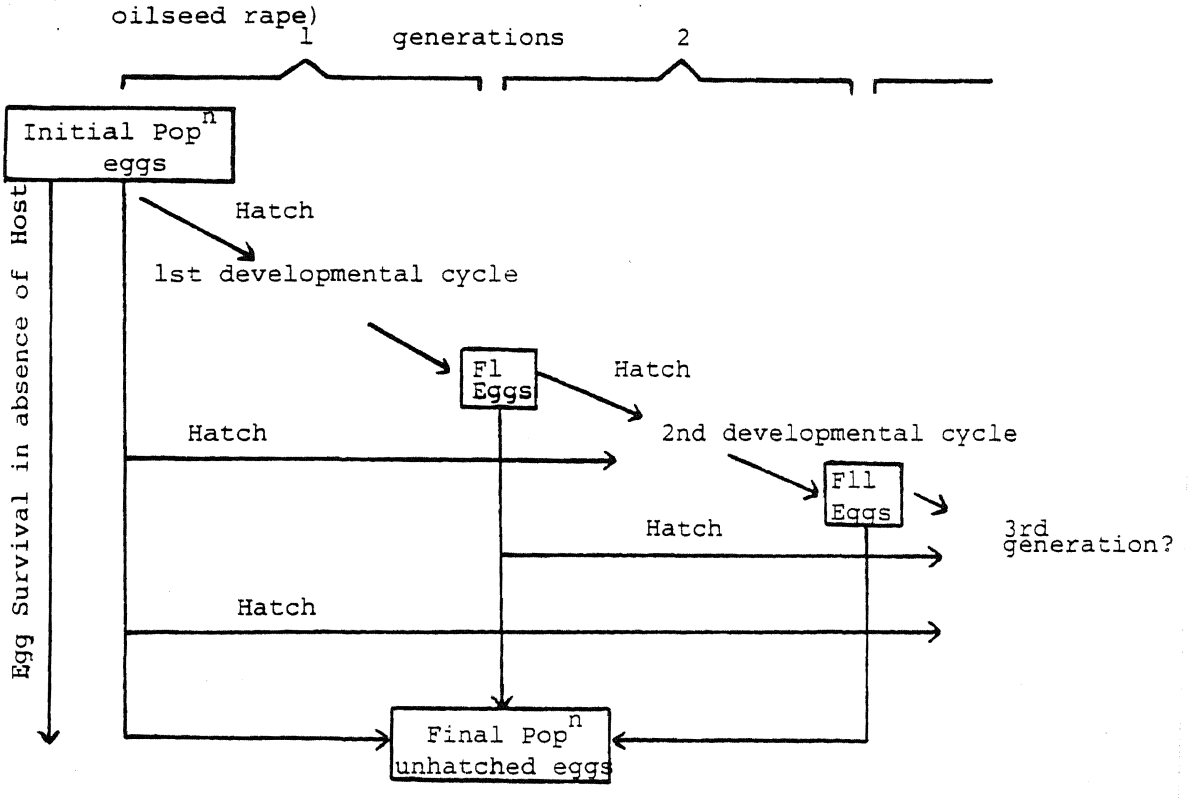
The life cycle of a cyst nematode is shown in Figure 2 a) and is divided into three principal phases: hatching; invasion; development/reproduction. The initial stages of the cyst nematode life cycle occur within the egg. After the eggs have been fertilised and gastrulation is complete the embryo increases in length to form the first stage juvenile (JJ1). These early stages of the development of H.schachtii are described by Raski (1950) and are similar to those of H.cruciferae. Characteristically, as with most nematodes, development to adult stages is punctuated by a series of moults, almost always four in number. The first moult occurs whilst the nematode is still in the egg to form the second stage juvenile (JJ2). This stage is distinguished from the JJ1 by the formation of the stylet at the anterior end. The JJ2 is the infective stage of the life cycle but may remain dormant within the egg for many years. The first two stages of development are non-feeding stages and juveniles utilize their own food (lipid) reserves. Dormancy ensures a very low rate of lipid utilisation and is a major factor contributing to the persistence of

Figure 2 Life cycle and population biology of cyst nematodes

a) Life cycle



b) Population biology (as proposed for the cyst nematodes of oilseed rape)



these nematodes in the soil when host plants are not growing (Atkinson, 1985).

HATCHING

The cessation of dormancy and hatching of second stage juveniles is usually dependent on a stimulus, usually in the form of root diffusates produced by a host plant. Field populations of cyst nematodes are composed of different aged eggs which may respond to root diffusates to varying degrees (Shepherd, 1962). The position of cysts in the soil in relation to the host plants will also influence the proportion of eggs in a population that hatch. Storey (1982) found that a greater proportion of G.rostochiensis eggs hatched close to the seed, reflecting the pattern of root growth and the time taken by roots to reach more distant cysts. Cyst nematodes hatch only when the soil temperature rises above a certain threshold increasing the probability of juveniles reaching the host roots and initiating development. Temperature may also be important for inducing and terminating dormancy in species such as H.avenae (Banyer & Fisher, 1971). The degree to which species depend on a host root stimulus varies according to the species (Winslow, 1955). In laboratory tests H.cruciferae is highly dependent on root diffusates for hatching to occur and only a few juveniles hatch in tap water. In contrast, H.schachtii is much less dependent on stimulation by root diffusates and hatches readily in tap water (Winslow, 1955). A mechanism of hatching for G.rostochiensis has been proposed by Perry & Clarke (1981). Evidence suggests that root diffusates initiate changes in the permeability of the egg shell membrane, allowing solutes in the perivitelline fluid surrounding the juvenile to move out of the egg. The importance of the solute content of perivitelline fluid, composed mainly of the diasaccharide trehalose, (Clarke & Hennessy, 1976) is discussed in a subsequent chapter. During dormancy the juvenile is in a state of incomplete hydration and the mechanism proposes that the juvenile can hydrate only when the osmotic stress imposed by the trehalose is removed. Ellenby & Perry (1976) showed that juveniles of G.rostochiensis hydrated after stimulation with potato root diffusate prior to eclosion. The stimulated juveniles do not rehydrate fully prior to eclosion due to the limits imposed by the egg shell membrane on the size of the juvenile (Ellenby, 1974). However, the stimulated yet unhatched juveniles are active as indicated by an increase in the

oxygen consumption and a decrease in the adenylate energy change of cysts (Atkinson & Ballantyne, 1977a, b) and they are able to emerge from the egg using their stylets to cut a slit and leave the egg (Doncaster & Shepherd, 1967). Once the juvenile is free of the egg the water content of G.rostochiensis increases immediately until the juvenile is fully hydrated (Ellenby, 1974). Although Perry (1977a) found that the water content of H.schachtii juveniles increased after eclosion there was no evidence of water uptake by the juvenile after stimulation by root diffusate but prior to eclosion. This suggests that root diffusate may have a direct influence on unhatched juveniles as well as indicating changes in egg shell membrane permeability. The hatching mechanism proposed for G.rostochiensis may therefore not apply to all species of cyst nematode.

INVASION

The potential infectivity of G.rostochiensis JJ2's is influenced by the extent of lipid reserves at the time of hatching and there is considerable variation in the time taken from stimulation to the eclosion of juveniles within a cyst. A delay in hatching after stimulation is associated with a loss in infectivity (Robinson et al., 1985). Once the juvenile has hatched and left the cyst, usually via the vulva, the ability to locate and invade a host is also strongly influenced by soil conditions. The movement and activity of juveniles depends on soil particle size, pore size, thickness of water film and the amplitude and frequency of wave forms along the body (Wallace, 1963). Juveniles in the soil may be attracted to hosts by either chemicals or gases originating in the host roots although such relationships are difficult to establish. Wallace (1958) found that root washings attracted H.schachtii and Johnson & Viglierchio (1969) indicated that CO_2 in water attracted H.schachtii. However, such attractive substances probably only act over short distances and are readily degraded by micro-organisms (Williams, 1978). A majority of host roots may be located by random exploratory movements.

The JJ2 usually invades the host just behind the growing tip and where lateral roots emerge. These probably represents either more penetrable regions or perhaps areas where attractive substances originate (Widdowson et al., 1958). The juvenile cuts through cell walls using

its stylet and migrates laterally through the root cortex until the head reaches the pericycle where the juvenile initiates feeding.

DEVELOPMENT AND REPRODUCTION

Species of Heterodera and Globodera feed within the roots by initiating a feeding site composed of large volumes of cytoplasm (syncytia) formed by the dissolution of cell walls (Endo, 1964). Syncytia are sites of intense metabolic activity and have a structure similar to that of a transfer cell. Jones & Northcote (1972) suggested that their role was to act as transfer cells increasing the efficiency of nematode feeding by facilitating the translocation of nutrients from a vascular vessel to the juvenile. The second moult of H.schachtii occurs shortly after feeding has been initiated. The JJ3 has a well developed rectum and genital primordia appear; female JJ3's having paired ovaries and male JJ3's developing a single testis (Raski, 1950). As male development continues the third and fourth moults occur within the cuticle of the third and fourth stage. The adult male, which usually develops slightly before the adult female, is elongated and is coiled with usually three flexures within the fourth stage cuticle. The adult male emerges from the old cuticle and leaves the roots. As the female develops the body size increases with the development of the ovaries. The fourth stage of female development is typically flask-shaped and the body is almost entirely occupied by ovaries. During the fourth moult the reproductive system of the female opens to the exterior via the vulva. At this time the body is swollen and full of eggs. The increase in body size causes the expanding female to break through the root cortex. Females secrete specific chemicals which attract the male and enhance the chances of successful fertilisation (Green, 1980). Many males may surround a female and multiple mating may occur (Green et al., 1970). Males of G.rostochiensis were found to be short-lived and remained active for only nine or ten days (Evans, 1970). After fertilisation, gastrulation and embryonation commence and the female eventually dies. Before the female dies and whilst still attached to the root a gelatinous egg sac is extruded by most Heterodera species into which eggs may be deposited. Franklin (1943) first described the presence of an egg sac attached to H.cruciferae females and found that they contained up to 200 eggs at various stages of development. Raski (1950) reported the occurrence of H.schachtii egg sacs and estimated from the number of

empty egg shells within the egg sac that they contain more than 100 eggs. After the female dies the egg sac may remain attached to the cyst but is easily dislodged into the soil. The immature female cyst is initially white in colour but due to the action of polyphenol oxidase on polyphenols in the cyst wall the cyst tans to form a brown cyst (Ellenby, 1946). The cyst, which becomes a shell enclosing a large number of eggs, may remain attached to the root for a short while but eventually is dislodged into the soil. The cysts of H.cruciferae and H.schachtii are morphologically similar, their lemon-shape typical of the Heterodera species. The cysts have a distinct anterior protuberance and a posterior vulval cone. In contrast with Heterodera cysts, Globodera cysts are more spherical in shape with only a clearly defined anterior protuberance. The size and shape of individual cysts varies considerably both within species and between species, making identification inaccurate when based on those characteristics. However, species may be identified more accurately by a more detailed examination of vulval cone characteristics supported by diagnostic features of the second stage juvenile.

(iv) Introduction to the population biology of cyst nematodes

Land which becomes infested with cyst nematodes and is frequently cropped with host plants provides the opportunity for cyst nematode populations to increase to levels at which damage may occur. An understanding of the population biology of a species of cyst nematode, when either a host crop is grown or when a non-host crop is grown or the land is left fallow, is a prerequisite for the sound management of land in areas were nematodes pose a threat. Such an understanding will aid the prediction of the growth of cyst nematode populations and, in conjunction with damage assessment studies, allow the potential yield losses of a crop to be estimated. Information may also be used to indicate the most efficient control measures taken against cyst nematodes.

Land may become infested with cyst nematodes through the transfer of contaminated soil which adheres to farm machinery and transplant material (Evans, 1984). Irrigation and flood-water may also influence the spread of nematodes on a local scale (Faulkner & Bolander, 1966). During a study of the spread and population density of H.schachtii on a vegetable growing farm between 1971 and 1977 the number of fields

with detectable nematode infestations increased from six to nine. The spread of H.schachtii was thought to be mainly a result of the movement of contaminated soil on farm machinery but in one field spread may have occurred by the movement of contaminated soil through surface-water run-off (Mai & Abawi, 1980). Bedi (1968) found that once an infestation of G.rostochiensis was established in a field, spread began from the first focus, speeding up as new foci were formed after the harvesting of successive potato crops. The rate of colonization may be rapid and Jones & Kempton (1978) point out that the widespread distribution of H.schachtii in the reclaimed fenlands of East Anglia has been achieved within a time scale of about 60 years.

The influence of 'founder effects' on cyst nematode populations and the aggregation of nematodes around the roots of host plants result in field populations of cyst nematodes being very spatially heterogeneous. The inherent tendency of populations to oscillate around their equilibrium density is affected by climatic factors and soil conditions and this may further influence the patchy distribution of a population within a field (Jones & Kempton, 1978). Whilst the population dynamics of the potato cyst nematode and the beet cyst nematode have been extensively studied (Fenwick & Reid, 1953; Evans, 1969; Jones, 1945; Jones, 1956) few studies have examined in detail the spatial population dynamics, both vertically and horizontally. Storey (1982) found that the first population changes of G.rostochiensis occurred close to the planting depth and that the timing and scale at other depths were related to the position of cysts relative to the seed. Cysts in the furrows between the rows of the potato crop were relatively uninfluenced by the host crop with approximately 70% of the eggs remaining unhatched.

After harvesting, nematode populations are most dense beneath rows or in soil scattered by roots when the crop is lifted. Assessing field populations is therefore more appropriate after ploughing and cultivation has re-distributed the nematodes more randomly. Methods which extract cysts from the soil such as the Fenwick can (Fenwick, 1940) and the fluidising column (Trudgill et al., 1972) are generally efficient although particular methods may suit different species and soil types (Seinhorst, 1964; Cooke et al., 1983). But as Sykes & Winfield (1966) point out, standard methods are not suitable for the extraction of cysts with attached egg sacs. Despite these generally

good extraction techniques, the assessment of field populations is often inaccurate due to the aggregated distribution of cyst nematode populations and may be unreliable in detecting lightly or partially infested fields (Cooke et al., 1983). Small plot and pot experiments suffer less from the effects of spatial heterogeneity of nematode populations and may allow more meaningful population studies to be made (Seinhorst, 1970).

Nematode multiplication is usually studied over a growing season, when Pi is the population at planting and Pf that after harvest. The large increase in the population density of cyst nemtodes on a host crop is offset by the decline in population density during the period in which non-host crops are grown. Evans (1984) suggests that a 1 year in 5 year rotation is usually sufficient to keep nematode populations below damaging levels. The population characteristics of cyst nematodes vary with the cyst nematode species and the type of host crop. The life cycle of cyst nematodes in relation to cyst nematode population biology is shown in Fig. 2a & b. This outlines the major characteristics expected of a cyst nematode population of either H.cruciferae or H.schachtii on a crop of oilseed rape. In contrast to the potato cyst nematode which completes one and a possible partial second generation on a potato crop (Evans, 1969) both H.cruciferae and H.schachtii are reported to complete two or more generations on hosts with which they are commonly associated (Jones 1950; Lewis, 1971). McCann (1979) found that H.cruciferae completed three generations on both winter and summer grown brassica crops and that reproduction continued on root debris remaining in the soil between crops. Recent work by Koshy & Evans (1986) has predic ted that H.cruciferae would be able to complete only two generations on an autumn sown crop of oilseed rape. However, the population biology of species which complete more than one generation on a crop such as oilseed rape which has a growing season as long as eleven months will be more complicated than species completing one generation (Fig. 2b). At the end of the growing season populations of such species will be composed of a greater mixture of old and new eggs of successive generations than species completing just one generation. Old and new egg cohorts may have different population characteristics and may complicate the population biology of such a species. The multiplication rate of cyst nematode populations per generation is usually much less than the reproductive potential as many eggs fail to hatch and many juveniles fail to locate and establish within hosts. Parasites and predators may

also eliminate a proportion of juvenile stages but the pyramidal age structure of populations ensures that losses of early juvenile stages simply removes juveniles surplus to the carrying capacity of the host. Conversely, the loss of female adult stages through parasites or predators such as either the fungal parasites of H.avenae females (Kerry & Crump, 1977) or the collembola predators of H.cruciferae cysts (Murphy & Doncastor, 1957) may have a greater effect on the growth of a population. The multiplication rate of cyst nematode populations on host plants also depends on the initial population density as shown in early work on H.rostochiensis by Fenwick & Reid (1953) and Peters (1961). At population densities above a certain level no further increases in multiplication occur, the multiplication rate being <1. This level is referred to as the equilibrium density. The amount of multiplication which occurs depends on the quality and the quantity of the food resource available to the developing nematode (Seinhorst, 1970). Although the food resource in growing plants is an expanding one for most of the growing season, it is not equal for all values of Pi but diminishes as increases in Pi lead to greater competition between developing nematodes for food resources. The multiplication rate is usually greatest at small nematode population densities therefore, and decreases with increasing Pi. This relationship between Pf and Pi on different crops is shown by Maas & Heijbroek (1982) for the host race of H.trifolii capable of parasitising oilseed rape. Multiplication rates decreased with increasing Pi on all the host crops tested although sugar beet, cabbage and broad bean supported more multiplication than other crops. In this way populations may be regulated through intraspecific competition for food resources between juveniles to develop into females. Sex expression in Heterodera species is almost always genetically controlled although the sex ratio may be influenced by environmental conditions (Triantaphyllou, 1973). Kerstan (1969) found that the numbers of maturing H.schachtii females on the roots of turnips was correlated with increases in degenerating and dead juveniles at all stages of development in the roots and concluded that the number of juveniles invading the roots and the thickness of roots were the major factors influencing the sex ratio. Similar results were found for H.schachtii on sugar beet by Steele (1975). Bridgman & Kerry (1980) established that sex ratios >1 were a result of a decreased recovery of females and were not associated with an increase in the number of males developing as reported for G.rostochiensis by Trudgill (1967) when the number of juveniles per centimetre of root increased.

However, different cyst nematodes may have different mechanisms for determining their sex. The results of Bridgman & Kerry (1980) suggest that the sex of H.cruciferae and H.schachtii is not labile but in unfavourable conditions such as intense intraspecific competition female juveniles may fail to establish syncytial transfer cells and be unable to develop to adulthood and produce eggs and so die. The main density-dependent mechanism therefore would seemingly act by regulating the sex ratio through differential mortality of the sexes.

The effect of damage to host roots by nematodes also influences multiplication rates (Chitwood & Feldmesser, 1948; Peters, 1961; Seinhorst, 1967). As root damage usually increases with increasing initial nematode densities it is at large densities that root damage will have the most pronounced effect on nematode multiplication whereas at small nematode densities root damage may have a negligable effect. The decrease in the multiplication rate with increasing Pi is therefore not only influenced by intraspecific competition between juveniles for food but also by the amount of damage inflicted by juveniles on the plant.

(v) The distribution and potential threat of H.cruciferae and H.schachtii as parasites of oilseed rape

The large increase in the acreage of oilseed rape, especially in the last decade means that a much greater area of host crops are available for parasitism by both H.cruciferae and H.schachtii than in previous years. In regions where the recent expansion of oilseed rape cultivation has overlapped with areas of vegetable brassica and sugar beet cultivation there will be much potential for populations to increase and damge to occur as (i) the cyst nematodes will be well established in these areas, and (ii) more than one host crop may be included in the rotation. The spread of cyst nematodes may be more rapid in these areas due to the frequent cropping of host crops.

The distribution of H.cruciferae in Britain is probably widespread (Jones & Jones, 1974) but reports of damage to brassica crops other than oilseed rape are few and usually only occur in areas of traditional intensive brassica cultivation (Savage, 1979) (Fig. 3). Sykes & Winfield (1966) found H.cruciferae to occur in at least 70% of fields in the vegetable growing area of SE Lincolnshire with 20% of

these fields having nematode population densities in excess of 25 eggs g^{-1} soil, population densities above which were considered damaging to winter cauliflowers. In Bedfordshire, where brassicas such as Brussels sprouts have a long history as crops H.cruciferae is also widely distributed, although the population densities were slightly smaller than those in SE Lincolnshire (Winfield et al., 1970). Lewis (1971) reported the occurrence of H.cruciferae in South Wales but found that in a study of four infested fields which had brassicas grown in them at least one year in four years that there was no large increase in population density. In three fields the population density fluctuated between 10 and 30 eggs g^{-1} soil whilst in the other the density remained below 5 eggs g^{-1} soil.

In 1984 there were eleven cases of damage to oilseed rape due to H.cruciferae infestation that were reported to ADAS. The majority of cases occurred in Kent and East Anglia, areas of intensive oilseed rape cultivation (Southey, 1984).

Oilseed rape is also a host of H.schachtii, which like H.cruciferae is probably widespread, but with distribution concentrated in areas where there is intensive sugar beet cultivation (Fig. 3), the major host crop of this species (Southey et al., 1982). Because of the national importance of the sugar beet crop, especially during the war, measures were made to reduce crop damage by H.schachtii by restricting the frequency with which infested land could be cropped with a host crop. This was initially implemented in contracts between sugar beet growers and the British Sugar Corporation and then later by successive Beet Eelworm Oders (Jones, 1951). Land affected by such restrictions comprised mainly the East Anglia black fen soils (Whiteway et al., 1982). Recent relaxation of the Beet Eelworm Order and the introduction of the Beet cyst nematode order in 1977 revoked the earlier statutory regulations on the growing of host crops in rotations enabling statutory controls to be introduced if and where evidence suggests that infestations are increasing to potentially damaging levels (Southey et al., 1982). Therefore, in areas not affected by such regulations, oilseed rape and sugar beet could be grown within the same rotation, so providing two host crops for H.schachtii and the potential for rapid population increase. Attention has been drawn to the importance of H.schachtii on brassica crops in the USA and work has shown that brassica crops in rotation with sugar

Figure 3 Major oilseed rape and Sugar beet growing areas in relation to recorded incidence of cyst nematode damage
(Data from Jones & Jones, 1974; Savage, 1979; Bunting, 1984)

Oilseed rape (most intensively cropped areas in heavier shading)

Sugar beet

Brassica-cyst nematode damage (traditional brassica-growing area encircled)

Beet-cyst nematode damage (area of greatest damage encircled)

beet crops result in large nematode populations and up to 50% reductions in both brassica and beet crops (Olthof et al., 1974; Abawi & Mai, 1980). These workers did not examine a rotation which included a beet crop alone in their experiments but it would be expected that both H.schachtii populations and crop losses would be smaller than when both a beet and brassica crop were grown. Southey (1984) reports that no cases of damage to oilseed rape in ADAS regions due to H.schachtii infestation have yet been made but warns of the potential threat of H.schachtii to oilseed rape crops. Workers in Italy and France (Preste, 1983; Caubel & Chauber, 1985) have also examined the multiplication of H.schachtii on oilseed rape and warned of the potential threat of this cyst nematode species to oilseed rape. Figure 3 summarises the general distribution of reports of damage by both H.cruciferae and H.schachtii to host crops and compares these to the major oilseed rape and sugar beet growing regions in England and Wales. The occurrence of reports of damage by the two species and the areas in which oilseed rape and beet are grown overlap extensively, especially in Eastern England and indicate the potential threat posed by these two species to crops in these areas. However, a recent survey of 47 fields in South Lincolnshire, North Norfolk, Cambridgeshire and Huntington where both sugar beet and oilseed rape crops were grown in the same rotation found a low incidence of both H.cruciferae and H.schachtii (Evans & Spaull, 1985). These workers reported that in one sugar beet factory area, of the 1649 sugar beet growers approximately 80 grow both oilseed rape and sugar beet in the same rotation. The consequences of these rotations on both nematode population multiplication and yields will not be known for some time.

(vi) Objectives and programme of research work on the cyst nematode parasites of oilseed rape

The population biology of H.cruciferae and H.schachtii and the relationship between population numbers and yield losses on cauliflower, cabbage and sugar beet crops have been well studied (Sykes & Winfield, 1966; Lewis, 1971; McCann, 1981; Jones, 1956; Cooke, 1984) whilst there is virtually no information concerning the population biology of these species on oilseed rape. The host-parasite relationship between cyst nematodes and oilseed rape is probably at a relatively early stage of development due to the comparatively recent, rapid expansion in the amount of oilseed rape grown. This presents a

unique situation in which a potential pest problem can be examined before widespread crop losses might occur and which can utilise the experience gained from the work on the more established cyst nematode pests of potato and sugar beet crops. The object of the research programme is to provide information which will be essential to the sound management of oilseed rape on land where cyst nematodes pose a threat. Information will also help to evaluate and forecast nematode population growth and yield losses.

The current project forms an integral part of a research programme on the nematode pests of oilseed rape being carried out at Rothamsted, MAFF laboratories and by a number of ADAS regional laboratories. The availability of resources dictated to a certain extent the division of research areas among the participants. Additionally, the small number of reports of damage to oilseed rape by cyst nematodes restricted the choice of sites for field trials and the source of nematode populations for experimental work. In 1984 field trials were already established by Rothamsted Experimental Station and ADAS on sites where crops had suffered from severe cyst nematode damage and were primarily concerned with providing data on yield losses, variety trials and control measures (Evans & Spaull, 1984; Harris & Winfield, 1985). Glasshouse and controlled environment studies were to form a major part of the work based at Luton College of Higher Education. The aim of these experiments is to provide data on aspects of the population biology of H.cruciferae and H.schachtii as parasites of oilseed rape which will lead to an understanding of field population dynamics and aid the interpretation of field trials data. As the project progressed certain aspects were identified as warranting further research and work was correspondingly directed into these areas. However, at the outset of the project the following aspects of the population biology were selected.

(a) The use of growth cabinet experiments to relate the hatching and multiplication of H.cruciferae and H.schachtii in the roots of oilseed rape to crop management, oilseed rape being a predominantly winter crop.

(b) To investigate, by glasshouse pot experiments, the resistance/tolerance within the gene pool of current, previous

and developmental cultivars of oilseed rape to H.cruciferae and H.schachtii.

(c) To employ glasshouse pot experiments to identify those weeds which may hatch H.cruciferae eggs, allow juvenile invasion and provide conditions suitable for nematode multiplication.

(d) To establish a regular sampling programme for the duration of the project from a bulk of infested soil, to relate the survival of nematode eggs, and their ability to subsequently infect oilseed rape seedlings, to crop rotation practices.

(e) To investigate competition for root space between developing H.cruciferae and H.schachtii juveniles in pot grown oilseed rape plants. Plants will be infected separately, and in combination, with the two species of nematodes at different population densities.

(f) To provide those parameters necessary to model the cyst nematode populations of oilseed rape similar to that for the potato cyst nematode (Jones & Perry, 1978).

2

The effect of temperature on the hatching and multiplication of *Heterodera cruciferae* and *H.schachtii*

INTRODUCTION

The oilseed rape crop in the UK is almost entirely autumn sown (Bunting, 1984) and may be in the ground for up to eleven months. Nematodes that invade the host roots in the autumn will be subject to a range of temperatures which follow both seasonal trends and daily fluctuations, although diurnal temperature changes 10cm below the soil surface are small and can usually be disregarded (Jones, 1975). Nematodes that invade spring sown crops probably experience a narrower and warmer temperature range and develop under conditions which get steadily warmer.

Jones (1975) points out that after entering the roots of host plants endoparasitic nematodes such as Heterodera spp. are able to obtain an uninterrupted supply of food and water and the rate at which they develop is a function of temperature. A study of the multiplication of nematodes on a crop should not just consider the endoparasitic phase but also the hatching and invasive phases. Wallace (1963) discusses factors that influence the infectivity of nematodes and identifies temperature, soil moisture and soil pore size as major factors.

Lewis (1971) made sequential plantings of cabbage seedlings throughout the year to measure the development rate of H.cruciferae by recording the time between planting and egg production. It was estimated that between two and three generations may occur on a crop between April and September. McCann (1979) found that three generations of H.cruciferae occurred on both summer and winter brassica crops and that development continued on root debris after harvest. Estimates of development rates can allow a harvest of crop to be timed to occur before a generation is completed thus limiting the increase in nematode population density (Webley & Jones, 1981). Harvest times based simply on dates would be inaccurate in years when temperatures deviated considerably from the average and the calculation of accumulated day degrees above a basal development temperature would be more accurate. This was found to model the development of H.schachtii on sugar beet fairly accurately (Jones, 1975). Koshy & Evans (1986) calculated the accumulated day degrees above a basal development temperature of 5°C by H.cruciferae on oilseed rape. In conjunction with information on the effects of temperature on hatching they predicted that two consecutive generations of H.cruciferae would be

possible on autumn sown oilseed rape in southern England. On the basis of the accumulated day degree requirements of each complete generation they calculated that the second generation would only mature fully sometime after the crop was harvested. However, an accurate prediction of nematode development using accumulated day degrees can only be achieved if the basal temperature is precisely known and if development is assessed by frequent sampling.

This study was undertaken to compare the hatching and multiplication of H.cruciferae and H.schachii on oilseed rape. Emphasis was directed towards comparing the relative numbers of developing nematodes and the hatching and development rates of the two species.

MATERIALS AND METHODS

i) Hatching test

The effects of temperature on the hatching of H.cruciferae and H.schachtii were examined by collecting diffusate produced by 28 day old oilseed rape plants cv. Jet Neuf and performing hatching tests at 5,10,15 and 20°C, using a range of dilutions of the diffusate.

Five pots, each containing one oilseed rape plant growing in a mixture of loam, sand and gravel (3:1:1), were leached with 50ml of distilled water. A further 50ml of distilled water were added 30 minutes later. The leachate from pots was collected in plastic trays and then filtered through Whatman No 1 filter paper. A dilution series was prepared by adding distilled water to give concentrations of 1:1, (i.e. 50%) 1:4, 1:16, 1:64 and 1:256. Diluted leachates were stored at 5°C until the hatching tests were performed.

Heterodera cruciferae cysts were extracted from soil in which oilseed rape was grown during 1983-84 and H.schachtii cysts were extracted from soil in which sugar beet was grown during 1984. Cysts were extracted by fluidising column (Trudgill et al.,1972) and one hundred batches of 50 cysts were prepared to give five replicates of each leachate concentration at either 5,10,15 or 20°C. Cysts were added without pre-soaking to 2ml of leachate in individual compartments of Repli-dishes and left in the dark at the desired temperature.

Additionally, five batches of 50 cysts were added to 2ml of distilled water at each temperature. The numbers of hatched juveniles were counted weekly and the leachate was replaced by 2ml of fresh leachate at the same time. After 3 weeks the numbers of unhatched juveniles were estimated by breaking open the cysts between a glass slide and an aluminium channel which prevented eggs and juveniles from becoming damaged (Southey, 1970). The eggs were separated from the cysts and released into suspension in 25ml of water by vigorous stirring with an electric stirrer. Counts of juveniles released by this process were made in one millilitre aliquot of the suspension and the number of unhatched juveniles were counted. The percentage hatch was calculated for each batch at each temperature and dilution.

ii) Nematode development

Nematode development was examined at 5,10,15 and 20°C. For each temperature sixty pots (10cm diameter) containing 500ml of a loam, sand and gravel mixture (3:1:1) were sown with three seeds of oilseed rape cv. Jet Neuf and placed in a glasshouse at 20°C day and 10°C night temperatures and with supplementary lighting at a 12 hour photoperiod. Plants were thinned to one healthy seedling per pot after 7 days, watered as required and fed weekly with a liquid N,P,K fertilizer. After 28 days the pots were transferred to Fisons Fitotron 600 growth cabinets set at the desired temperatures and with a 12 hour photoperiod. After 24 hours acclimatisation plants were inoculated with juveniles.

Two thousand juveniles were released from eggs by vigorously crushing a predetermined number of cysts between a glass slide and aluminium channel. The freshly released juveniles were added to 2ml of distilled water and were pipetted into the soil of each pot at four locations at a depth of 2-4cm. For each temperature thirty pots were inoculated with H.cruciferae juveniles and a further thirty pots with H.schachtii juveniles.

The extent of nematode development in the roots was examined 15,30,45, 60 and 70 days after inoculation. The root systems of six plants inoculated with each species were examined on each occasion. Plants were gently removed from the soil and the roots were washed, blotted dry and the fresh root weight of each plant recorded. Nematode

development in the roots was examined by staining each root system for 3 minutes in a boiling solution of acid fuchsin in glycerol, lactic acid and distilled water (Marks & McKenna, 1981). The root system was carefully removed from the staining solution and blended for 15-30 seconds, depending on the size of the root system, in a MSE "Ato-mix" in 100ml of water. The root suspension was made up to 150ml with distilled water and the nematodes in three 20ml aliquots were counted. The stage of development of each nematode was recorded.

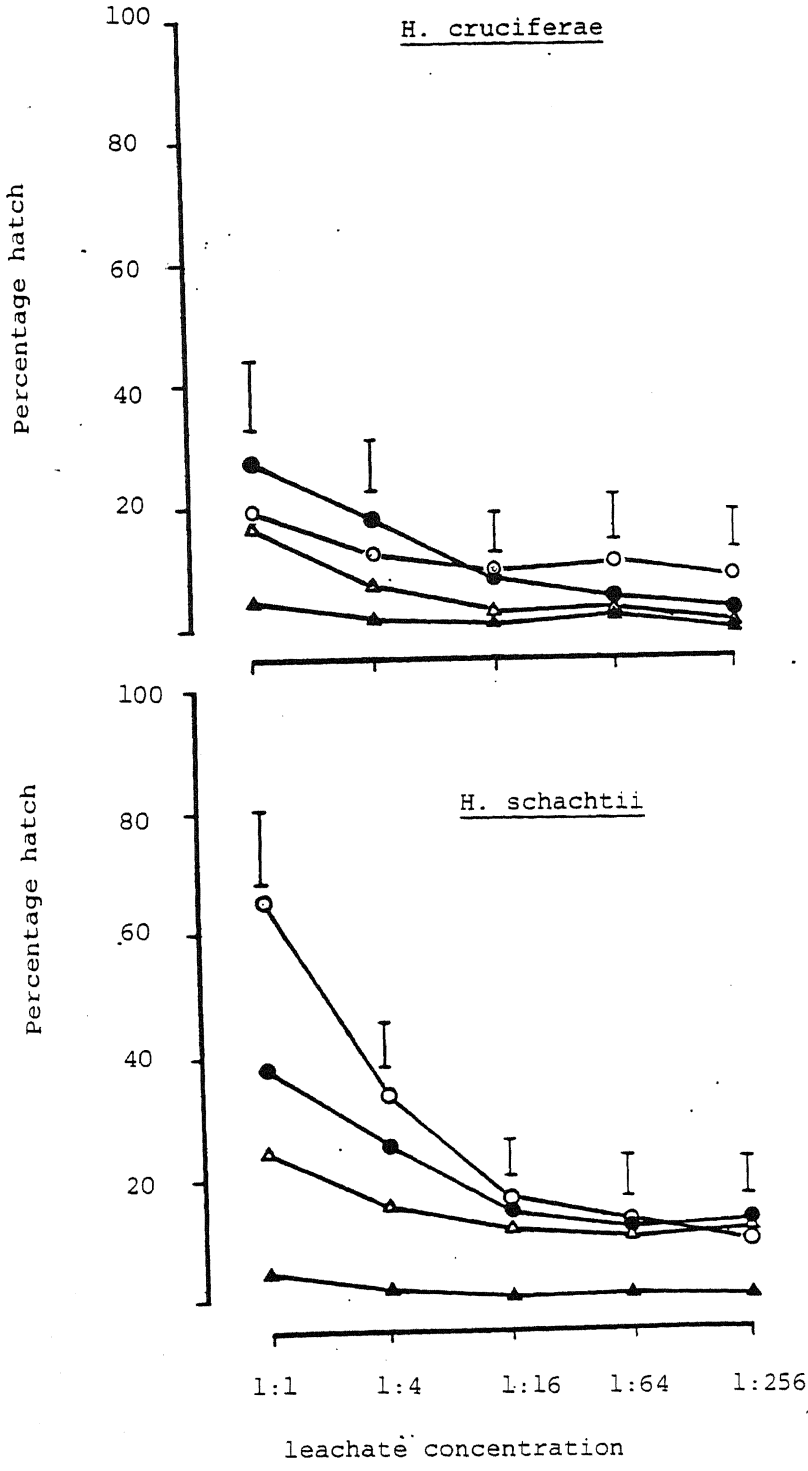
At 30,45,60 and 70 days the soil from each pot was retained, thoroughly mixed and any cysts were extracted from a 200g sample by fluidising column. The numbers of cysts were counted and the egg contents of the cysts estimated by releasing the eggs between a glass slide and aluminium channel and counting the total numbers of eggs in aliquot samples. Additionally, females and egg sacs were extracted from a further 200g sample of soil from those pots sampled at 30 days. The females and egg sacs were extracted by decanting and sieving soil in a suspension of 500ml of water. A number of females with attached egg sacs were picked from the material retained on a 250µm sieve and were added to 2ml of root leachate and placed at the temperature at which they had been developing. The cysts and egg sacs were kept in oilseed rape leachate at this temperature until the first juveniles hatched.

RESULTS

i) Hatching test

The effects of temperature and the dilution of root leachate on the percentage hatch of each species are shown in Fig. 4. At 5°C there was virtually no hatch by either H.cruciferae or H.schachtii in any leachate solution or in distilled water. At 10°C in the most concentrated leachate 17.2% and 23.8% of H.cruciferae and H.schachtii juveniles hatched respectively, and hatching decreased with dilution of the leachate solutions to a percentage hatch similar to that of H.cruciferae (2.3%) and H.schachtii (10.6%) in distilled water. The largest number of H.cruciferae juveniles (27.9%) hatched in the most concentrated leachate at 15°C. Fewer (19.2%) H.cruciferae juveniles hatched in the most concentrated leachate at 20°C. The mean percentage hatch of H.cruciferae juveniles in distilled water at 15 and 20°C was

Figure 4 Percentage hatch of H. cruciferae and H. Schachtii after three weeks in five concentrations of oilseed rape leachate at four temperatures; ▲ 5°C, △ 10°C, ● 15°C, ○ 20°C. Vertical bars represent the L.S.D. (P = 0.05)



3.7% and was slightly less than the mean percentage hatch in the 1:256 dilution at 15 and 20°C (8.3%). The greatest percentage hatch (66.8%) of H.schachtii occurred at 20°C. Fewer H.schachtii juveniles hatched in the 1:1 leachate dilution at 5,10 and 15°C and less than 20% of juveniles hatched in 1:16, 1:64 and 1:256 dilutions at these temperatures. In distilled water at 10,15 and 20°C a mean of 12.3% of juveniles hatched. The weekly counts showed that the maximum hatch of both species at 10,15 and 20°C occurred in the second week of the hatching test.

ii) Nematode development

Figures 5 & 6 show the numbers of nematodes at each developmental stage in the roots 15,30,45,60 and 70 days after inoculation at each temperature.

There was little root penetration at 5°C by either species with less than 0.5% of the juvenile inoculum invading the roots. A second experiment was performed in which juveniles were inoculated at 15°C for a 48 hour period before the plants were transferred to 5°C. This permitted limited juvenile invasion and the examination of any subsequent development at 5°C. During the 48 hour period at 15°C a mean of 68.4 H.cruciferae juveniles and 130.0 H.schachtii juveniles penetrated the roots; 60.8% and 94.8% of the numbers of second stage juveniles in the roots at 15°C after 15 days. This indicates that a large number of H.schachtii juveniles were able to penetrate the roots earlier than H.cruciferae juveniles at 15°C.

At 5°C third stage juveniles of H.cruciferae and H.schachtii had developed by 45 days but no further development had occurred by 70 days. At 10°C, similar numbers of both species had penetrated the roots and had developed to third stage juveniles by 30 days. Fourth stage H.cruciferae juveniles were first recorded at 45 days whereas H.schachtii fourth stage juveniles were not recorded until 60 days. A larger number of H.cruciferae than H.schachtii had developed to the fourth stage by 70 days at 10°C but no adult stages were seen in these roots over this period. The invasion rate of H.schachtii juveniles increased at the higher temperatures with the greatest numbers

Figure 5 **Numbers of H.cruciferae second stage juveniles (a), third stage juveniles (b), fourth stage juveniles (c) and adult stages (d) in the roots at 5, 10, 15 and 20°C**

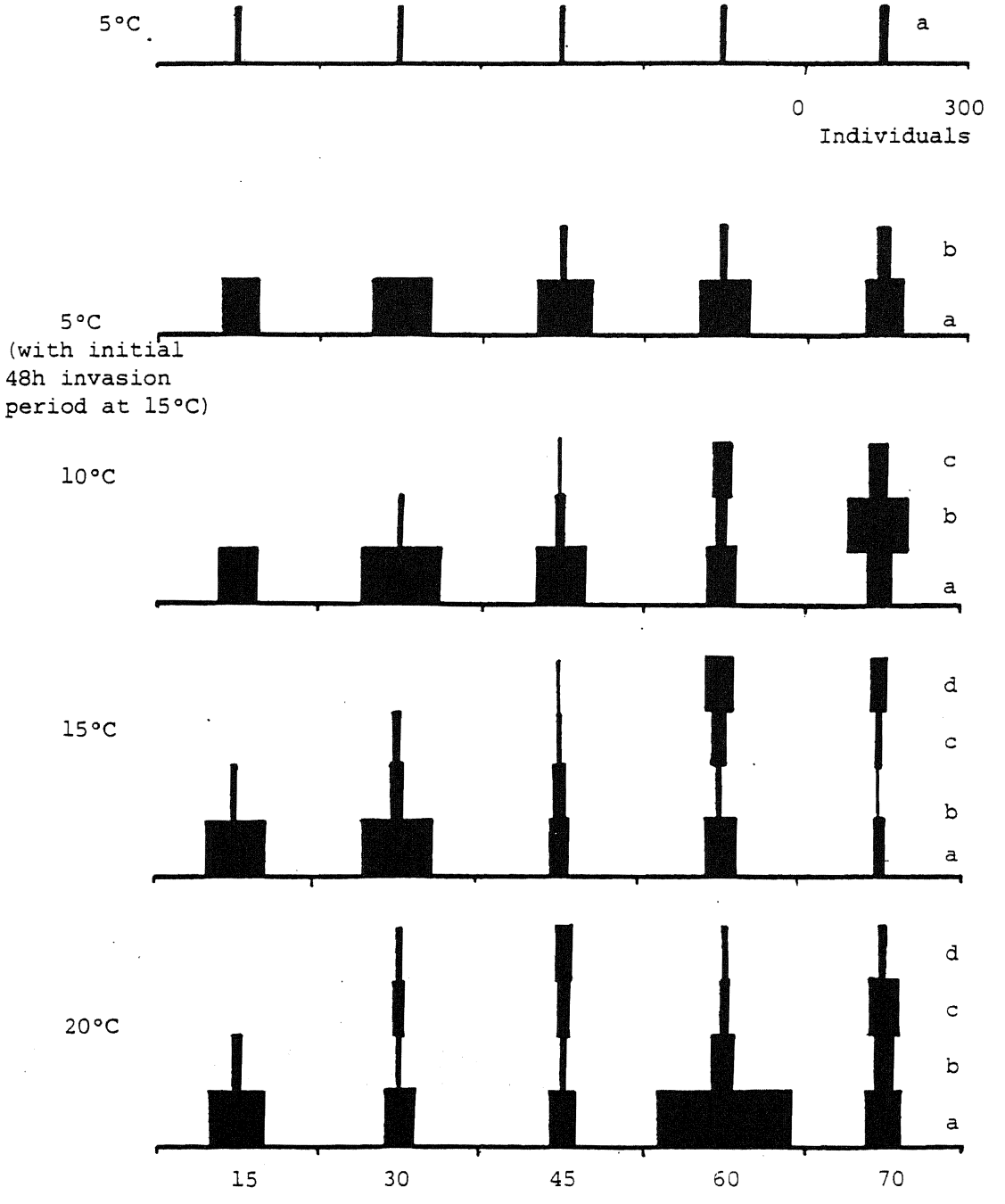
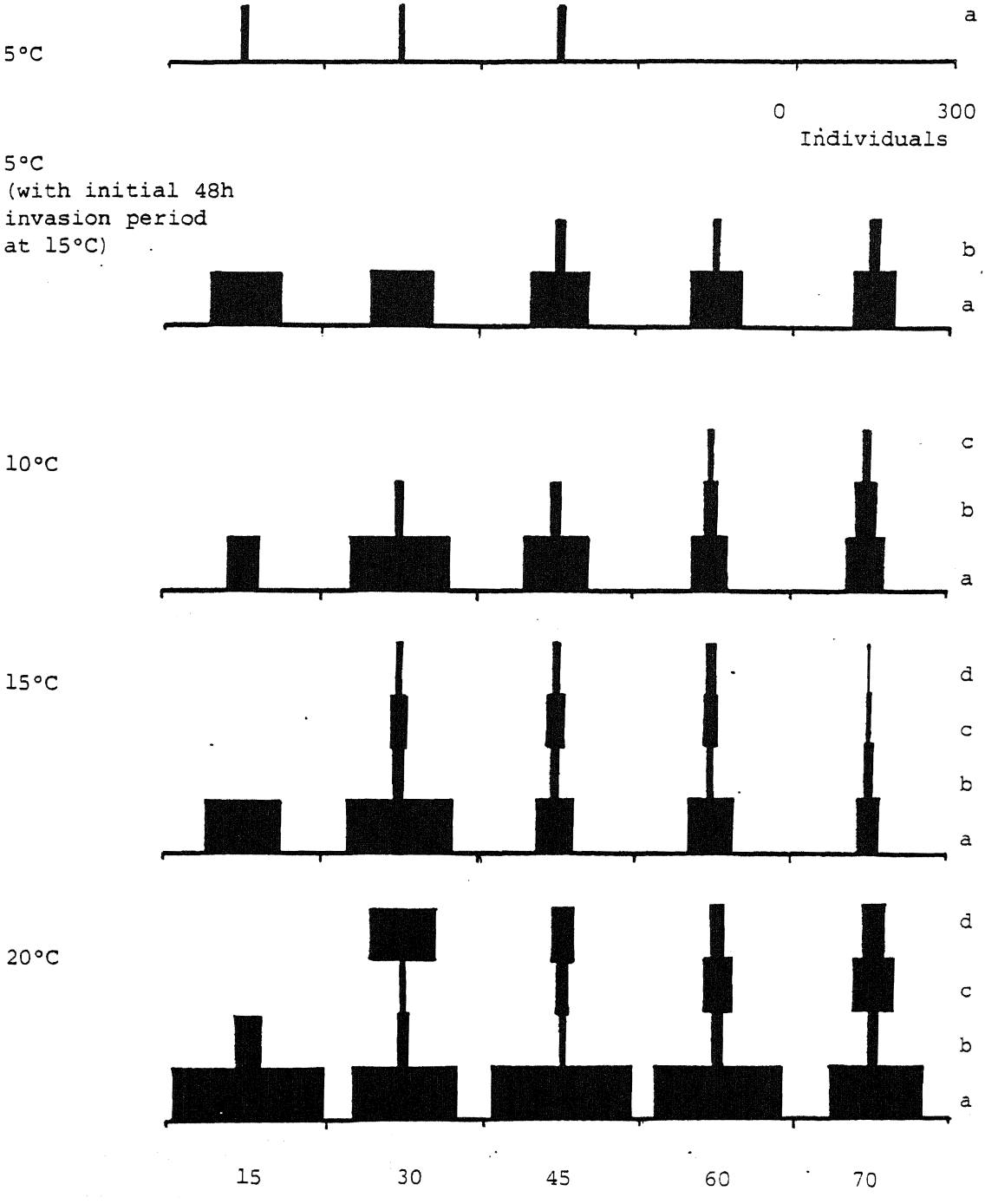


Figure 6 **Numbers of H. schachtii second stage juveniles (a), third stage juveniles (b), fourth stage juveniles (c) and adult stages in the roots at 5, 10, 15 and 20°C**



invading at 20°C. Heterodera cruciferae invasion rates did not increase much at higher temperatures and were similar at 15 and 20°C.

Individuals of all stages of H.schachtii were present after 30 days at 15°C and all stages of H.cruciferae after 45 days at this temperature. At 20°C, a larger number of H.schachtii than H.cruciferae adult stages had developed by 30 days, and indicating that H.schachtii develops more rapidly in the roots at this temperature.

Table 1: Development of H.cruciferae and H.schachtii at two temperatures

(Figures are the number of days elapsed between inoculation and the hatching of F1 juveniles)

	15°C	20°C
H.cruciferae	71	52
H.schachtii	66	46

Table 1 shows that F1 juveniles in eggs of females developing at 20°C hatched on average 19.5 days earlier than those developing at 15°C. The number of days elapsed between inoculation and the hatching of F1 juveniles represents the time taken to complete one generation. In Fig. 5 at 20°C an increase in the number of H.cruciferae second stage juveniles at 60 days is probably due to the invasion of roots by juveniles hatching from eggs produced by the first generation, which would be completed by this time. Evidence for a second generation in the H.schachtii population is less clear as large numbers of second stage juveniles were recorded throughout the 70 day period.

No cysts had been produced at either 5 or 10°C by 70 days but Fig. 7 shows the number of cysts produced per plant by both species at 15 and 20°C and Table 2 shows the numbers of eggs per cyst and the multiplication rates of H.cruciferae and H.schachtii at the two temperatures.

Figure 7 Mean number of cysts produced per pot by *H. cruciferae* and *H. schachtii* at 15°C and 20°c.

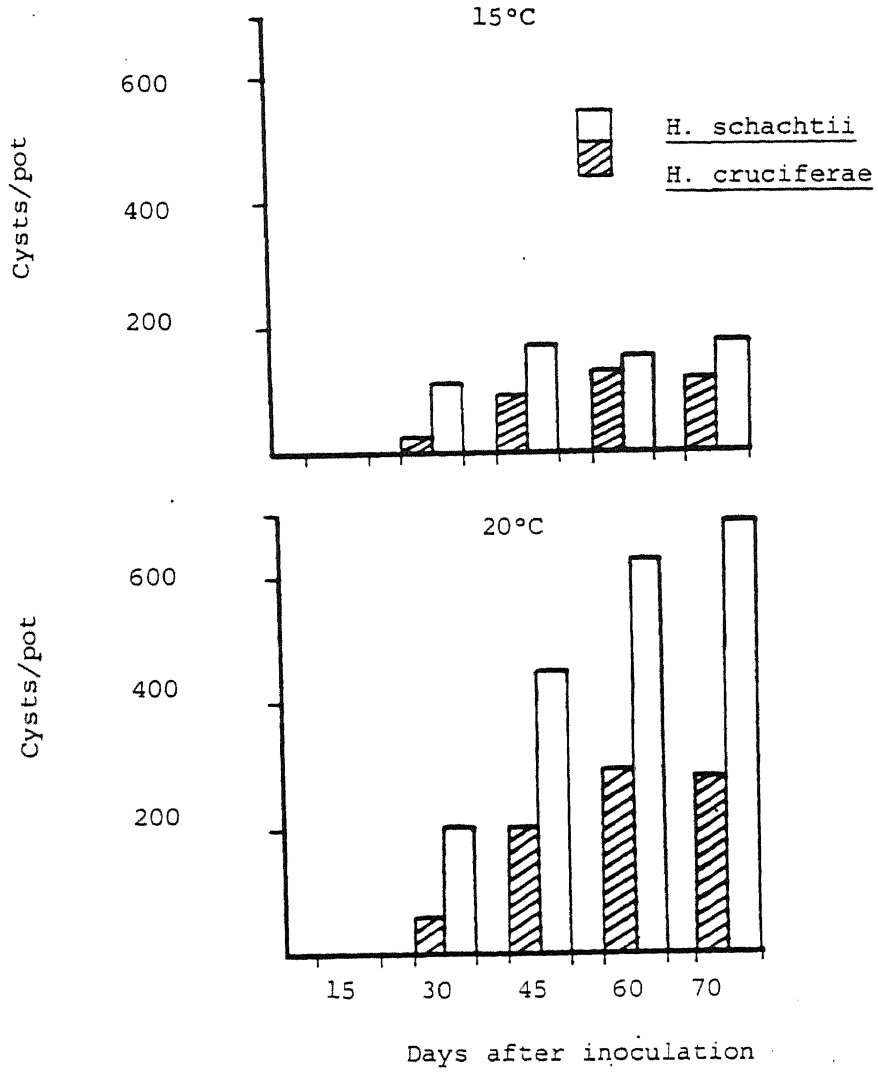


Table 2: Numbers of eggs/cyst produced by H.cruciferae and H.schachtii after 70 days at 15 and 20°C. Multiplication rates

(total eggs produced per plant/number of juveniles inoculated) in brackets.

	15°C	20°C
H.cruciferae	80.0 (3.3)	102.5 (10.6)
H.schachtii	88.1 (7.7)	136.5 (47.6)

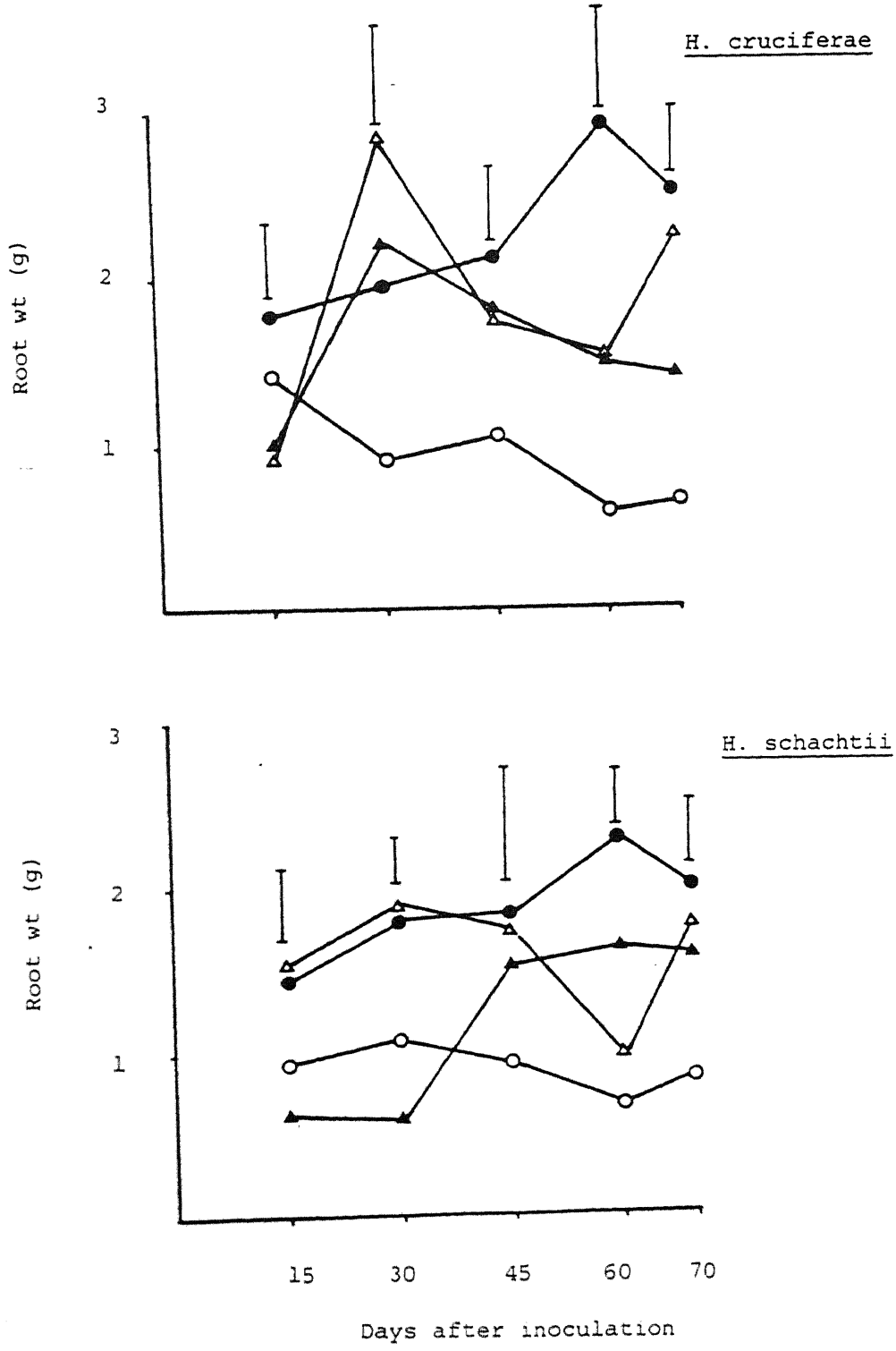
Cysts were found in the soil at both 15 and 20°C by 30 days. The eggs would still have been immature at this time. A larger number of cysts containing more eggs were produced at 15°C by H.schachtii than H.cruciferae. This difference was much greater at 20°C, with 697 H.schachtii and 299 H.cruciferae cysts being recorded. The H.schachtii cysts contained the most eggs and the population had a multiplication rate 4.49 x that of the H.cruciferae population.

Figure 8 shows the relative growth of infected roots at the four temperatures. Uninfected plants could not be included as controls at each temperature as the size of growth cabinets restricted the number of pots in each trial. A large variation in root weight was found among plants and few consistent differences could be identified. Many of the fine fibrous roots may have been lost on removing the plants from pots, resulting in smaller root weights. Roots infected with either species generally grew better at 15°C than did infested roots at 20°C. The root growth of infected plants growing at 5 and 10°C was intermediate.

DISCUSSION

The hatching tests showed that H.schachtii hatched more readily than H.cruciferae in oilseed rape leachate. This readiness to hatch was further shown by H.schachtii having a large hatch in distilled water and probably reflects the ability of H.schachtii to utilise a wide host range whereas H.cruciferae has a narrower host range (Winslow, 1954). The greatest number of H.schachtii juveniles hatched at 20°C although the optimum temperature for hatching is probably above 20°C

Figure 8 Root weights of plants infested with nematodes at four temperatures; ▲ 5°C, △ 10°C, ● 15°C, ○ 20°C. Vertical bars represent the L.S.D. (P = 0.05)



(Wallace, 1955; Maas & Heijbroek, 1982; Cooke, 1985). The greatest number of H.cruciferae juveniles hatched at 15°C and this compares to the optimum hatching temperature between 16 and 20°C reported by Koshy & Evans (1986). In the most dilute leachate more H.cruciferae juveniles hatched than in distilled water whereas a similar number of H.schachtii juveniles hatched in either the most dilute leachate or distilled water. The small hatch of H.cruciferae in distilled water agrees with the results of Winslow (1955) and indicates that H.cruciferae is strongly dependent on host root diffusates for hatching to occur. The dilution of root leachate may imitate the reduction in the production and activity of diffusate by older plants (Widdowson, 1958; Perry et al., 1980; Tefft & Bone, 1985). The readiness of H.schachtii to hatch would ensure that larger numbers of juveniles could hatch in the presence of a weaker stimulus later in the growing season whereas comparatively fewer H.cruciferae juveniles would hatch. However, the relationship between diffusate production and different age oilseed rape plants requires further study.

Table 3: Ten year monthly mean soil temperatures (°C at 10cm depth) (Humberside, E Anglia, S England regions)

S	O	N	D	J	F	M	A	M	J	J
13.8	9.9	6.3	3.8	2.6	2.7	4.6	7.3	11.5	15.5	17.6

Both hatching and invasion are strongly influenced by temperature. Hatching and invasion will occur in the warm soils of September and October, when the oilseed rape crop is establishing (Table 3). Over winter, when soils are below 5°C for long periods, hatching and invasion will be minimal except in response to any brief warmer periods although soil temperatures are unlikely to rise much in response to brief periods of higher air temperatures. Lewis (1971) reported little invasion of cabbage seedlings over winter by H.cruciferae and Koshy & Evans (1986) found invasion rates to be low at 8°C. When soil temperatures increase in the spring hatching and invasion rates will increase accordingly, and Koshy & Evans (1986) point out that this probably results in a flush of second stage juveniles in the roots which come from the residual soil population rather than a generation which has matured on the crop.

The development of H.cruciferae and H.schachtii proceeds very slowly at 5°C, which is below the basal temperature assumed by Koshy & Evans (1986). Lewis (1971) also reported that H.cruciferae developed over winter on cabbage, as indicated by an increase of the number of cysts on the roots. Similar reports of over winter development by H.avenae and H.goettingiana have been made (Kerry & Hague, 1974; Beane & Perry, 1984). Soil temperature may be below 10°C for two-thirds of the growing season and the ability of H.cruciferae to develop slightly faster at 10°C may be more important than the superior rate of development of H.schachtii at higher temperatures. The shorter generation time of H.schachtii is a result of earlier initial invasion and indicates that H.schachtii juveniles become physiologically active earlier than H.cruciferae. The larger number of eggs recorded per H.schachtii female suggest that this species may be more fecund and this would contribute to its superior multiplication rate. However, it is unlikely that H.schachtii will be able to complete more than two generations on winter oilseed rape in the UK although the second generation may be more mature than that of a second H.cruciferae generation at harvest. Vinduska (1972) predicted that three generations of H.schachtii may develop on winter oilseed rape but a longer period of plant growth allowed time for more nematode development.

The results obtained suggest that H.schachtii is better able to exploit warmer soils than H.cruciferae. This is similar to the different responses to temperature of Globodera pallida and G.rostochiensis (Franco, 1979). In New Zealand this was related to an adaptation by a G.pallida population to the cool conditions when potatoes were grown as a winter crop. Heterodera schachtii may be better adapted to the warmer regime of the sugar beet growing season whereas H.cruciferae has a history of association with traditionally grown brassica crops (Savage, 1979) many of which like cauliflowers may be winter sown. In other laboratory experiments H.schachtii invades and develops most rapidly at soil temperatures of 21-27°C (Raski & Johnson, 1959; Thomason & Fife, 1962) and multiplication may not have been studied at the optimum temperature for this species in this experiment. Heterodera schachtii may prove to be the more damaging of the two species to oilseed rape, especially if soils are warm for long periods after the crop is sown in September, and when seedlings are at their most vulnerable to nematode infection. The prediction of the development of H.cruciferae on oilseed rape agrees

closely with that of Koshy & Evans (1986). However, the continued development of H.cruciferae at 5°C suggests that the basal development temperature is below that suggested by these workers. If the basal temperature was assumed to be 4°C then 773 day degrees would be accumulated per generation rather than 680 day degrees. At 8°C and 12°C H.cruciferae took 224 and 105 days respectively to complete a generation (Koshy & Evans, 1986). In this experiment sufficient time was not allowed for the completion of a generation at either 5°C or 10°C. However, at 15°C and 20°C H.cruciferae required a mean of 806 day degrees above a basal temperature of 4°C per generation whilst H.schachtii required 731 day degrees per generation.

Between 15 September and 15 July, (the date at which root invasion is likely to begin and the earliest likely date of harvest) 1298 day degrees would be accumulated (based on soil temperatures in Table 3). On this basis H.cruciferae and H.schachtii could complete 1.61 and 1.77 generations respectively. A large proportion of the time taken to complete a generation is that required for eggs to mature, so second generation cysts would have been produced by harvest time but no juveniles would hatch from them. In warmer years (when more day degrees are accumulated) or with later harvest, the second generation will have matured and juveniles hatched leaving smaller population densities in the soil. Therefore, synchrony (or lack of it) between the completion of a generation and the harvest of a crop in some years may complicate the prediction of population growth and potential yield losses.

3

The contribution of egg sacs to the population dynamics of *Heterodera cruciferae* and *H.schachtii*

INTRODUCTION

The egg sac, consisting of a gelatinous matrix into which eggs are deposited, is a feature of many species of Heterodera (Jones 1950). Franklin (1943) first reported the occurrence of egg sacs on cysts of H.cruciferae and found that they contained up to 200 eggs. The significance of egg sacs produced by H.schachtii was discussed by Thorne (1935) and Raski (1950) who reported a maximum of 131 eggs to be deposited in the egg sac (about 17% of the total eggs produced). Jones (1950) ranked the common cyst nematodes found in the UK on the basis of the numbers of eggs extruded into egg sacs. Heterodera carotae and H.cruciferae had the largest egg sacs with up to half of the eggs found in egg sacs. Large numbers of H.schachtii eggs were only found in egg sacs that were attached to the larger cysts. Large numbers of eggs have also been reported in the egg sacs of H.glycines, H.trifolii, H.cajani and H.mothi (Hirschmann, 1956; Koshy & Swarup, 1971; Khan & Jairajpuri, 1975). Despite the many reports of the occurrence of egg sacs on Heterodera spp. little information is available on the development and fate of eggs contained within egg sacs. In H.cruciferae the egg sac is produced by the cells of the uterine wall (McKintosh, 1960). This contrasts with production of the gelatinous matrix by the rectal glands in Meloidogyne (Maggenti & Allen, 1960). Juveniles hatch more readily from eggs within egg sacs of H.cruciferae than from cysts (Koshy & Evans, 1986) but it is not known whether this is due to differences in the maturation rates of juveniles in cysts and egg sacs or to differences in the physiology of juveniles in cysts and egg sacs. Juveniles from egg sacs of H.glycines hatch readily and in favourable conditions were assumed to be a source of infective juveniles for consecutive progeny (Ishibashi, et al., 1973). This is also shown by the increase in the number of H.schachtii second stage juveniles in the roots of sugar beet that occurred after the formation of egg sacs (Tacconi, 1979). The rapid hatch of H.carotae and H.cruciferae juveniles from egg sacs is also suggested to facilitate the earlier re-invasion of the roots (Greco, 1981; Koshy & Evans, 1986).

The egg content of egg sacs in the soil probably contributes greatly to H.cruciferae population density (Harris & Winfield, 1985). However, attempts to measure the contribution of egg sacs to the population dynamics have rarely been made due to difficulties in their

extraction. Sykes & Winfield (1966) failed to find a reliable method for their extraction and Koshy & Evans (1986) suggested that a method was needed to extract egg sacs from the soil, either free or attached to cysts, to enable their persistence to be assessed.

A method suitable for extracting egg sacs from the soil would probably have to accompany a standard method of cyst extraction to give a reliable estimate of population density. For advisory work the increase in cost and time per sample might be unacceptable. This study was undertaken to provide information on the contribution of egg sacs to the population biology of H.cruciferae and H.schachtii on oilseed rape and to evaluate the need for another extraction method.

MATERIALS AND METHODS

Newly formed females and egg sacs were used in all experiments. One hundred and twenty pots (10cm diameter) were filled with a mixture of loam, sand and gravel (3:1:1) and sown with three seeds of oilseed rape cv. Jet Neuf. Plants were grown for 28 days in a glasshouse with a day temperature of 20°C and night temperature of 10°C. Seven days after sowing plants were thinned to one healthy seedling per pot. After a further 21 days, the pots were transferred to growth cabinets at 15°C and 12 hour day and night periods. Each plant was then inoculated with 1000 juveniles which had been released from freshly macerated field cysts. The juveniles were added to each pot in 2ml of distilled water by pipetting the suspension into the soil at four locations to a depth of 2-4cm. Sixty pots were inoculated with H.cruciferae juveniles and 60 with H.schachtii juveniles. The plants were watered as required and fed weekly with a liquid N,P,K fertiliser.

i) Development of nematodes and egg deposition in egg sacs

Two pots containing H.cruciferae and two containing H.schachtii were removed from the growth cabinets 42 days after the juveniles had been added and thereafter at 5 day intervals on a further seven occasions. The soil and root material was removed from each pot and gently mixed by hand. A 200g sample of soil and root material was added to 400ml of water in a 500ml beaker and stirred gently with a glass rod before

pouring the suspension through a 250 micron sieve. The soil and root material was washed with a further two x 400ml aliquots of water before transferring material retained on the sieve into a filter funnel containing a Whatman No. 1 filter paper and allowing the water to drain away. Preliminary tests confirmed that females and egg sacs accumulated in the sediments towards the centre of the filter paper (Sykes & Winfield, 1966). The sediments were therefore removed with a spatula and added to 2-4ml of water in a 9cm petri-dish. Females with attached egg sacs were then picked from the resulting suspension of sediment and root material using fine forceps under a strongly illuminated binocular microscope.

On each occasion, the number of eggs contained within either the female or the egg sac and the proportion of these eggs that contained fully developed second stage juveniles were recorded in each of 25 randomly selected females and attached egg sacs. Juvenile development was assessed by examining at least 100 eggs. Photographs were taken to record the size, form and positioning of egg sacs relative to the female and the roots.

ii) Hatching from cysts and egg sacs

Sixty two days after inoculation, cysts and egg sacs were collected from 10 plants infected with H.cruciferae and 10 plants infected with H.schachtii. Brown cysts with egg sacs were selected and stored for 12 hours in cold water (5°C) to prevent any premature hatching. The egg sacs were separated from the cysts by gently teasing them apart with fine forceps.

Leachate was taken from a bulk solution stored at 5°C which had been collected from 28 day old oilseed rape plants cv. Jet Neuf by the method described previously. A dilution series was prepared by adding distilled water to give concentrations of 1:1, 1:4, 1:16, 1:64 and 1:256. Cysts and egg sacs were counted into batches of 25 and three replicate batches were added to 2ml of each concentration. In addition, three replicate batches were added to 2ml of distilled water. Hatching tests were carried out in the dark at 15°C.

The numbers of juveniles which hatched from each batch of cysts or egg sacs were counted daily for 4 weeks. The leachate solutions were replaced with fresh solutions weekly. After 4 weeks the numbers of unhatched juveniles in each batch was counted by the standard method and the percentage hatch calculated.

iii) Invasion from cysts and egg sacs

Cysts and egg sacs were collected from 20 plants 62 days after inoculation infected with either H.cruciferae or H.schachtii and stored in cold water (5°C) to prevent juveniles hatching prior to inoculation. Cysts and egg sacs of each species were separated and grouped into batches as follows: 15 batches of 25 cysts; 15 batches of 25 egg sacs; 15 batches of 25 cysts with attached egg sacs. This provided inocula of 1625, 875 and 2500 H.cruciferae eggs and 1750, 625 and 2375 H.schachtii eggs respectively.

Twenty-eight days prior to preparation of the inocula, 90 pots (10cm diameter) were filled with a mixture of loam, sand and gravel (3:1:1) and sown with three seeds of oilseed rape cv. Jet Neuf, subsequently thinned to one plant per pot and grown in a glasshouse with a 20°C day and 10°C night temperature. For inoculation, the cysts and/or egg sacs were added to 2ml of distilled water before pipetting them at four locations, 2-4cm deep, around the root system. Plants were left in the glasshouse and were watered as required.

After 3,6,9,12 and 15 days, three plants of each treatment were gently removed from their pots and the roots washed, blotted dry and weighed. The numbers of juveniles that had invaded the root systems were counted by staining and releasing the nematodes from the roots by the standard method.

iv) Persistence of cysts and egg sacs

The soil and root material from the remaining 14 pots of each species was bulked and gently mixed 62 days after inoculation with juveniles. The numbers of full eggs/cyst and full eggs/egg sac of each species were estimated from the contents of cysts and egg sacs collected from three x 100g soil samples by the standard method. The bulked soil

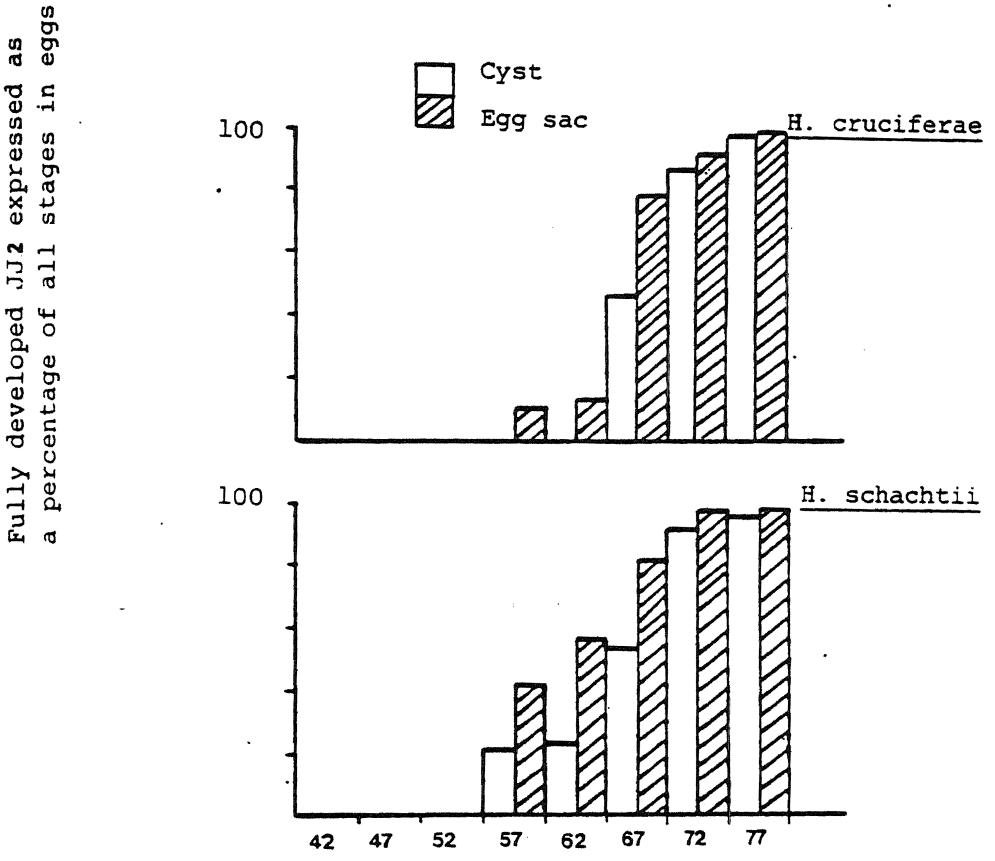
containing each species was divided into two fractions each of approximately 4Kg of soil. The fractions were then stored at either 15°C or 5°C. After 2,4,6,8 and 10 weeks of storage the numbers of full eggs/cyst and full eggs/egg sac of each species were again estimated from three 100g soil samples on each occasion.

RESULTS

i) Development of nematodes and deposition of eggs in egg sacs

Eggs were found in both females and egg sacs at 42 days but none contained fully embryonated juveniles. The percentage of eggs containing fully-formed second stage juveniles is shown in Fig. 9. Second stage juveniles were first recorded in H.schachtii females and egg sacs and in egg sacs of H.cruciferae 57 days after juveniles had been inoculated. Second stage juveniles of H.cruciferae were not observed in H.cruciferae females until 67 days. More H.schachtii second stage juveniles were found in egg sacs than in females at 57 days, suggesting that juvenile development was slightly advanced in egg sacs. The earlier appearance of juveniles in H.schachtii reflects the slightly shorter period that it takes to complete a generation as shown in the previous chapter. This shorter period was mainly due to faster initial invasion and may not represent differences in maturation rates of the two species. However, there are differences between the juvenile maturation within females and egg sacs. Fig. 10 shows the percentage change in the mean numbers of eggs/female and eggs/egg sac. The mean number of eggs within females and egg sacs at 42 days is represented as 100% because there were no large increases in the number of eggs produced after this time and none of the immature juveniles they contained were able to hatch before this time. The largest percentage decrease between samplings in the egg content of H.schachtii females and egg sacs was recorded for the period between 57 and 62 days. The number of juveniles in H.cruciferae egg sacs also began to decline after this time whereas the number of juveniles within H.cruciferae females did not begin to decline until after 62 days. These changes occurred at the same time as second stage juveniles matured within females and egg sacs as shown in Fig. 9. The largest overall decreases were 71.8% and 63.0% for H.schachtii and H.cruciferae respectively and were recorded for eggs within egg sacs, because a large number of juveniles had hatched from them.

Figure 9 Deposition and development of eggs in cysts and egg sacs



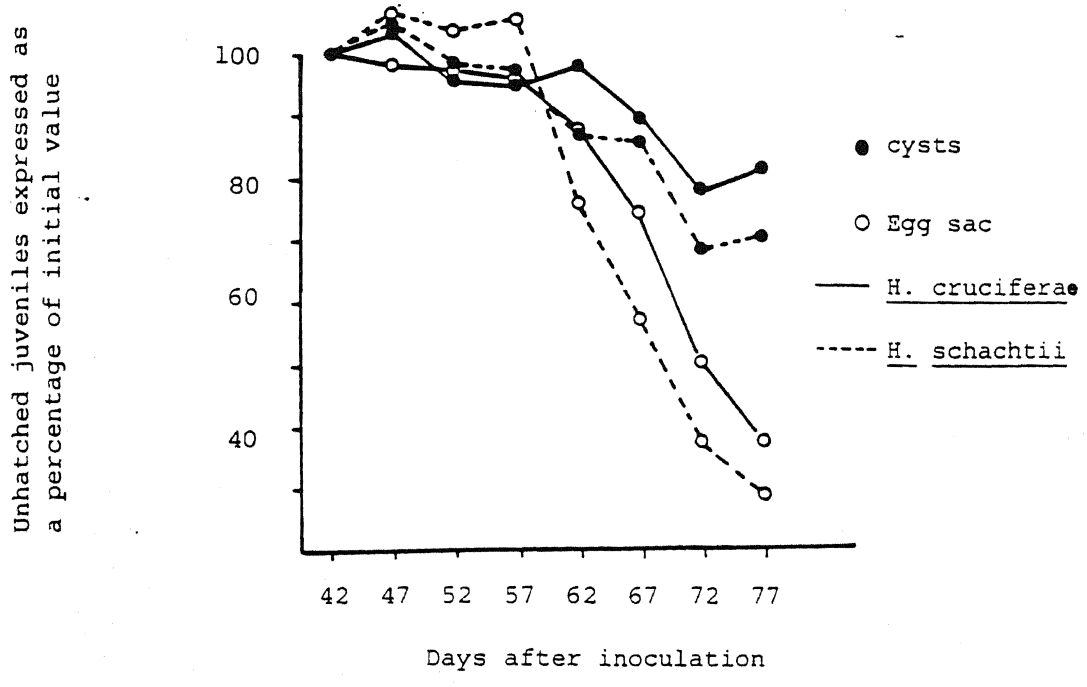


Figure 10 Changes in the number of eggs/cyst and eggs/egg sac.

Approximately 70% of the juveniles within H.cruciferae and H.schachtii females remained unhatched.

Table 4 gives the mean number of eggs recorded within either females or egg sacs and expresses the number of eggs found within the egg sac as a percentage of the total number of eggs produced. The mean values were based on counts recorded at 42,47,52 and 57 days. Data from 62 days onwards was discarded because a large number of juveniles matured and hatched after 57 days. The means are therefore, based on counts from 100 individual females and egg sacs of each species.

Table 4: Distribution of eggs between cysts and egg sacs of H.cruciferae and H.schachtii (Means ± S.E.)

	Eggs/Cyst	Eggs/egg sac	Percentage in egg sac
H.cruciferae	103.7 ± 21.6	68.2 ± 15.0	39.7
H.schachtii	139.5 ± 17.4	70.9 ± 19.6	33.7

ii) Hatching from cysts and egg sacs

The percentage of juveniles which had hatched after four weeks from eggs, in cysts and in egg sacs at each concentration of leachate are shown in Fig 11 and the rate of hatching from cysts and egg sacs in the most concentrated leachate solution is given in Fig 12.

Few juveniles of either species hatched from cysts in any leachate solution: the maximum hatches were 10.1% and 12.0% from H.cruciferae and H.schachtii cysts respectively, which was small in comparison to hatches of 27.9% and 38.1% from H.cruciferae and H.schachtii field cysts respectively described in Chapter two. In contrast, large numbers of juveniles hatched from egg sacs with maximum hatches of 73.2% and 71.5% from H.cruciferae and H.schachtii egg sacs in the 1:1 leachate concentration. The dilution of leachate decreased hatching from egg sacs by a small amount. Between 45% and 60% of juveniles hatched in the 1:16, 1:64 and 1:256 leachate concentrations. In distilled water 57.7% and 41.3% of juveniles hatched from H.cruciferae and H.schachtii egg sacs respectively. Juveniles hatched rapidly from egg sacs of both species (Fig. 12), a large percentage hatch occurring

Figure 11 Percentage hatch after four weeks of H. cruciferae and H. schachtii from egg sacs and cysts in five concentrations of oilseed rape leachate. (● cyst, ○ egg sac, ——— H. cruciferae, ----- H. schachtii) Vertical bars represent the L.S.D. (P = 0.05)

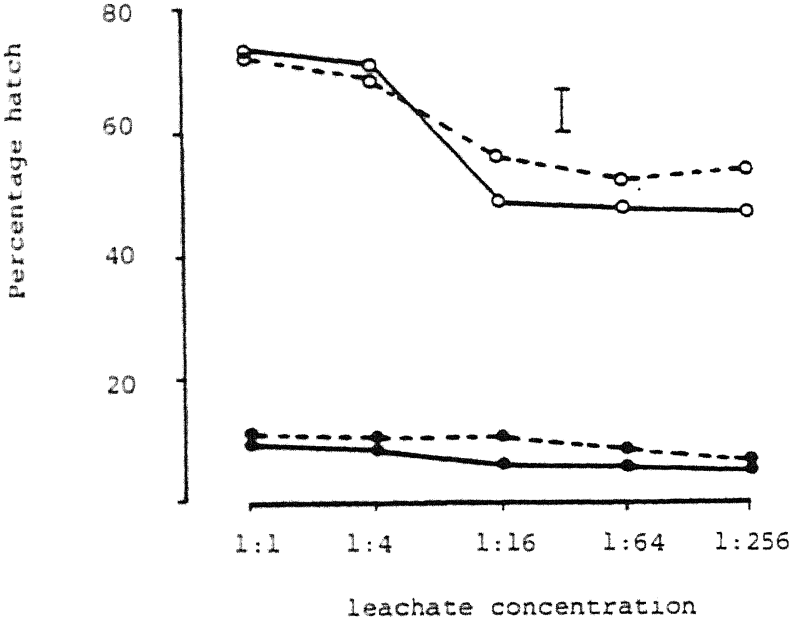
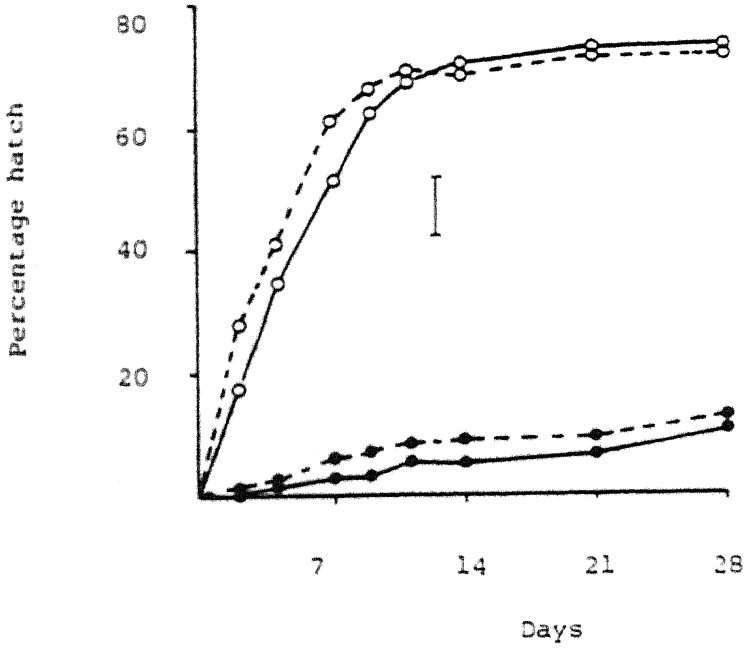


Figure 12 Hatching rate of eggs in Cysts and egg sacs in 1:1 root leachate concentration (legend as for Fig. 11)



within 14 days. The hatching rate of juveniles in cysts was low; few juveniles had hatched within the 28 day period.

To compare the hatching of juveniles from young and old cysts a further identical hatching test using a 1:1 leachate concentration was performed 4 months later with cysts from the same populations. Percentage hatches of 24.7% and 33.5% from H.cruciferae and H.schachtii respectively demonstrated that juveniles hatched more readily from older cysts.

iii) Invasion from cysts and egg sacs

The numbers of juveniles that had invaded the roots at each date were expressed as percentages of the eggs added in cysts, egg sacs or cysts with egg sacs, and are shown in Fig. 13 for H.cruciferae and H.schachtii. Invasion by juveniles of both species occurred both earlier and in larger numbers from egg sacs. The largest numbers of juveniles were observed in roots harvested at 15 days, although 2.5% and 2.1% of the H.cruciferae and H.schachtii inoculum were detected in the roots only 3 days after adding egg sacs to plants. Invasion from H.cruciferae cysts was first recorded 9 days after cysts had been added but invasion from H.schachtii cysts was earlier at 6 days. At 15 days 3.8% and 10.2% of the H.cruciferae and H.schachtii juveniles from cysts had invaded the roots. Invasion from cysts with attached egg sacs was intermediate for both species as the greater invasion from egg sacs represented a smaller percentage of the overall larger inoculum. The root weights of plants collected on each occasion did not vary between treatments and was considered not to influence the extent of invasion from any source.

iv) Persistence of cysts and egg sacs

The number of full eggs/cyst and full eggs/egg sac in soil bulked from the remaining 20 pots of each species was taken as representing 100%. Subsequent changes in the numbers of full eggs were calculated as percentages of this initial value and are shown in Fig. 14. The number of full eggs/egg sac of each species in soil stored at 15°C declined more rapidly than the number of full eggs/cyst of each species at 15°C between 2 and 10 weeks. After 10 weeks at 15°C, only 15.0% of

Figure 13 Invasion of roots by juveniles from Cysts and egg sacs of H. cruciferae and H. schachtii (● cysts, ○ egg sacs, △ cysts and egg sacs). Vertical bars represent the L.S.D. (P = 0.05)

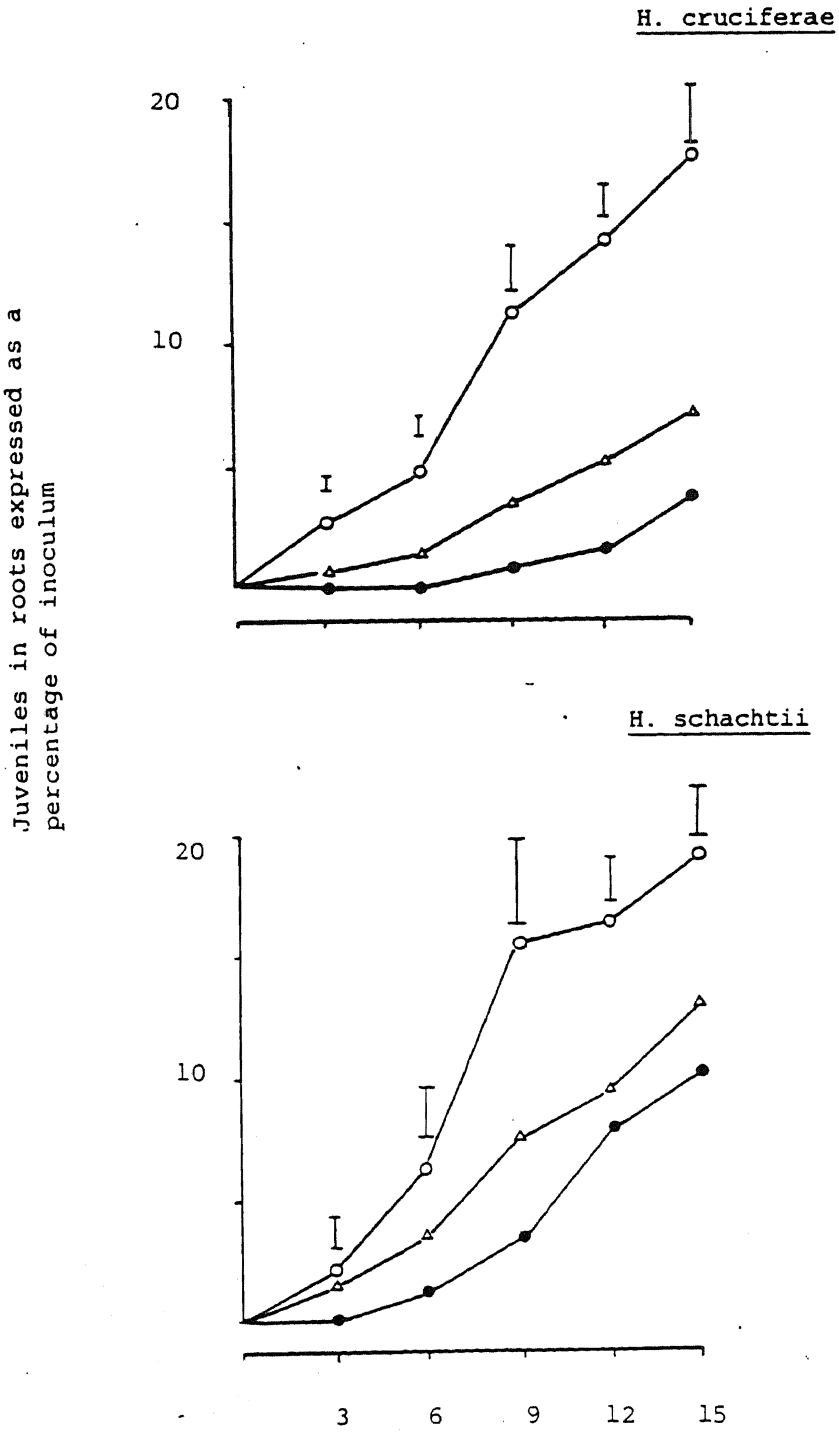
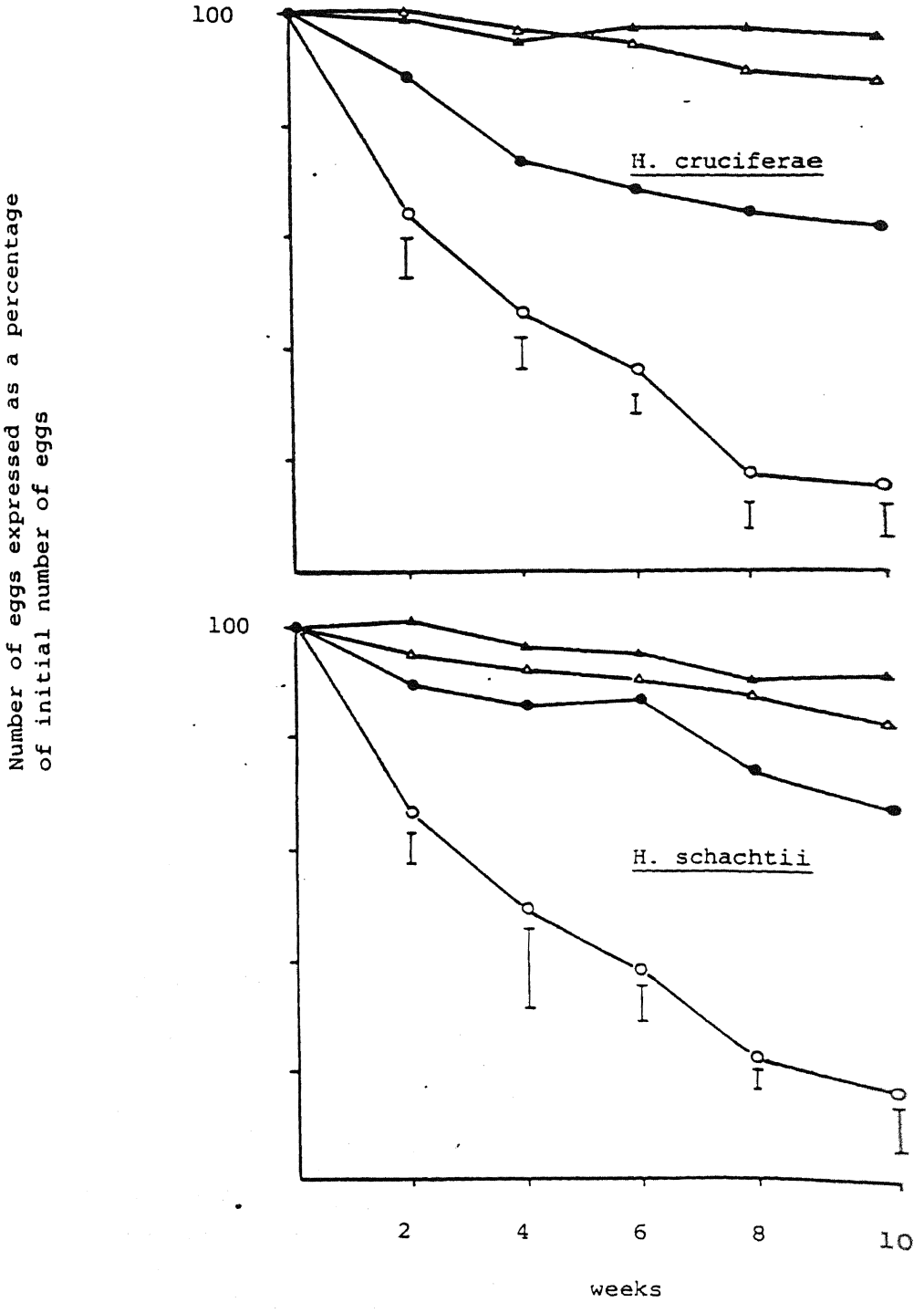


Figure 14 Changes in the number of eggs/cyst and eggs/egg sacs in fallow soil at 5°C and 15°C. (○ egg sacs at 15°C, ● cysts at 15°C, ▲ cysts at 5°C, △ egg sac at 5°C). Vertical bars represent the L.S.D. (p = 0.05).



H.cruciferae and 16.5% of H.schachtii eggs within egg sacs still contained juveniles whilst 61.0% of H.cruciferae and 67.0% of H.schachtii eggs within cysts contained juveniles after this time. After 10 weeks at 5°C only small changes in the numbers of full eggs of each species were detected in either cysts or egg sacs.

DISCUSSION

Egg sacs that are produced by females of H.cruciferae and H.schachtii on oilseed rape can be extracted from the soil and the roots of the host by a careful decantation and sieving technique. Standard methods of cyst extraction result in the egg sac becoming dislodged and left behind either on the root or in the soil. For this reason it is difficult to ascertain the fate of juveniles in egg sacs. A less vigorous extraction technique allowed many of the egg sacs to remain attached to cysts. Egg sacs were recorded on approximately 60-80% of all the cysts, suggesting that a large proportion of egg sacs were extracted. Adding cysts and egg sacs to soil in order to test the efficiency of the technique would give inaccurate results because the adhesion of egg sacs to roots would not be mimicked.

Egg sacs are found on both immature and mature females (Plates 1 and 2) with the size of the egg sac varying from a small gelatinous extrusion to almost twice the size of the cyst. The size of egg sacs was comparable to those of H.glycines, H.trifolii and H.cajani (Hirschmann, 1956; Koshy & Swarup, 1971). The egg sacs lie parallel to the root axis (Plate 3) and adhere quite strongly to both the cyst and the root. Very often the egg sac may become obscured by grains of soil which stick to it and occasionally two or more cysts may become clumped together by the coalescence of their egg sacs (Plate 4).

The more rapid hatch of H.cruciferae juveniles from egg sacs than from cysts agrees with the results of Koshy & Evans (1986). Hatching tests described here have shown that H.schachtii behaves similarly and that juveniles from egg sacs of both species hatch more readily than those from cysts in either distilled water or in weak diffusate. This is similar to reports for H.cajani, H.glycines, H.mothi, H.oryzae and H.carotae (Koshy & Swarup, 1971; Ishibashi et al., 1973; Khan & Jairajpuri, 1975 and Rao & Jayaprakesh, 1977; Greco, 1981). The ability of juveniles in egg sacs to hatch more readily and earlier

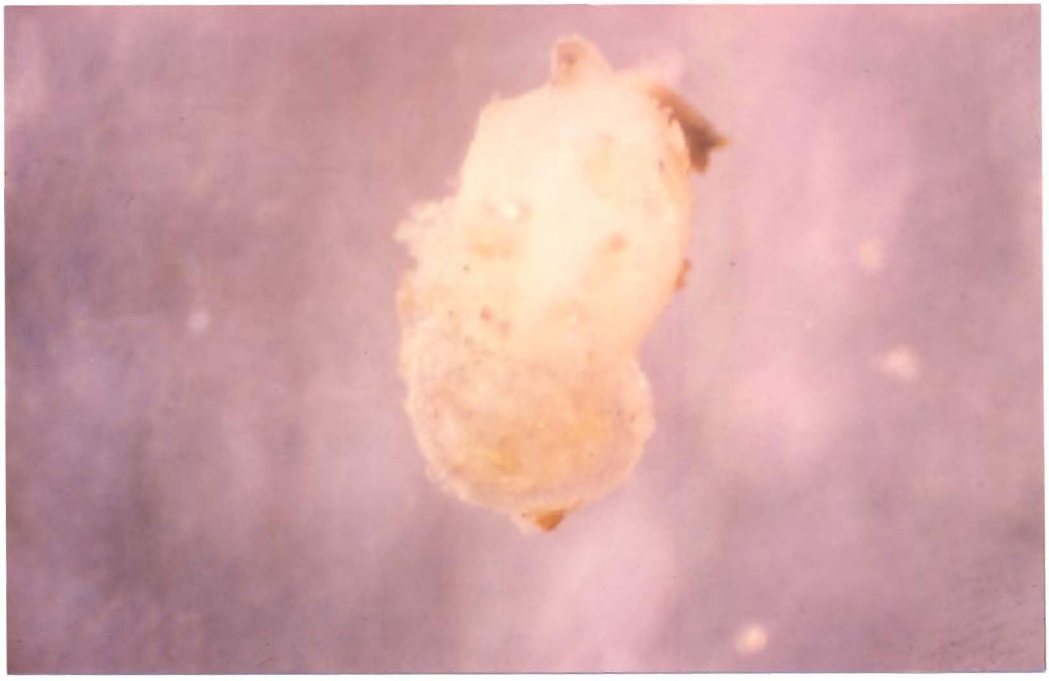


Plate 1. Immature H. cruciferae female with egg sac.

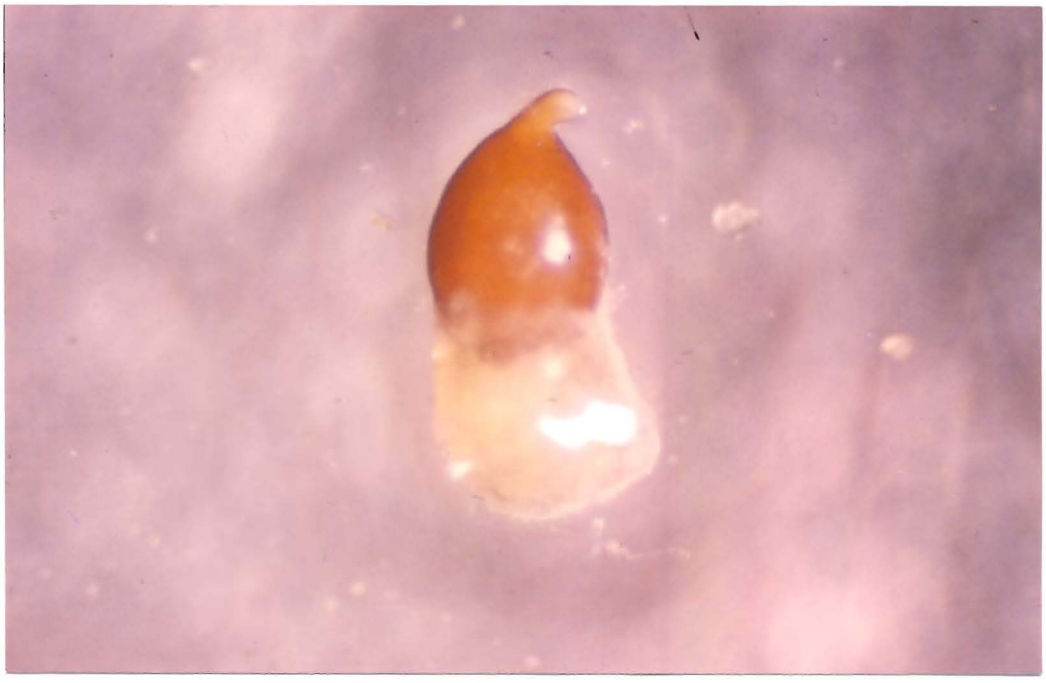


Plate 2. Mature H. cruciferae female with egg sac.

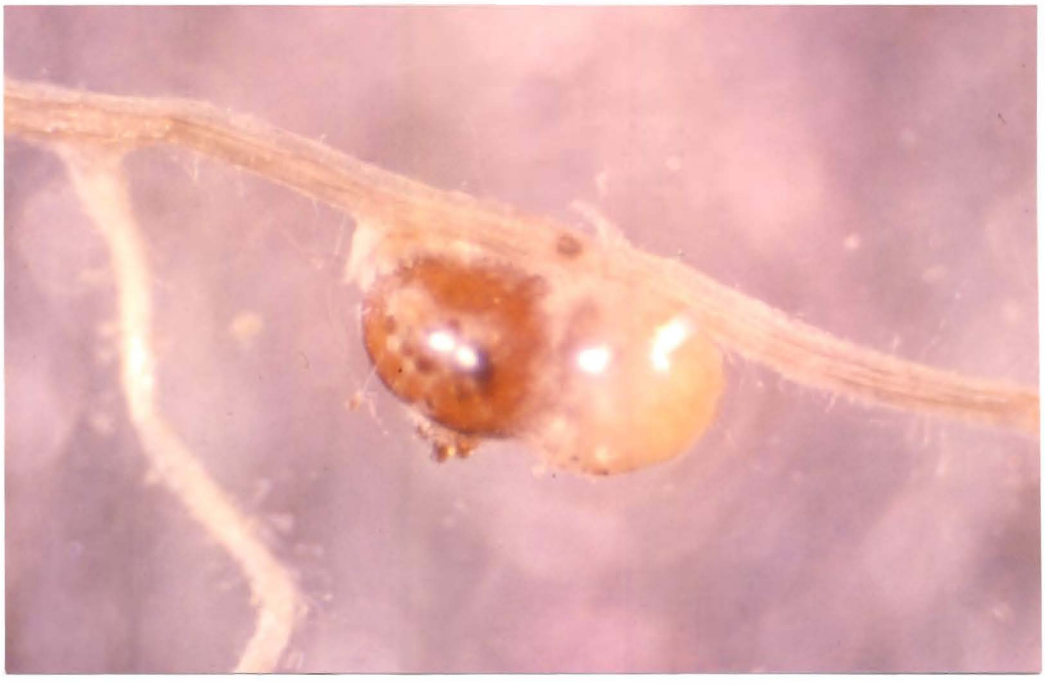


Plate 3. Position of H. cruciferae female with egg sac on the root.

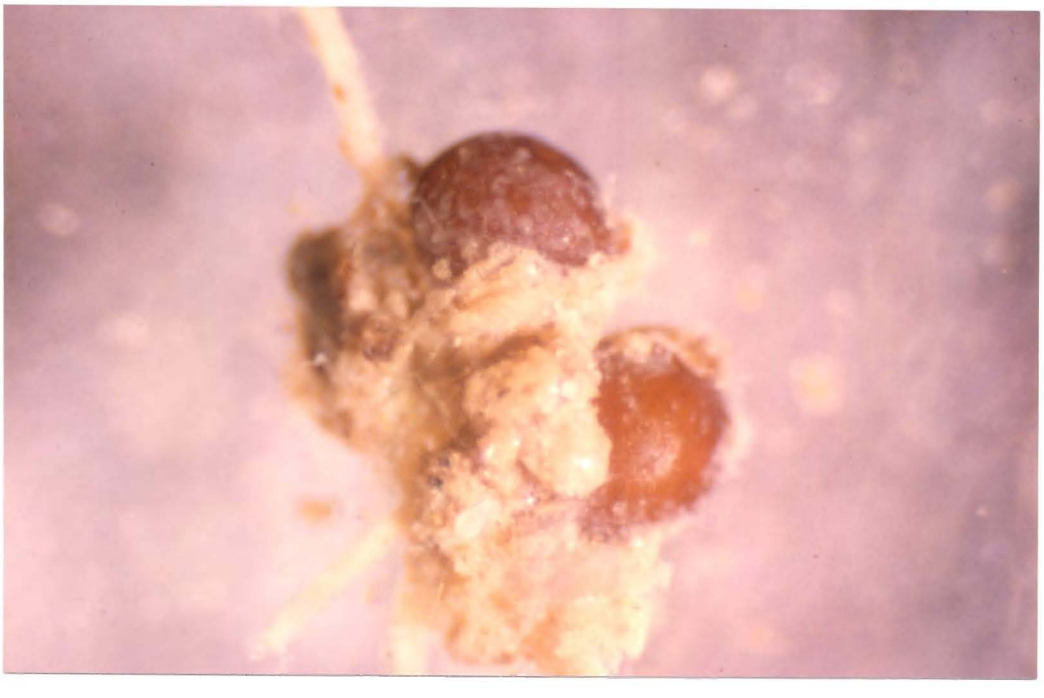


Plate 4. Coalescence of two H. cruciferae females with egg sacs. Egg sacs obscured by soil particles.

than juveniles in cysts means that a large number of juveniles will be able to quickly re-invade the host. This was shown by the results of the invasion tests for both H.cruciferae and H.schachtii. Juveniles from the egg sacs of H.glycines were also found to invade the host roots earlier than juveniles from cysts (Ishibashi et al., 1973). Egg sacs, therefore, provide a method by which consecutive generations may occur rapidly, one after another. In favourable conditions this will allow much nematode multiplication. On a winter sown crop of oilseed rape an invasion from F1 egg sacs would allow most H.cruciferae and H.schachtii juveniles to complete two generations within the growing season. Few juveniles hatched from newly formed females exposed to root diffusate in comparison to four month old cysts and older field cysts. Greco (1981) found that H.carotae behaved similarly in this respect and only after two months was there a substantial hatch from eggs in newly formed cysts. This may be a mechanism for the longer term persistence of the nematode. Juveniles within egg sacs matured more rapidly than those within cysts. The temporal advantage gained by the earlier developing juveniles of H.schachtii was small whereas the developing juveniles of H.cruciferae gained an advantage of 10 days over those juveniles in cysts. This earlier maturation of juveniles in egg sacs will contribute to the more rapid hatching of these juveniles and also suggests that the first eggs produced by the female are extruded into the egg sac. The absence of a large hatch by juveniles within cysts suggests that the physiology of juveniles in cysts and egg sacs are different and an examination of their hatching mechanisms may explain their different hatching reponses.

The number of eggs deposited in the egg sac of H.glycines was small when plants were grown without fertiliser and under a short photoperiod (Ishibashi et al., 1973). The effect of environmental conditions on the extrusion of eggs into egg sacs of H.cruciferae and H.schachtii developing on oilseed rape is unknown but has been reported to influence egg sac development of H.cruciferae on cabbage (Mugniery, pers.comm.). This warrants further investigation on a winter crop such as oilseed rape when females are developing over winter months. However, a large proportion of H.cruciferae and H.schachtii populations may be contained within egg sacs. The number of eggs in H.cruciferae egg sacs did not approach the 50% suggested by Jones (1950) but was similar to that found by Koshy & Evans (1986). Larger numbers of eggs were found in H.schachtii egg sacs than was found by Jones (1950). Juveniles do not remain long in egg sacs if

temperatures are conducive to hatching with the results that egg sacs contain only a few juveniles after 10 weeks in the soil. If soil temperatures remain at below the threshold for hatching then the juveniles would be able to overwinter and contribute to the population which would then threaten a spring sown host crop or contribute to continual invasion of a winter sown crop.

For advisory purposes, egg sacs can probably be ignored in terms of predicting crop damage, and estimates of population densities based on counts of cyst-bound eggs will be reliable. However, assessment of multiplication rates of H.cruciferae and H.schachtii on oilseed rape and other crops should take the number of eggs within egg sacs produced by F1 and subsequent generations into consideration.

4

Comparison of the water content of unhatched H.cruciferae juveniles in cysts and egg sacs and the role of trehalose during the hatching process

INTRODUCTION

Juveniles only hatch readily from encysted eggs of H.cruciferae when stimulated by host root diffusates whereas a large number of juveniles may hatch from eggs within egg sacs in the absence of any host stimulus. The reliance of encysted eggs on a hatching stimulus is shown by a hatch of less than 3% in water whilst the numbers of juveniles hatching from egg sacs in water exceeds 50%. Hatching from egg sacs may be further increased by root diffusates. Recent work on G.rostochiensis and H.goettingiana shows that when encysted eggs are immersed in root diffusates, the water content of unhatched juveniles increases before eclosion (Ellenby & Perry, 1976; Perry et al., 1983). A further increase in the water content occurs immediately after hatching (Ellenby, 1974). By contrast, unhatched H.schachtii juveniles show no water uptake prior to eclosion (Perry, 1977). Differences between species have been discussed in relation to the amount of osmotic stress imposed on the unhatched juvenile by trehalose in the egg fluid.

As with H.cruciferae females, H.schachtii and H.goettingiana females also produce egg sacs into which a large number of eggs may be extruded (Jones, 1950). The large hatch that occurs from egg sacs in the absence of a host stimulus is a much reported characteristic of egg sacs yet the differing hatching characteristics of eggs in cysts and egg sacs and their underlying mechanisms have not been previously studied. Changes in water content of G.rostochiensis, H.schachtii and H.goettingiana juveniles during the hatching process have been examined using interference microscopy (Ellenby & Perry, 1976; Perry, 1977; Perry et al., 1980; Perry et al., 1983). Interference microscopy gives an accurate determination of the refractive index of individual nematodes from which the water content estimations may be derived (Ellenby, 1968). This technique is used in the present work to compare the water dynamics before and after hatching of H.cruciferae juveniles from cysts and egg sacs.

MATERIALS AND METHODS

Cysts and egg sacs of H.cruciferae were produced by inoculating pot grown seedlings of oilseed rape cv. Jet Neuf with H.cruciferae juveniles. Plants were grown for 56 days at 15°C before the tops of

plants were cut off and the soil containing newly formed cysts and egg sacs was removed and stored at 5°C for a period not exceeding 2 months. Oilseed rape root diffusate was taken from a stock solution which had been previously leached from 28 day old plants cv. Jet Neuf and stored at 5°C.

When cysts and egg sacs were required for experimental work they were extracted from the soil by the method described in the previous chapter.

i) Determination of the water content of unhatched juveniles after stimulation by root leachate and distilled water.

Cysts and egg sacs were taken from the soil and where possible comparisons were made between cysts and their attached egg sac. A batch of cysts with their attached egg sacs was placed in a cavity dish containing either full strength root leachate or distilled water at 15°C. The water content of 10 unhatched juveniles from cysts and egg sacs was determined after 0,1,2,3 and 4 days. On each occasion eggs were carefully released from a different cyst and egg sac and batches of eggs from each were placed on a glass slide in a drop of water. A cover slip was placed over the eggs and the slide positioned under a Vickers MAI interference microscope. Each egg was located under the microscope and the juvenile artificially hatched by applying pressure on the cover slip with a mounted needle. When necessary the juvenile was orientated by rotating the microscope stage so that the longitudinal axis of the juvenile was at right angles to the interference fringes. After focusing, a photograph was taken of the juveniles; all photographs were taken within 30 seconds of releasing the juvenile from the egg. The anterior of the juvenile was always photographed as the fringes were particularly distinct in this region (Ellenby, 1968).

ii) Determination of the water content of juveniles immediately after eclosion

Cysts and egg sacs were separated from one another by careful dissection and a batch of each were placed in cavity dishes containing root leachate at 15°C. Cysts were kept in root leachate for 5 days and

egg sacs for 4 hours and 3 days. After each period, five eggs were taken from a different cyst and egg sac and artificially hatched as previously described. Individual juveniles were then photographed at 0.5,1,2,4,6,8 and 10 minutes after being released from the egg to determine the change in water content immediately after eclosion.

iii) Determination of the water content of hatched juveniles under osmotic stress

Solutions of trehalose at different concentrations were used to test the influence of osmotic stress on the water content of hatched juveniles. The concentration of trehalose solutions were prepared as 0.2, 0.4 and 0.6m and their refractive index was checked with a Bellingham & Stanley sugar refractometer. Juveniles were hatched from cysts in root leachate at 15°C and only those which had hatched within the first 48 hours were used for experimentation. Batches of hatched juveniles were placed in cavity dishes containing the experimental solutions and a control batch was kept in glass distilled water at 15°C. The water content of 10 juveniles was determined immediately before they were transferred to the experimental solutions to give a water content value fore zero time. After 6, 24 and 48 hours, batches of 10 juveniles were taken from each of the experimental solutions and the glass distilled water control. The juveniles were placed on a glass slide and photographed under an interference microscope.

Measurements of optical displacement and juvenile diameter were made from contact prints. The centre of a selected interference fringe was pinpointed and the displacement within the nematode was measured. The diameter of the juvenile was measured at the point to which the fringe was displaced within the nematode. The refractive index of the juvenile (Rn) was calculated according to the formula:

$$Rn = Rm + \frac{\text{fringe displacement in } \mu m}{\text{diameter of juvenile in } \mu m}$$

where Rm is the refractive index of the medium surrounding the juvenile. The main substances of which juveniles are composed, such as proteins, lipo-proteins and amino acids have similar specific refractive increments when in solution, having an average value of

0.0018 (Perry, 1977b). The weight/volume of the total solids in the juvenile were obtained from the formula:

$$\text{concentration of total solids} = \frac{\text{R.I. juvenile - R.I. water}}{0.0018}$$

The water content of each juvenile was then estimated by subtracting the concentration of cell solids from one hundred.

RESULTS

i) Water content of unhatched juveniles after stimulation by root leachate and distilled water.

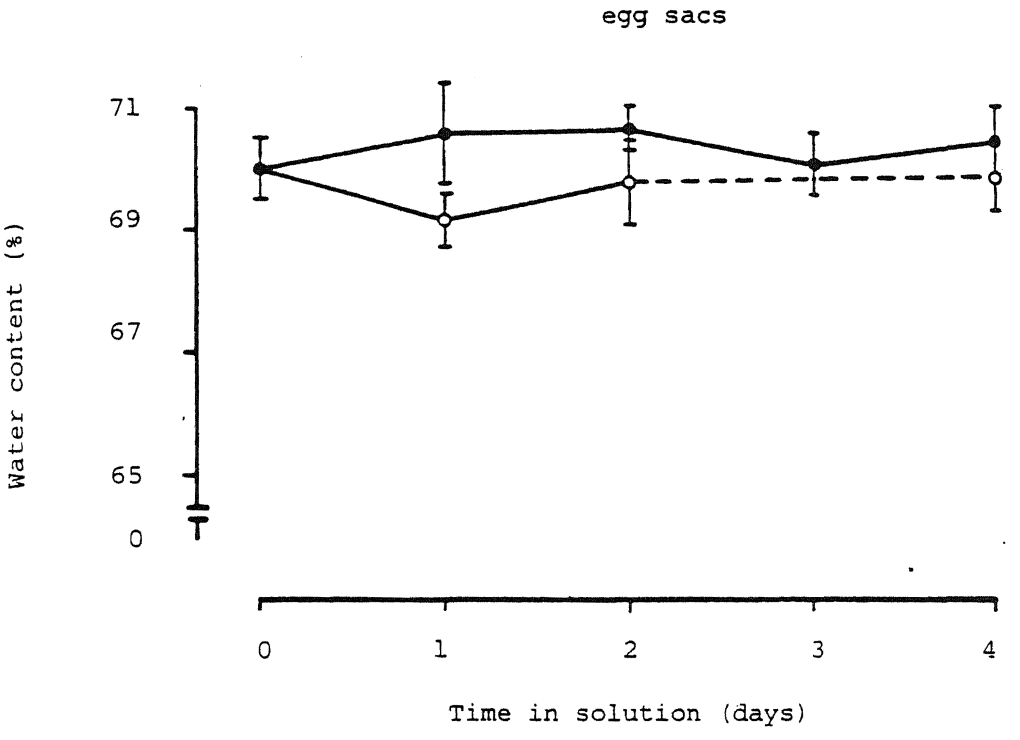
Changes in the water content of unhatched juveniles of H.cruciferae within egg sacs and cysts are shown in Figure 15. The initial water content of juveniles in egg sacs was 70.0%±0.49 and juveniles showed no increase in their water content when exposed to either root leachate or distilled water for different periods. The water content of juveniles placed in distilled water decreased to slightly smaller values than those in root leachate, however statistical analysis of the refractive index values from which the water content is derived was carried out (Ellenby & Perry, 1976) and showed that these values were not significantly different ($p<0.005$).

The initial water content of juveniles within cysts was 66.7%±0.84, a smaller water content than those in egg sacs. The water content of juveniles increased significantly ($p<0.05$) after 2 days exposure to root leachate and after 12 days the water content was 69.7%±0.41. Juveniles in cysts exposed to distilled water showed no significant changes ($p<0.05$) in their water content throughout the duration of the experiment.

ii) Water content of hatched juveniles immediately after eclosion.

After hatching, there was a significant increase in the water content of juveniles from both egg sacs and cysts over the 10 min period to give a final water content of about 73% (Fig. 16). There were no

Figure 15 Water content of unhatched second stage juveniles after hatching stimulus by root diffusate (●) and in glass-distilled water (○). Vertical bars represent S.E. mean.



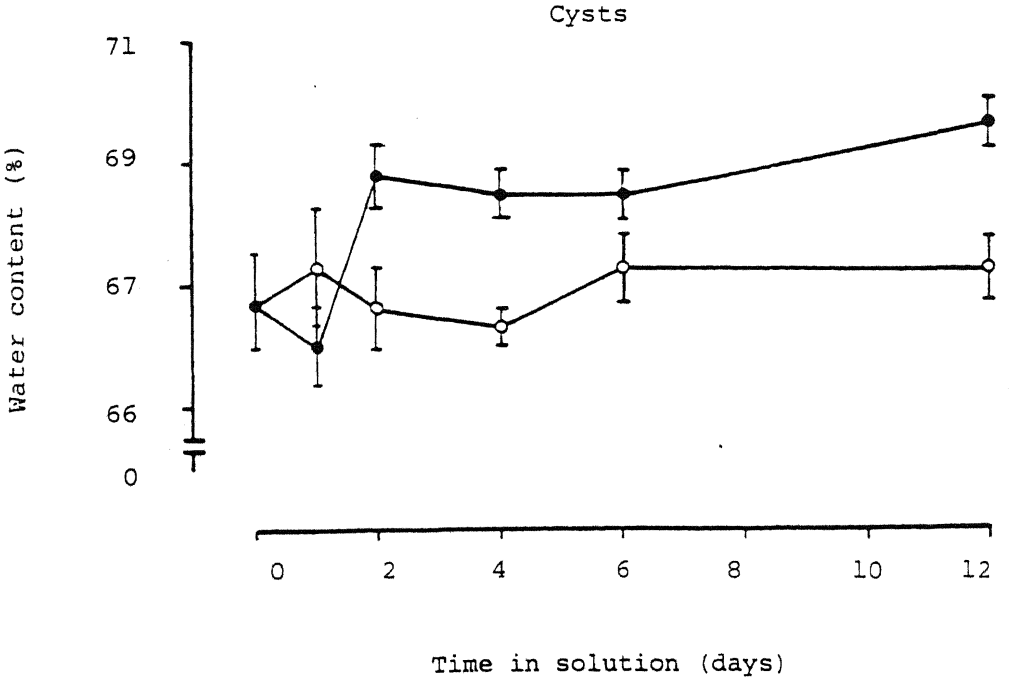
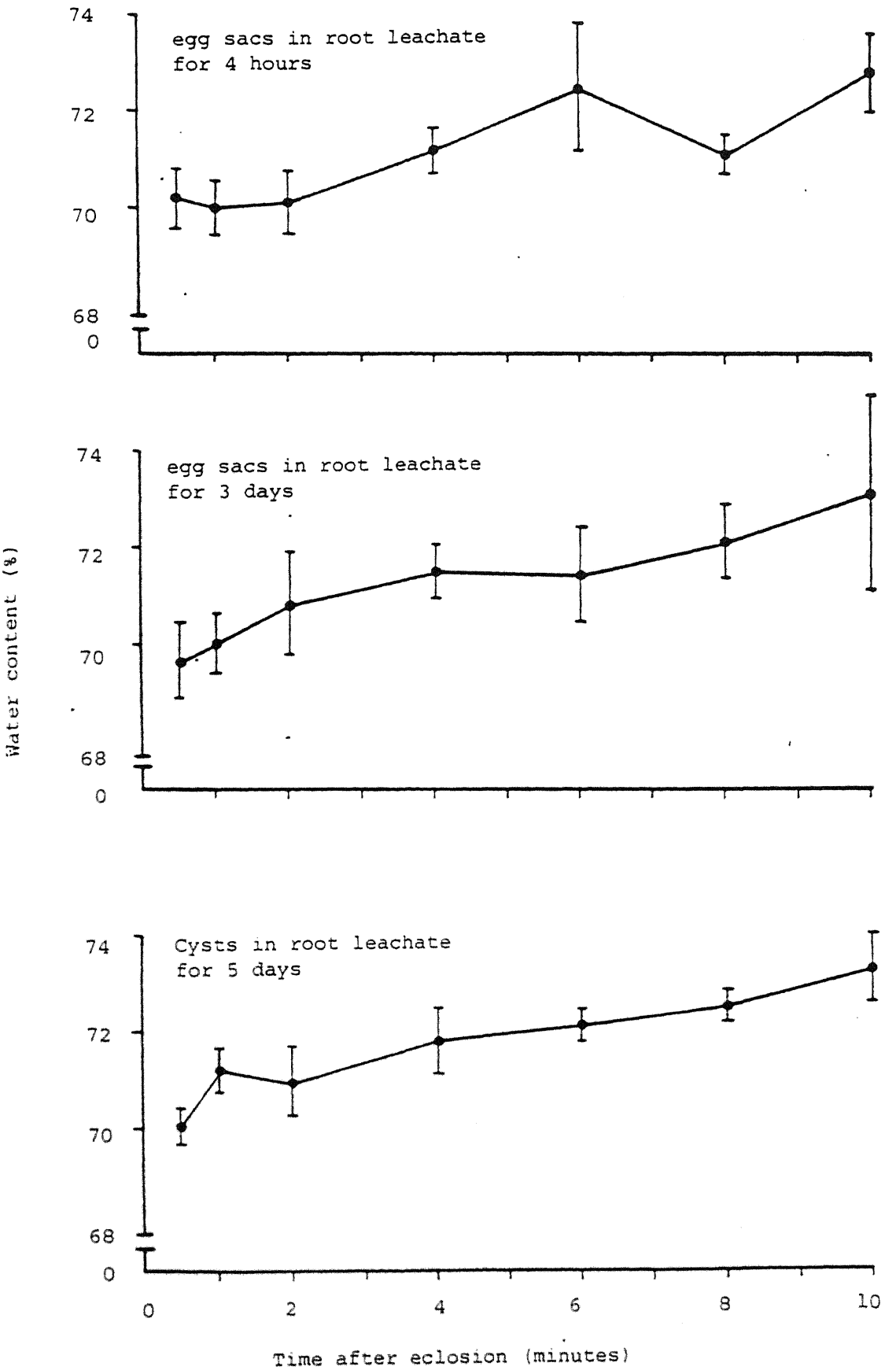


Figure 16 Water content of hatched second stage juveniles immediately after eclosion (mean of 5 individuals followed continuously). Vertical bars represent S.E. mean.



significant ($p>0.05$) differences between either the initial or final water content of juveniles in each of the three treatments. Additionally, there was no significant difference ($p>0.05$) between the water content of juveniles in egg sacs at any time when immersed in root leachate for either 4 hours or 3 days.

iii) Water content of hatched juveniles under osmotic stress.

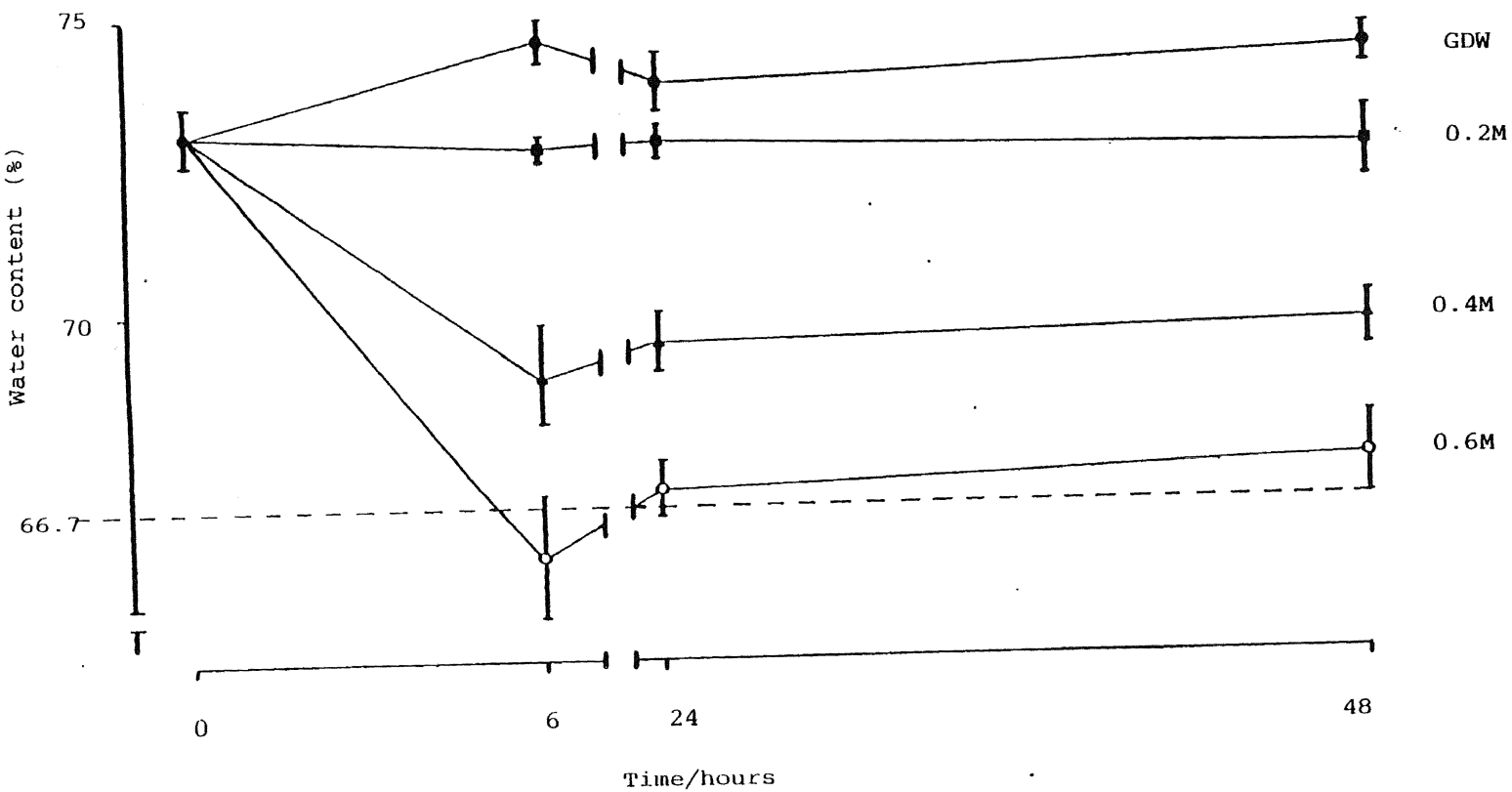
Figure 17 shows the changes in the water content of hatched juveniles after transfer from the hatching medium to trehalose solutions. Water loss increased with the concentration of the solute reaching a minimum value after 0-6 hours, then increasing to a more constant value after 6-48 hours in the two most concentrated solutions. After 48 hours the water content of juveniles was significantly different ($p>0.05$) when in different test solutions. In the 0.2 m solution there was little change in the water content of juveniles throughout the experiment whilst in distilled water the water content of juveniles increased from 73.0% to 74.2%. The water content of juveniles in distilled water was not significantly different ($p>0.05$) from the value obtained in the previous experiment 10 minutes after being artificially hatched, although the value was slightly larger.

The water content of unhatched juveniles in cysts (66.7%) as determined in the first experiment is shown on Figure 16, a trehalose concentration of 0.6 m resulted in free juveniles having a similar water content to unhatched juveniles.

DISCUSSION

Differences in the water content between unhatched H.cruciferae juveniles from cysts and egg sacs indicate that there are physiological differences between eggs in cysts and egg sacs which result in the different hatching responses of cysts and egg sacs. After stimulation by root diffusates, unhatched juveniles from cysts take up water until juveniles contain a similar amount of water to that of juveniles in egg sacs prior to eclosion. However, there is no detectable water uptake by unhatched juveniles in egg sacs on stimulation and prior to eclosion. The increase in water content of encysted H.cruciferae juveniles after stimulation by root diffusates

Figure 17 Water content of second stage juveniles in trahalose solutions at three concentrations and glass distilled water (GDW). Vertical bars represent S.E. mean.



is similar to that of G.rostochiensis and H.goettingiana (Ellenby & Perry, 1976; Perry et al., 1983), whereas the absence of any water uptake by juveniles from egg sacs of H.cruciferae is similar to H.schachtii (Perry, 1977). The water content of unhatched juveniles in cysts is maintained at low levels by the osmotic pressure of the egg fluid which is removed by the action of root diffusates. Heterodera cruciferae juveniles contained most water 12 days after exposure to hatching stimulus, thus taking longer to hydrate to a maximum water content than G.rostochiensis and H.goettingiana which take 2 and 7 days respectively (Ellenby & Perry, 1976; Perry et al., 1983). This indicates that H.cruciferae juveniles are either exposed to a greater osmotic stress, from which juveniles take longer to hydrate or that the initiation and rate of permeability changes of the eggshell associated with the hatching stimulus take longer.

Perry & Beane (1982) found that the hatching of G.rostochiensis was stimulated by exposure to potato root diffusate for 5 minutes per week for 5 weeks and that extending the period of stimulation did not increase hatching. However, H.goettingiana requires 18 hour periods of stimulation by pea root diffusate before hatching was equivalent to that occurring when cysts were exposed continually to root diffusate. It would be useful to compare the duration of stimulation required for hatching of H.cruciferae from cysts. A chemical stimulus does not appear to be required for the initiation of hatching from eggs in H.cruciferae egg sacs. Juveniles in egg sacs contained more water than in cysts and are already in a suitable condition for hatching. Other factors such as changes in temperature may stimulate hatching from egg sacs (Greco, 1981). However, the enhanced hatch from egg sacs in the presence of root leachates indicates that root diffusates may have some influence.

The proposed hatching mechanism for cyst nematode suggests that the lipid layer of the egg shell probably determines the permeability characteristic of an egg (Perry & Clarke, 1981). Structural differences in the membranes of different species may account for differences in the trehalose concentration in the egg fluid and thus the water content of juveniles and rate of change of water content after hatch stimulation. Ultrastructural examination of the egg shell of H.schachtii has shown that the inner lipid layer is not always present (Perry & Trett, 1986). Furthermore, the presence of the inner

lipid layer in encysted eggs of H.schachtii correlates with a small hatch in water; a large water hatch occurring only when this layer has been disrupted. Disruption of membrane structure in H.cruciferae eggs in egg sacs may cause leakage of trehalose from the egg fluid and an increase in the water content of juveniles. Such changes in membrane structure may occur as a result of either the loss of protection from environment by the cyst or by the action of substances in the gelatinous matrix. The gelatinous matrix is secreted by cells of the uterine wall and eggs are surrounded by the gelatinous material (McKintosh, 1960). A detailed examination of the chemical composition of this material may help to elucidate which substances influence changes in membrane structure and thus egg physiology. The enhancement of hatching from egg sacs in root leachates perhaps indicates that whilst the physiology of most eggs is altered by an unknown factor, some remain unchanged and still require root diffusate stimulation. The continued water uptake by H.cruciferae juveniles from both cysts and egg sacs after eclosion indicates the constraint imposed on the water content of juveniles by the egg shell (Ellenby, 1974). Juveniles can only fully hydrate once they are free of the eggs. This pattern and the final water content of H.cruciferae juveniles is similar to that of G.rostochiensis and H.schachtii (Clark et al., 1978; Perry, 1977).

The effect of osmotic pressure on the water content of H.cruciferae shows some differences from that reported for G.rostochiensis (Clarke et al., 1978), H.schachtii (Perry et al., 1980) and H.goettingiana (Perry et al. 1983). The water content of H.cruciferae juveniles in 0.6 M trehalose solution is similar to that of unhatched juveniles suggesting that there may be a similar concentration of trehalose in the egg fluid. However, experiments on free juveniles may not relate exactly to conditions within the egg. This value is greater than those derived by similar experiments for other species. The concentration of trehalose in the egg fluid of G.rostochiensis, H.schachtii and H.goettingiana has been determined as 0.4, 0.3 and 0.5 M respectively. A high concentration of solutes in the egg fluid which maintains a low juvenile water content will favour the long term persistence of H.cruciferae whilst the deposition of a large number of eggs into an egg sac ensures a continual source of juveniles for nematode multiplication. The hatching process of encysted eggs of H.cruciferae resembles that in other species in that a host stimulus is required to initiate the hatching process. It appears that juveniles of

H.cruciferae, like juveniles of G.rostochiensis and H.goettingiana cannot hatch until the osmotic pressure imposed by trehalose in the egg fluid is removed. Therefore, eggs of H.cruciferae in egg sacs are physiologically different from those in cysts, their physiology being probably altered as a consequence of being extruded into the egg sac. Juveniles in egg sacs, like juveniles of H.schachtii in cysts are in a suitable condition for hatching, a stimulus for which may or may not be of host origin.

5

Preliminary screening of oilseed rape for resistance to *H.cruciferae* and *H.schachtii*

INTRODUCTION

It is not known whether there is any resistance to either H.cruciferae or H.schachtii among cultivars and breeding lines of oilseed rape. However, recently some nematode-resistant cruciferous crops such as oil radish and mustard have been developed and have been shown to reduce H.schachtii populations by 50-60% (Cooke, 1985). Current cultivars and breeding lines of oilseed rape have a fairly narrow genetic base. Cultivars Primor, Jet Neuf, Bienvenu and Rafal have all been extensively grown since 1975 but all come from the same French breeder. Kimber (1981) points out that cultivars from the same breeder tend to have an even narrower genetic base. Resistance is more likely to be found if a range of cultivars and lines with different genetic bases are screened. Cultivars and breeding lines of oilseed rape are classified according to the amount of erucic acid found in the oil from the crushed rape seed and on the amount of glucosinolate left in the seed after crushing. Erucic acid is believed to be linked with heart lesions in experimental animals so plant breeders have attempted to reduce the erucic content giving rise to so called single-low cultivars. The glucosinolates in rape seed are harmless in that form but when they are hydrolysed by the enzyme myrosinase, which is also present in the seed, toxic compounds are released. Plant breeders therefore have attempted to develop cultivars with low glucosinolate content as well as low erucic acid content and these are double low cultivars. The glucosinolates are secondary nitrogenous compounds and have a significant role in plant defence against insects (Edwards & Wratten, 1980). Ellenby (1951) suggested that on hydrolysis glucosinolates in the roots of crucifers release phenyl isothiocyanate which, may suppress the hatching of nematodes. If this is the case with oilseed rape, then diffusates from the roots of low glucosinolate cultivars may hatch more juveniles and result in greater invasion than single low cultivars. Cultivars which produce root diffusates that stimulate only a few juveniles to hatch will permit only limited nematode multiplication and so will be better able to tolerate the presence of nematodes (Evans, 1983). Korunic (1976) found differences between hatching of H.cruciferae in root leachates collected from different cabbage cultivars and Widdowson (1958) and Evans (1983) found differences between hatching of the potato cyst nematode in leachates collected from different potato cultivars. The age of plants also influences the stimulative action of root diffusates (Winslow, 1955; Widdowson, 1958; Perry & Beane, 1981). Root diffusate from

soybean plants caused a greater hatching of H.glycines eggs during vegetative growth, but the activity declined with plant senescence (Tefft & Bone, 1985). Therefore, the maturation rates of cultivars may influence the period in which plants produce an active root leachate.

Preliminary screening of cultivars for nematode multiplication is usually carried out in glasshouses providing favourable conditions for nematode development and reducing plant to plant variation (Shepherd, 1958; Krause et al., 1984). The use of a juvenile inocula excludes differences in the hatching efficiency of cultivars but allows the development of a reasonably uniform cohort to be assessed. Multiplication is usually assessed by the numbers of cysts and eggs produced per plant but as Shepherd (1959) and Doney & Whitney (1969) point out invasion and development may be strongly influenced by the size and quality of the root system. The relationship between multiplication and root growth is likely to be complex; a cultivar which produces a large number of eggs per plant may not necessarily be a better host than a cultivar that produces fewer eggs per plant but which has a smaller root system. However, expressing multiplication as the number of eggs produced per gram of root will compensate for some of the difference.

This experiment examines the hatching of H.cruciferae and H.schachtii in root leachates from different cultivars and lines of oilseed rape and the multiplication of both species on the same cultivars and lines.

MATERIALS AND METHODS

i) Hatching and multiplication of H.cruciferae

On 1 May 1984, 17 cultivars and breeding lines were sown (three seeds per pot) in 10cm pots, each containing 300g of a mixture of five parts sterilised loam to one part of sand/gravel. Nine pots were prepared for each cultivar or line and each was subsequently thinned one week after the shoots had emerged to a single healthy plant per pot. Throughout the experiment the plants were watered as required, fed weekly with a liquid fertilizer (20:20:20, N:P:K) and maintained in a

glasshouse at 20°C day and 10°C night temperatures with a 16 hour day length.

When plants were 28 days old each pot was inoculated with 4000 second stage juveniles added at four positions, 2-3cm deep around the root system. After 14, 50 and 85 days root diffusates were leached out of three pots of each cultivar or line with two x 50ml distilled water, added 30 min apart. The leachates were filtered and stored at 10°C in the dark for up to 48 hours. Three ml of each leachate were added to three replicate batches of 75 freshly extracted cysts of H.cruciferae in Repli-dishes which were kept in the dark at 20°C for 21 days. Each week the leachate was replaced with 3ml of fresh leachate taken from stock solutions kept at 5°C. Replicate batches of cysts were also added to controls using 0.6mM flavianic acid as an artificial hatching agent (Southey, 1970) and distilled water. The numbers of hatched juveniles were counted after 21 days. After root leachate collection at 14, 50 and 85 days, plants were gently removed from the soil and the roots washed, blotted dry and weighed. The numbers of nematodes in the root systems were counted by staining and releasing the nematodes from the roots by the standard method.

The soil around each plant in the 50 and 85 day observations was retained and cysts therein extracted by fluidising column. The number of eggs produced were counted by the standard method.

ii) Hatching and multiplication of H.schachtii

On 19 February 1985, 18 cultivars and breeding lines were screened for resistance to H.schachtii by a similar procedure to that described for H.cruciferae. However, root leachates were collected and hatching tests performed on only one occasion; 14 days after juvenile inoculation. A distilled water control but no artificial hatching agents were included. Nematode invasion, egg production and plant growth were assessed at 14 and 105 days after juvenile inoculation in four pots of each cultivar or line. Plant growth was also assessed with reference to the growth stage key (Sylvester-Bradley & Makepeace, 1984).

RESULTS

i) Hatching and multiplication of H.cruciferae

Heterodera cruciferae was found to invade and multiply on all cultivars and lines tested. Table 5 lists the cultivars and breeding lines screened and shows nematode multiplication and root growth. Cultivars and lines with similar genetic bases are grouped together in the list. Root growth was assessed by calculating root growth scores (root weight at 85 days/root weight at 14 days). On warm days glasshouse temperatures could not be controlled and often reached in excess of 32°C.

Considerable differences were found between the numbers of juveniles that hatched (expressed as percentage hatch) in diffusate produced by cultivars and lines 14 days after inoculation. The smallest (5.9%) percentage hatch occurred in diffusate produced by Diander whilst the largest (15.4%) in diffusate produced by Korina. In distilled water 1.2% of juveniles hatched and in Flavianic acid 22.6% of juveniles hatched. To adjust the results of the hatching tests for differences in the size of root systems that produced the diffusate, the number of juveniles that were hatched per g root weight were calculated (Turner & Stone, 1982). Root diffusate from Elvira, Korina and Norli hatched most juveniles per g root whilst Jet Neuf and Diander hatched fewest. Table 6 gives the results of hatching tests using leachates collected at 14, 50 and 85 days. There were no significant differences between the numbers of juveniles that hatched in either distilled water or Flavianic acid on the three occasions whereas the numbers of juveniles that hatched in diffusate collected from older plants declined. Between 14 and 50 days, hatching in diffusate from Elvira, Darmor and Korina decreased more than from other cultivars. The largest reductions in hatch between 14 and 85 days occurred in diffusate from Elvira, Norli and Korina. Diffusate from Mikado, Jet Neuf, line 2/84 and Diander resulted in smallest reductions in hatch during this period. Between 50 and 85 days, the largest reduction in hatch was in diffusate from Midas.

At 14 days the largest numbers of nematodes per g root were found in Rafal and Tandem (Table 5). A large number of juveniles per root system was found in Jet Neuf because this cultivar had a large

Table 5

Assessment of root growth and multiplication of H.cruciferae in oilseed rape cultivars and breeding lines

Cultivar or line	Percentage hatch at 14 days	Juveniles hatched/g root at 14 days	Root weight at 14 days (g)	Root growth score	Nematodes/ g root at 14 days	Nematodes/ root system 50 days	Nematodes/ root system 85 days	Cysts produced per plant 50 days	Cysts produced per plant 85 days	Eggs produced per plant at 85 days
Jet Neuf	9.4	15.1	11.6	1.1	7.2	25	41	37	43	234
Bienvenu	6.7	21.0	6.0	6.5	8.3	10	19	13	24	257
Rafal	14.6	29.4	9.3	1.7	10.6	5	18	28	36	457
Lingot	13.5	29.4	8.6	2.4	6.2	44	32	8	21	251
Fiona	11.0	23.2	8.9	3.6	4.5	9	48	20	40	323
Korina	15.4	35.8	8.1	2.3	7.9	30	18	22	43	616
Mikado	9.8	18.6	9.8	1.6	2.7	10	22	22	83	1288
1/84	12.3	25.5	9.0	3.3	5.9	25	13	20	45	741
2/84	9.4	19.6	9.0	3.1	6.7	40	31	33	40	251
Belinda	12.6	26.9	8.8	2.8	4.9	24	64	20	38	264
Elvira	12.6	38.4	8.2	4.7	7.3	15	18	19	28	331
Norli	15.3	34.5	8.3	3.2	6.8	30	16	41	71	436
Diander	5.9	15.7	7.1	4.5	7.5	35	48	16	32	468
Tandem	13.1	22.9	10.9	2.8	10.6	18	33	21	36	257
Darmor	12.6	29.2	8.1	3.3	5.3	68	60	17	19	214
Westar	6.8	18.1	7.2	1.0	3.7	8	15	11	9	52
Midas	7.4	21.9	6.2	1.0	4.3	16	13	15	11	62
Mean	11.1	24.3	8.5	2.9	6.5	24.2	29.9	21	36	382
SD	3.1	8.0	1.4		2.2	16.2	16.6	9.3	20.5	292.3
SE	0.7	1.9	0.3	0.5	3.9	4.0	2.2	4.8	70.9	

Table 6

The hatching of H.cruciferae in leachate collected from cultivars and breeding lines of oilseed rape on three occasions

Cultivar or line	Juveniles hatched/g root weight		
	14 days	50 days	85 days
Jet Neuf	15.1	7.4	5.3
Bienvenu	21.0	4.9	1.0
Rafal	29.4	20.1	4.5
Lingot	29.4	11.5	3.5
Fiona	23.2	16.6	1.4
Korina	35.8	16.1	6.0
Mikado	18.6	10.2	8.6
1/84	25.5	14.3	2.7
2/84	19.6	16.9	8.6
Belinda	26.9	13.5	2.1
Elvira	38.4	15.3	1.2
Norli	34.5	17.4	2.1
Diander	15.7	9.5	3.2
Tandem	22.9	15.5	0.8
Darmor	29.2	8.8	4.6
Westar	18.1	13.4	1.5
Midas	21.9	24.3	1.3
Mean	24.3	13.9	3.4
SE	1.9	1.2	0.6
% hatch			
Flavianic acid	22.6	24.7	22.1
Distilled water	1.2	0.8	1.6

corresponding root weight. Fewer nematodes were found per g root of Mikado despite an above average root weight. Root growth scores indicated that Bienvenu, Elvira and Diander grew better than other cultivars and lines. Tandem, Fiona and line 1/84 also developed larger root systems by 85 days but this was not reflected in the root growth scores as these plants had already established large root systems by 14 days. At 50 days, significantly more cysts were produced by Norli, Jet Neuf and line 2/84 than by other cultivars ($p > 0.05$). However, many of the nematodes had probably not completed adult development by this time and at 85 days significantly more cysts were found in Mikado and Norli ($p > 0.05$). In most cultivars and lines a larger number of juveniles per root system were found at 85 days than at 50 days. The largest increases in the numbers per root system between 50 and 85 days were found in Fiona and Belinda. At 85 days, Mikado, line 1/84 and Korina produced significantly more eggs per plant than other cultivars ($p > 0.05$) whereas Westar and Midas produced significantly fewer eggs ($p > 0.05$).

ii) Hatching and multiplication of H.schachtii

Heterodera schachtii invaded and multiplied on all cultivars and lines tested (Table 7). The numbers of juveniles that hatched in diffusate produced by plants 14 days after inoculation were very different between cultivars and lines. The hatching test was not completed for Korina due to fungal contamination. Slight contamination was also noticed in hatching tests in diffusate produced by Tandem and Bienvenu. The smallest percentage hatch occurred in diffusate produced by Tandem and may have been a result of this contamination. The largest percentage hatch occurred in diffusate produced by Midas and an average of 7.2% of juveniles hatched in distilled water. Adjusting the hatching for root weights showed that Jet Neuf, Westar and Norli hatched significantly more juveniles per g root whilst Tandem hatched fewest ($p > 0.05$). At 14 days the root systems of plants were smaller than those at 14 days in the H.cruciferae screening experiment. Overall, more H.schachtii than H.cruciferae juveniles were found per root system at 14 days. The largest numbers of juveniles per g root at 14 days were in Jet Neuf and Primor with the smallest numbers in Elvira and Lingot. At 105 days there were smaller numbers of juveniles per g root than at 14 days but these represented much larger numbers of juveniles per root system. The largest numbers of nematodes per

Table 7

Assessment of root growth and multiplication of H.schachtii in oilseed rape cultivars and breeding lines

Cultivar or line	Percentage hatch at 14 days	Juveniles hatched/g root at 14 days	Root weight at 14 days (g)	Root growth score	Nematodes/ g root at 14 days	Nematodes/ root system at 105 days	Cysts produced per plant at 105 days	Eggs produced per plant at 105 days
Jet Neuf	28.4	304.7	2.1	14.7*	61.9	155	69	2941
Bienvenu	6.6	83.3	1.8	14.2	43.9	178	84	3360
Rafal	13.6	80.7	3.8	7.3	34.1	688	248	11884
Lingot	11.4	91.7	2.8	10.8	17.5	30	247	1621
Primor	8.2	102.7	1.8	21.1	58.3	265	155	8357
Fiona	9.4	35.2	6.0	5.6	20.5	204	118	8242
Korina	-	-	4.0	7.4	25.4	296	99	8419
Mikado	7.2	55.8	2.9	12.1	23.7	423	241	23384
1/84	7.4	34.7	4.8	6.0	19.9	116	71	1869
2/84	6.2	31.8	4.4	7.4	29.2	162	102	7762
Belinda	10.4	57.3	4.1	9.0	22.8	517	87	3291
Elvira	8.3	45.6	4.1	6.7	16.5	336	61	4557
Norli	23.3	138.1	3.8	11.1	29.6	424	216	19783
Diander	6.5	42.0	3.5	11.1	25.5	860	228	16459
Tandem	2.5	16.2	3.5	9.7	32.1	238	187	13907
Darmor	10.1	43.6	5.2	5.4	20.1	838	129	5516
Westar	26.5	207.6	5.6	2.4**	22.6	345	51	2001
Midas	36.8	106.6	4.0	2.9**	46.4	180	85	4123
Mean	13.3	86.9	3.8	9.1	30.5	363.8	135.7	8193.6
SE	2.9	17.8	0.3	1.7	3.2	53.7	16.1	1527.8

105 day old plants were at stem extension stage except * flowering, ** seed development

root system at 105 days were found in Diander and Darmor and the smallest number in line 1/84. Root growth scores indicated that Primor, Jet Neuf and Bienvenu grew better than other cultivars and lines. Westar and Midas did not grow as well and this was probably a result of reduced root growth during the later stages of plant growth in the experiment. Rafal, Mikado and Diander produced significantly more cysts per plant at 105 days than other cultivars and lines ($p > 0.05$) whilst significantly more eggs per plant were produced by Mikado, Norli, Diander and Tandem ($p > 0.05$). Lingot and line 1/84 had produced significantly fewer eggs per plant at 105 days ($p > 0.05$).

Table 8 gives the multiplication factor (number of eggs produced per plant/number of juveniles inoculated per plant) of both H.cruciferae and H.schachtii on each cultivar and line. The multiplication of each species is also expressed as the number of eggs produced per g root of each cultivar and line at either 85 or 105 days. Overall, H.schachtii achieved greater multiplication than H.cruciferae and produced more eggs per g root. Most H.cruciferae eggs per g root were produced on cultivars Mikado, Korina, Rafal and line 1/84 whilst Bienvenu produced fewest H.cruciferae eggs pe g root. Most H.schachtii eggs per g root were produced by Mikado, Norli, Rafal and Diander whereas Lingot and line 1/84 produced fewest.

Cultivars and lines were ranked according to (i) numbers of cysts produced per plant, (ii) number of eggs produced per g root, and (iii) the multiplication factor of each species. They were scored in each category on a scale of 1-17 for H.cruciferae and 1-18 for H.schachtii, with the largest value in each category scoring 1 whilst those which had the smallest value scored either 17 or 18. The scores from each category were totalled for each cultivar and line and are given in Table 9. Joint scores for each cultivar and line were calculated by adding the scores for H.cruciferae and H.schachtii and these are also given in Table 9.

DISCUSSION

No cultivar or breeding line showed resistance to either H.cruciferae or H.schachtii but differences were found in both the multiplaction that occurred and in the growth of the cultivars and lines used. Hatching of H.cruciferae and H.schachtii varied and their percentage

Table 8

Multiplication of H.cruciferae and H.schachtii on cultivars and breeding lines of oilseed rape

Cultivar or line	Multiplication factor		Eggs produced per g root at 85 days	at 105 days
	H.cruciferae	H.schachtii	H.cruciferae	H.schachtii
Jet Neuf	0.06	0.73	18.1	94.8
Bienvenu	0.06	0.84	6.5	131.7
Rafal	0.11	2.97	29.2	430.5
Lingot	0.06	0.40	12.1	53.5
Primor	-	2.08	-	220.5
Fiona	0.08	2.06	10.1	242.4
Korina	0.15	2.10	33.2	283.4
Mikado	0.32	5.84	82.5	662.4
1/84	0.19	0.46	24.7	64.4
2/84	0.06	1.94	9.1	238.8
Belinda	0.07	0.82	9.5	88.9
Elvira	0.08	1.13	8.6	162.7
Norli	0.11	4.94	16.6	466.5
Diander	0.11	4.11	14.5	420.9
Tandem	0.06	3.47	8.3	409.0
Darmor	0.05	1.37	7.9	197.0
Westar	0.01	0.50	8.6	355.4
Midas	0.01	1.03	8.6	148.2
Mean	0.09	2.04	18.1	259.5
SE	0.018	0.38	4.46	39.4

Table 9

Scores accumulated by cultivars and breeding lines of oilseed rape ranked according to the number of cysts produced per pot, eggs per g root and multiplication factors of H.cruciferae and H.schachtii

H.cruciferae		H.schachtii		Joint Scores *	
Mikado	(3)	Mikado	(4)	Mikado	(7)
Korina	(9)	Norli	(8)	Norli	(20)
1/84	(9)	Rafal	(9)	Rafal	(23)
Norli	(12)	Diander	(10)	Diander	(30)
Rafal	(14)	Tandem	(14)	Korina	(31)
Jet Neuf	(16)	Korina	(23)	Fiona	(41)
Fiona	(19)	Primor	(23)	Tandem	(42)
Diander	(20)	Fiona	(24)	2/84	(47)
Belinda	(22)	2/84	(27)	1/84	(54)
2/84	(23)	Darmor	(28)	Jet Neuf	(58)
Elvira	(27)	Midas	(37)	Belinda	(60)
Lingot	(27)	Elvira	(39)	Darmor	(60)
Tandem	(28)	Westar	(39)	Elvira	(63)
Bienvenu	(33)	Bienvenu	(40)	Midas	(69)
Darmor	(35)	Belinda	(41)	Bienvenu	(70)
Midas	(35)	Jet Neuf	(45)	Westar	(74)
Westar	(37)	1/84	(48)	Lingot	(79)
		Lingot	(54)		

* Scores for Primor excluded

hatch values were rather small compared with those found in a previous experiment (Chapter 2). Adjusting the numbers of juveniles that hatched for the root weight of each cultivar or line made the results more uniform and more accurately assessed intrinsic differences between cultivars and lines. Heterodera schachtii hatched more readily in root diffusates than in distilled water but very large differences were found in the hatching test. These may have resulted from fungal contamination, the extent of which was unknown for all hatching tests. The source of contamination may have come from within the cysts of H.schachtii (Burnsall & Tribe, 1974) or may have been introduced by root leachates.

Turner & Stone (1981) found that diminished hatching activity was not associated with resistant potato clones and therefore did not contribute towards resistance to potato cyst nematodes. However, Evans (1983) suggested that potato cultivars which caused fewer juveniles to hatch could expect to be less heavily invaded and to suffer less damage. This would also apply to cultivars and lines of oilseed rape which hatched fewer juveniles. No difference was found between the numbers of juveniles hatched by either high or low glucosinolate cultivars suggesting that if glucosinolates were present in root leachates they had little effect on the hatching of either H.cruciferae and H.schachtii. The decrease in the hatching activity of diffusates produced by older plants may be an important aspect of the host-parasite relationship. By 85 days all cultivars and lines had flowered and were producing seed. The hatching activity of diffusates produced at 50 and 85 days had decreased considerably and was probably related to physiological changes that occur in plants at the time of flowering (as reported for potato plants by Evans (1982b)). The rate of decrease in the hatching activities of diffusates was different between cultivars and lines and would presumably be related to differences in their maturation rates. Widdowson (1958) found that the root diffusates of 2-3 week old potato plants caused more juveniles to hatch than did diffusate from older plants but was unable to show that differences in the maturation rates of early and late cultivars were reflected in the activity of diffusates. Cultivars of oilseed rape which produced a much less active diffusate at 50 days (Elvira, Darmor and Korina) would hatch fewer juveniles than other cultivars and lines and restrict the amount of nematode multiplication that could occur. However, this would only apply to eggs within cysts as Fl juveniles within the egg sacs of H.cruciferae and H.schachtii require very

little hatching stimulus and would be able to hatch. Differences between the maturation rates of these cultivars and those of other cultivars and lines were not apparent. This relationship warrants further investigation using plants growing under natural conditions to determine more accurately the time at which these changes occur in the growing season.

Multiplication of H.cruciferae on cultivars of oilseed rape was poor in comparison to that of H.schachtii. The large difference between the two species may be partially explained by the detrimental effects of high glasshouse temperatures on the development of nematodes (Trudgill, 1970; Thomason & Fife, 1962). The H.cruciferae screening was carried out between May and July whereas the H.schachtii screening was carried out betweeen February and June. The development of H.cruciferae may have been influenced more by inhibitory high temperatures than that of H.schachtii. The duration of the two screening experiments was also different and this would have influenced the extent of the development of each species. The development of H.schachtii was probably advanced and the second generation already well established. This was indicated by a large number of H.schachtii juveniles in the roots at 105 days and comparisons between the multiplication of both species on cultivars and lines should take the extent of development of each into consideration. The addition of a large number of juveniles to the soil in four locations will cause intense competition between juveniles for the root space available in young plants. Therefore, the numbers of juveniles that successfully invade and initiate feeding sites will be smaller than with a more gradual invasion from cysts and nematode multiplication would be expected to be less than on hosts grown in soil infested with cysts. The results indicated that the number of juveniles invading a particular cultivar was not always correlated with the number of cysts produced. The proportions of juveniles that invade the roots and become females are represented by the number of cysts found on a host. The numbers of cysts are often used to distinguish between good and poor hosts. However, a host which produced a large number of cysts may not necessarily be a good host if the cysts contain a small number of eggs. In the experiments the numbers of eggs produced per plant may be under-estimated as some juveniles may have hatched by the time the soil was sampled. The numbers of eggs in egg sacs of H.cruciferae and H.schachtii were not measured and this will also under-estimate multiplication. Ranking

cultivars and lines on the basis of the three categories will more reliably identify either good or poor hosts than using any one category. Greater reliance can be put on cultivars and lines which accumulate either high or low scores whereas intermediate scores may be a result of compounding high and low scores. Mikado, Norli and Rafal were identified as good hosts of both H.cruciferae and H.schachtii and Midas, Bienvenu, Westar and Lingot as poor hosts. Some cultivars showed different host status for either H.cruciferae or H.schachtii. Line 1/84 and Jet Neuf were good hosts of H.cruciferae but were poor hosts of H.schachtii. Jet Neuf is a good host of H.cruciferae in the field (Evans, 1984) but there is no information concerning the multiplication of H.schachtii on Jet Neuf in the field.

It is important that if hosts are to be grow in infested fields that they are not intolerant of nematode damage. Bienvenu, Diander and Elvira grew well in both screening experiments and may be expected to tolerate nematode damage better than other cultivars and lines. Damage to 28 day old plants by a juvenile inoculum would have been largely restricted to the regions where juveniles were added and the overall effect of localised damage on plant growth was unknown as no inoculated controls were included. Plants are likely to be more sensitive to nematode damage when sown directly into infested soil. Pot tests can accurately assess nematode multiplication but the growth of plants may be very different from in the field. Nematode multiplication should also ideally be studied over a range of initial nematode population densities as the amount of multiplication that can occur on a particular cultivar or line may vary with initial nematode density. There is a paucity of information concerning the multiplication of either H.cruciferae or H.schachtii on different cultivars in the field. Lear (1971) found differences between cultivars of brussels sprouts as hosts of H.schachtii but no differences as hosts of H.cruciferae although Lewis (1971) suggested that the increase in H.cruciferae populations is affected by the use of different cultivars of cabbage and cauliflower. Oilseed rape cultivar Jet Neuf was found to increase populations of H.cruciferae more than Bienvenu at one trial site (Evans & Spaull, 1985) but at another site Bienvenu produced more eggs g^{-1} soil than either Jet Neuf or Mikado (Evans, et al., 1987). Tolerant cultivars such as Bienvenu may increase populations more than less tolerant cultivars by providing a larger root system for greater nematode development.

6

Multiplication of H.cruciferae and H.schachtii on oilseed rape and their effects on the growth of different cultivars

INTRODUCTION

Preliminary screening of cultivars and breeding lines of oilseed rape indicated that whilst no cultivar or line showed complete resistance to either H.cruciferae or H.schachtii there were differences in the amount of nematode multiplication that occurred and in tolerance, as measured by root growth scores. Little is known of the relationship between oilseed rape root growth and the multiplication of either H.cruciferae or H.schachtii and whether differences in the growth characteristics of cultivars and lines may account for the amount of nematode multiplication which they support. The amount, rate and type of root growth has been found to influence the amount of multiplication of other cyst nematodes on different hosts (Golden & Shafer, 1958; Shepherd, 1959; Evans et al., 1977). The rate at which different cultivars mature may influence the amount of nematode multiplication either by providing a shorter growing period in which nematodes can develop or due to physiological changes in the roots associated with maturation shortening the period in which roots represent a more favourable environment for nematode development. Early maturing potatoes and rapidly maturing varieties of pea do not support such large populations of Globodera rostochiensis or H.goettingiana respectively as main crop and late maturing varieties (Jones & Jones, 1975). Steele (1975) found that the amount of development of H.schachtii on tomato and sugar beet decreased with plant age and Storey (1982) suggested that the invasion of potato roots by G.rostochiensis late in the growing season may have been more difficult in older roots. Therefore, rapidly maturing cultivars and lines of oilseed rape may provide a shorter period in which either H.cruciferae or H.schachtii may develop.

Plants may be expected to suffer more damage when seeds are sown directly into infested soil than when older plants are inoculated with juveniles as in the preliminary screening experiment. Seedlings of cabbage transplanted into infested soil were less damaged by H.schachtii than directly-drilled plants (McCann, 1979). Similarly, the threshold value above which the inoculum density of H.avenae had an effect on oat seedlings increased with the age of plants (Rawsthorne & Hague, 1986). The ability of older plants to tolerate damage caused by nematode invasion will indicate the benefits of any pre-sowing nematicide treatment to the soil which delays the invasion

of roots and associated damage. Storey (1984) found that pre-sowing treatments of oxamyl increased potato yields because the nematicide delayed the hatching of G.rostochiensis and the invasion of roots until the older plants were more tolerant of damage.

The first experiment in this section compares the growth of four cultivars of oilseed rape when either sown directly into infested soil or inoculated with nematodes after being grown in uninfested soil for 28 days. The multiplication of H.cruciferae is examined in relation to the growth of each cultivar in an attempt to explain differences in the amount of nematode multiplication which each supports. The following cultivars were selected because they differed in characteristics which had been identified in a preliminary screening.

1) Jet Neuf: a commonly grown cultivar which, despite showing good initial root growth prior to inoculation, grew little after juveniles had been added to the roots. Jet Neuf supported much H.cruciferae multiplication.

2) Mikado: this cultivar had a low growth score when infested with nematodes but supported more H.cruciferae multiplication than any other cultivar or line.

3) Elvira: grew well when infested with H.cruciferae but did not support as much multiplication as either Jet Neuf or Mikado. Elvira gave a similar response to that of Bienvenu, a more commonly grown cultivar, but was selected in preference because there was a large decrease in the hatching activity of diffusate it produced from older plants.

4) Midas: this cultivar grew less well and matured more rapidly than other cultivars and lines. Fewer eggs were produced by H.cruciferae on this cultivar which may be a direct result of rapid maturity.

A second experiment examines the growth of three cultivars (Bienvenu, Mikado, Ariana) when sown directly into soil infested with either H.cruciferae or H.schachtii and the multiplication of the two species on these three cultivars. Bienvenu is currently one of the most

commonly grown cultivars in Britain but Mikado and Ariana have only recently been released. They will probably occupy an increasing proportion of the oilseed rape acreage in the future. Ariana is a double low variety.

MATERIALS AND METHODS

i) Effect of H.cruciferae on the growth of four cultivars of oilseed rape

On 5 January 1985 eighty pots (10cm diam.) were filled with 500ml of a loam, sand and gravel mixture (3:1:1). Twenty pots were sown with one seed per pot of each of the four cultivars Jet Neuf, Mikado, Elvira and Midas. Ten pots of each cultivar were then inoculated with H.cruciferae cysts to give an inoculum of approx. 5000 eggs per pot. Cysts were pipetted into the soil in 5ml of distilled water at 4 locations at a depth of 2-4cm around the seed. Ten plants of each cultivar were left as uninoculated controls. Plants were then placed in a glasshouse at 15°C day and 10°C night temperatures, with supplementary lighting over a 12 hour photoperiod. Plants were watered as required and fed weekly with a liquid N,P,K fertilizer. Twenty-eight days after sowing, plants from five infested pots and from five uninfested pots of each cultivar were gently removed from the soil and washed clean. The plants were blotted dry and the shoot and root fresh weights were measured. Additionally, the leaf area of each plant was measured by passing the individual leaves of each plant through a CSP portable area meter and the length of each root system estimated by the method described by Spaull (1981). The numbers of nematodes in the root systems of each plant were then counted by staining and releasing juveniles from the roots by the usual method. Nematodes were extracted from the soil of each infested plant by the method described by Whitehead & Hemming (1965). One hundred and thirteen days after sowing the remaining plants of each cultivar were removed from pots and shoot weight, root weight and leaf areas were measured as before. The root systems were too extensive to be measured by the method described previously and were measured by a Comair root length scanner.

ii) Multiplication of H.cruciferae on four cultivars of oilseed rape inoculated at 28 days after sowing

Concurrently with experiment (i) two hundred pots (10cm diam.) were filled with 500ml of a loam, sand and gravel mixture (3:1:1) and fifty pots were sown with three seeds per pot of each of the four cultivars of rape Jet Neuf, Mikado, Elvira and Midas and placed in the same glasshouse. Five days after sowing plants were thinned to one healthy seedling per pot. Plants were watered as required and fed weekly with a liquid N,P,K fertilizer. Twenty eight days after sowing twenty five pots of each cultivar were inoculated with H.cruciferae cysts giving an inoculum of approx. 5000 eggs per pot. Cysts were pipetted into the soil of each pot as described previously. Twenty five plants of each cultivar were left as uninoculated controls. At 10, 28, 35, 50 and 85 days after inoculation five plants from infested pots and five from uninfested pots of each cultivar were gently removed from the soil and washed clean. The plants were blotted dry and the shoot and root fresh weights and leaf areas of each plant measured as described previously. At 35, 50 and 85 days the tap roots of plants were separated from the lateral roots and the two types weighed. The total lengths of the root systems of two plants were measured by a Comair root length scanner. Because the roots of these plants had to be cut into small sections for this measurement the numbers of nematodes within could not be counted accurately due to the risk of releasing nematodes from the roots. The numbers of nematodes in the roots of the other three plants were counted by the standard method and the numbers of juveniles at each development stage were recorded. After the roots had been removed the soil from each infested pot was retained at 10, 28, 35, 50 and 85 days. The soil of each pot was thoroughly mixed and halved; nematodes were extracted from one half of the soil by the tray method (Whitehead & Hemming, 1965) and at 50 and 85 days cysts were extracted from the other half of the soil by fluidising column. The egg content of cysts were counted by the standard method.

iii) Multiplication and effects of infestation of H.cruciferae and H.schachtii on three cultivars of oilseed rape

On 21 March 1986 ninety pots (10cm diam.) were filled with 500ml of a loam, sand and gravel mixture (3:1:1) and thirty pots were sown with one seed per pot of each of the three cultivars Ariana, Bienvenu and

Mikado. Ten pots of each cultivar were then inoculated with H.cruciferae cysts and a further ten pots inoculated with H.schachtii cysts giving an inoculum of approx. 5000 eggs per pot. Cysts were pipetted into the soil of each pot as described previously. Ten pots of each cultivar were left as uninoculated controls. Plants were placed in a different glasshouse but again were given 15°C day and 10°C night temperatures with supplementary lighting over a 12 hour photoperiod. Plants were watered as required and fed weekly with a liquid N,P,K fertilizer. Twenty eight days after sowing the pots in which three plants of each cultivar were growing in uninfested soil were leached with 50ml of distilled water. A further 50ml of distilled water were added 30 minutes later. The leachate from pots was collected in plastic trays and filtered through Whatman No. 1 filter paper. Three ml of the leachate were added to each of three batches of 75 freshly extracted cysts of either H.cruciferae or H.schachtii. Leachates were replaced with 3ml of fresh leachate each week and the numbers of hatched juveniles were counted after 3 weeks. After root diffusate collection five plants from uninfested pots and five plants from pots infested with either H.cruciferae or H.schachtii were gently removed from the soil. The root systems of each plant were blotted dry and the root fresh weight measured. The numbers of juveniles in the roots were counted by staining and releasing juveniles from the roots by the standard method. One hundred and five days after sowing the remaining plants were gently removed from the soil of each pot, brushed clean and root fresh weights measured and the numbers of nematodes in the roots of infested plants were counted as before. The soil of infested pots was retained, thoroughly mixed and cysts extracted from 200g of soil by fluidising column. The egg content of cysts were counted by the standard method.

RESULTS

i) The root weights and root lengths of all the cultivars were smaller than those of uninfected plants after 28 days in soil inoculated with H.cruciferae cysts at sowing (Table 10). The shoot weights and leaf areas of Mikado and Midas were also smaller than those of uninfected plants at this time. At 28 days more nematodes were found in the soil in which Jet Neuf and Midas had been growing (Table 11). The largest numbers of nematodes were found in the roots of Mikado and Elvira. Second and third stages were present in the

Table 10 The growth of four cultivars of oilseed rape after 28 and 113 days when grown in soil either infected (+) or uninfected (-) with H.cruciferae

	Shoot fresh wt (g)		Leaf area (cm2)		Root fresh wt (g)		Root length (m)	
	+	-	+	-	+	-	+	-
28 days								
Jet Neuf	1.24	1.56	41.6	59.3	0.07*	0.11	1.22*	1.78
Mikado	1.19*	1.92	50.2*	94.0	0.17*	0.21	1.49*	2.36
Elvira	1.55	1.29	53.6	45.0	0.17*	0.24	1.47*	1.78
Midas	1.81*	2.37	48.3*	83.3	0.09*	0.28	1.41*	2.59
113 days								
Jet Neuf	42.7	47.8	946.1	975.4	6.1*	8.4	33.9*	40.1
Mikado	33.6*	42.5	547.8*	820.3	6.3*	8.2	35.4*	42.3
Elvira	36.7	40.1	912.8	930.7	5.7*	6.5	31.7*	33.3
Midas	32.5	28.5	698.5	662.8	3.6*	4.9	22.7*	29.1

* significantly different from uninfested plants, $p<0.05$

Table 11 The numbers of nematodes in the soil and roots of four cultivars of oilseed rape 28 days after pots were inoculated with cysts at sowing

	JJ2 in soil	JJ2 in roots	JJ3	JJ4	Total JJ in roots	Total JJ /g root
Jet Neuf	1122	49	30	-	79	1128.5
Mikado	836	66	40	9	114	670.5
Elvira	672	67	23	-	90	529.4
Midas	1062	38	10	-	48	533.3

roots of all cultivars and a small number of fourth stages in the roots of Mikado. At 113 days the root weights and lengths of infested plants of all cultivars were smaller than those of uninfected plants. The shoot weight and leaf area of Mikado was smaller than those of uninfected plants. Jet Neuf grew better than other cultivars whereas Midas grew less well.

ii) The changes in the shoot weight, leaf area, root weight and root length of Jet Neuf, Mikado, Elvira and Midas after cysts were added to 28 day old plants are shown in Fig. 18a-d. There were few significant differences between infected and uninfected plants although almost invariably, the infected plants did not grow as well. All cultivars grew most rapidly in the first 50 days after inoculation after which time the growth rates of Mikado, Elvira and Midas declined. Jet Neuf and Mikado grew better than other cultivars as indicated by a larger overall plant fresh weight at 85 days. Midas grew less well with only a small increase in either shoot or root weight after 50 days. However, this cultivar matured more rapidly than others and had flowered by 85 days. The other cultivars were still growing vegatatively at this time, but the growth rate of shoots and roots of Mikado also declined somewhat (more than in Jet Neuf and Elvira) after 50 days, suggesting this cultivar may also have been more mature with declining growth rates associated with the onset of flowering. Tap roots were small and indistinguishable from fibrous roots at 10 days and had only just started to develop by 28 days. Between 28 and 35 days tap roots grew rapidly in all cultivars and accounted for a large proportion of total root weight by 35 days. Table 12 shows that tap roots accounted for a larger proportion of total root weight in infected plants than in uninfested plants at 35 days (eg. 81.5% of the total root weight in Elvira). At 50 days the differences in the proportions of total root weight accounted for by the tap root between infected and uninfected plants were smaller in Jet Neuf and Midas whilst tap roots still accounted for a larger proportion of the total root weights of infected plants of Elvira and Mikado. The proportion of total root weight acounted for by tap roots decreased in both infected and uninfected plants of all the cultivars between 35 and 85 days. At 85 days Elvira and Midas had larger tap roots than Mikado and Jet Neuf.

Figure 18 Comparison of the growth of four infested (●) and uninfested (○) cultivars of oilseed rape

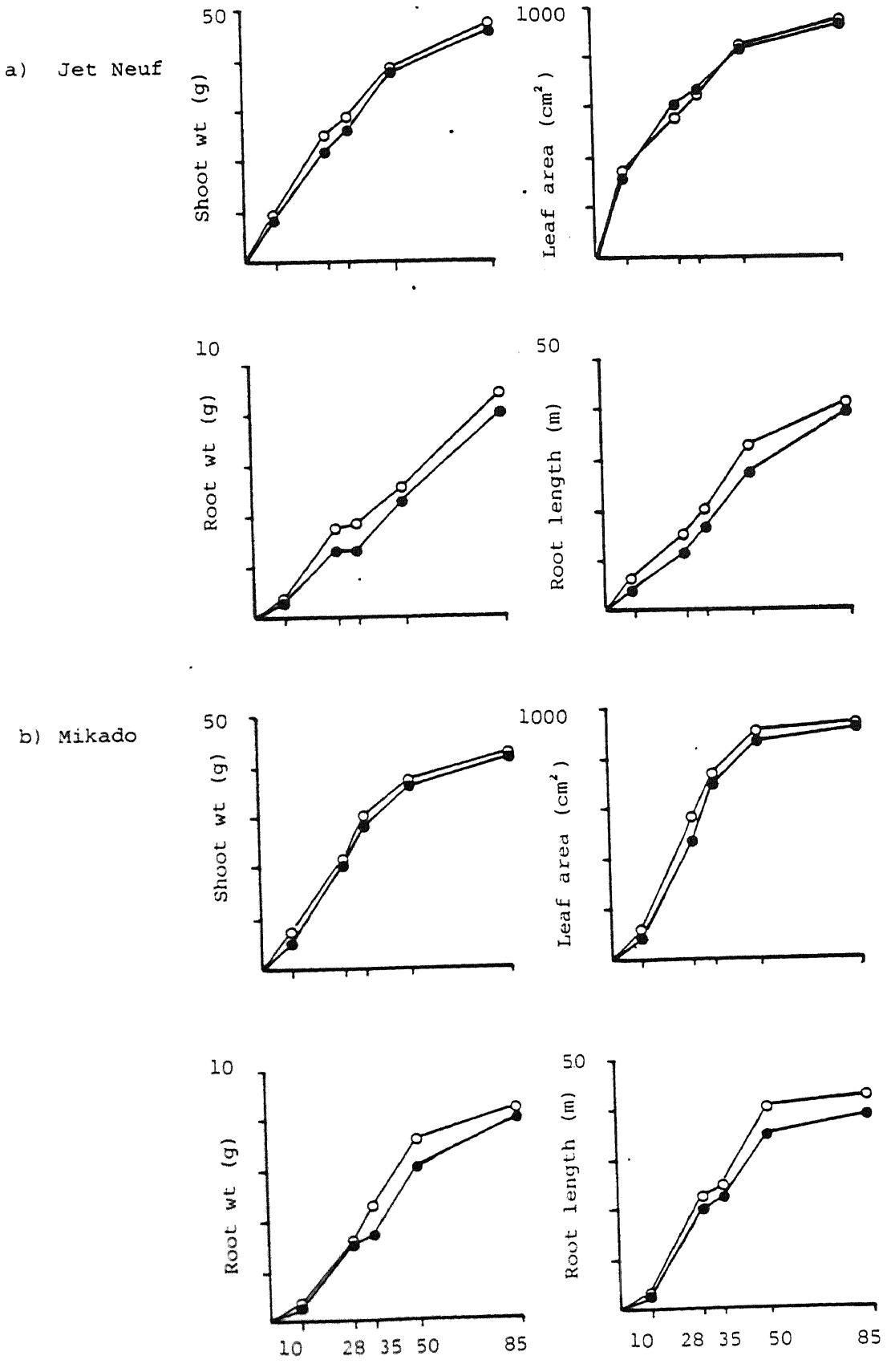
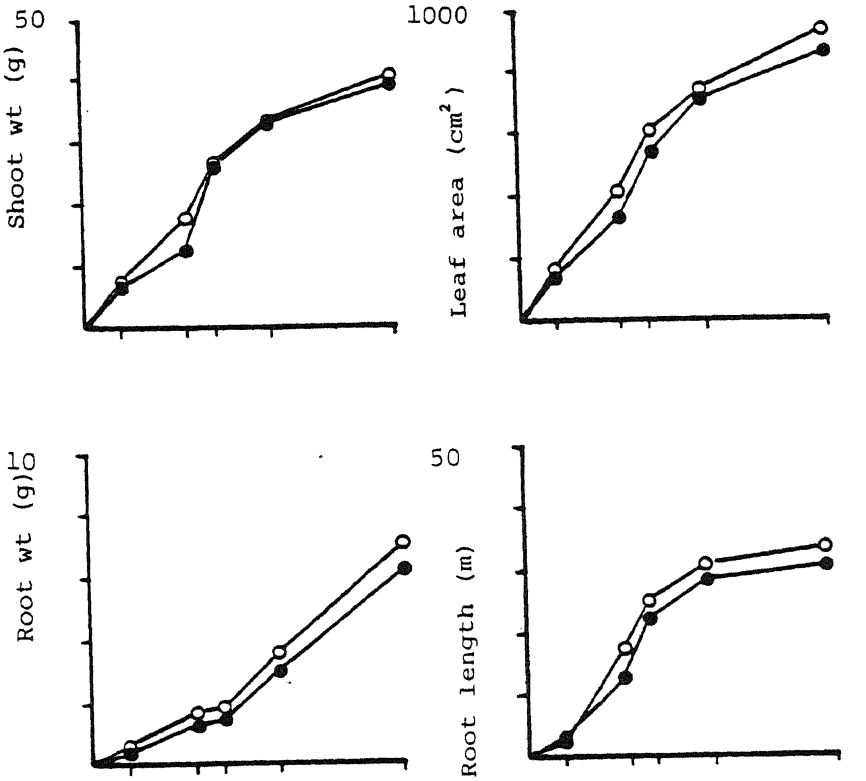


Figure 18 (continued)

c) Elvira



d) Midas

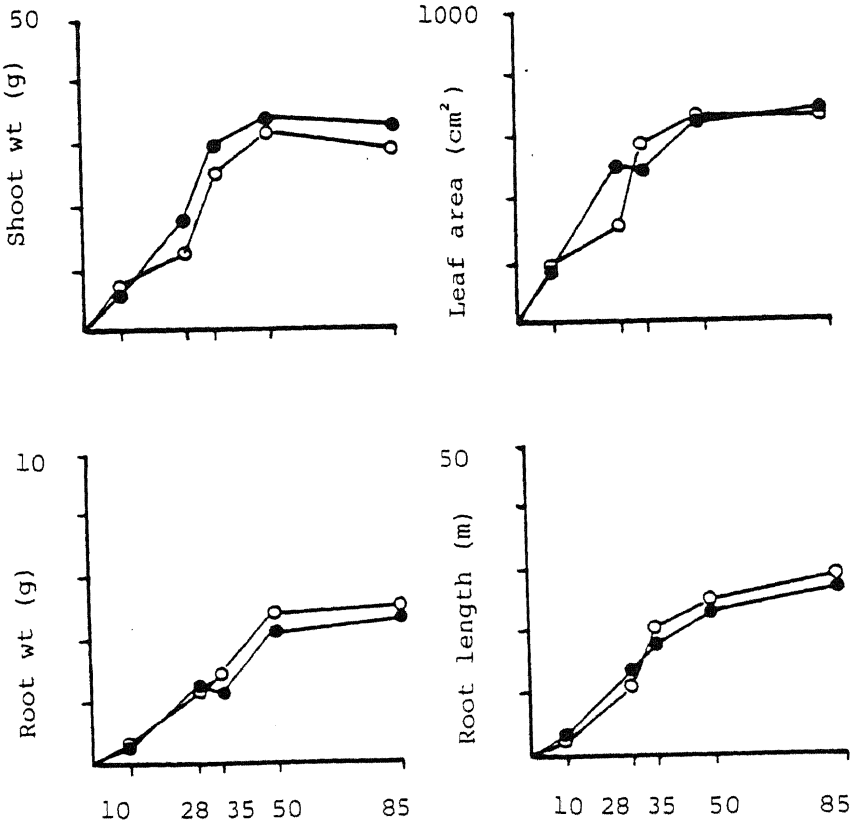


Table 12 Fresh weight of tap roots expressed as a percentage of total fresh root weight of infected (+) and uninfected (-) plants

	35 days	50 days	85 days
Jet Neuf +	57.58*	34.3	27.8
Jet Neuf -	38.8	26.8	24.1
Mikado +	46.7*	34.6*	21.7
Mikado -	33.6	22.7	18.
Elvira +	81.3*	56.2*	50.1*
Elvira -	65.1	42.3	39.7
Midas +	61.1*	43.2*	35.8
Midas -	41.1	34.0	31.1

* Significantly different from uninfected plants ($p < 0.05$)

Table 13 The total numbers of nematodes in the roots and in the soil of each cultivar

Days after	Jet Neuf		Mikado		Elvira		Midas	
inoculation	roots	soil	roots	soil	roots	soil	roots	soil
10	20	1524	28	2953	17	758	13	1231
28	95	1440	67	1763	107	2480	225	3825
35	153	2475	84	1780	65	2385	170	1780
50	159	890	205	205	212	706	165	165
85	226	1122	355	2051	183	815	166	603

Table 14 Egg production by H.cruciferae on four cultivars of oilseed rape

	Eggs produced /plant		Eggs produced /g root	
	50 days	85 days	50 days	85 days
Jet Neuf	957±241	4081±316	222.6±84.6	516.9±114.1
Mikado	461±145	5548±607	89.3±32.1	697.6±105.3
Elvira	471±112	645±192	162.0±24.7	124.0±55.7
Midas	1313±314	1610±253	301.6±67.3	368.4±72.0

Means ± S.Error

The development of H.cruciferae on the four cultivars is shown in Fig.19. The numbers of second stage juveniles and adult males extracted from the soil on each occasion are given in Table 13. By 10 days juveniles had hatched and invaded the roots of all cultivars although no third stage juveniles had developed in the roots by this time. The largest number of juveniles were found in the soil of pots containing Mikado, representing 59.1% of the number of eggs added to the soil. The smallest number of juveniles in the soil at this time were found in pots containing Elvira (15.2% of the number of eggs added to the soil). At 28 and 35 days the largest numbers of second stage juveniles were found in the roots of Midas and this corresponded to a large number of juveniles found in the soil at 28 days. Second, third and fourth stage juveniles and adult males were found in the roots of all cultivars by 28 days and adult males were present in the soil of pots containing Jet Neuf, Mikado and Elvira. At 35 days a large number of juveniles were found in the soil of pots containing Jet Neuf and Elvira and the numbers of juveniles in the roots of Jet Neuf were greatest at this time. The numbers of third and fourth stage juveniles in the roots of Jet Neuf, Mikado and Midas were greater at 35 days than at 10 and 28 days. No fourth stages were found in the roots of Elvira at 35 days. At 50 and 85 days the number of second stage juveniles in the roots of Jet Neuf, Elvira and Midas had decreased whilst the number in the roots of Mikado had increased. Fewer juveniles were found in the soil of all cultivars at 50 days than on previous occasions but the numbers had increased by 85 days. Adult females were found in the roots of all cultivars at 50 days and larger numbers had developed by 85 days in Jet Neuf, Mikado and Elvira. This corresponded to a large number of cysts in the soil of Jet Neuf and Mikado at 85 days. Table 14 gives the numbers of eggs produced by H.cruciferae on each cultivar at 50 and 85 days. At 50 days the largest numbers of eggs per plant and eggs per g root were produced on Midas and Jet Neuf. At 85 days Mikado and Jet Neuf had produced more eggs per plant and eggs per g root than other cultivars. Elvira produced fewest eggs per plant at85 days and the number of eggs produced per g root had decreased between 50 and 85 days.

iii) Root difusate produced by Ariana hatched more H.cruciferae juveniles per g root than other cultivars whilst diffusate produced by Mikado hatched more H.schachtii juveniles (Table 15). At 28 days the roots of Bienvenu and Mikado infected with H.cruciferae weighed

Figure 19 Development of H. cruciferae in the roots of four cultivars of oilseed rape

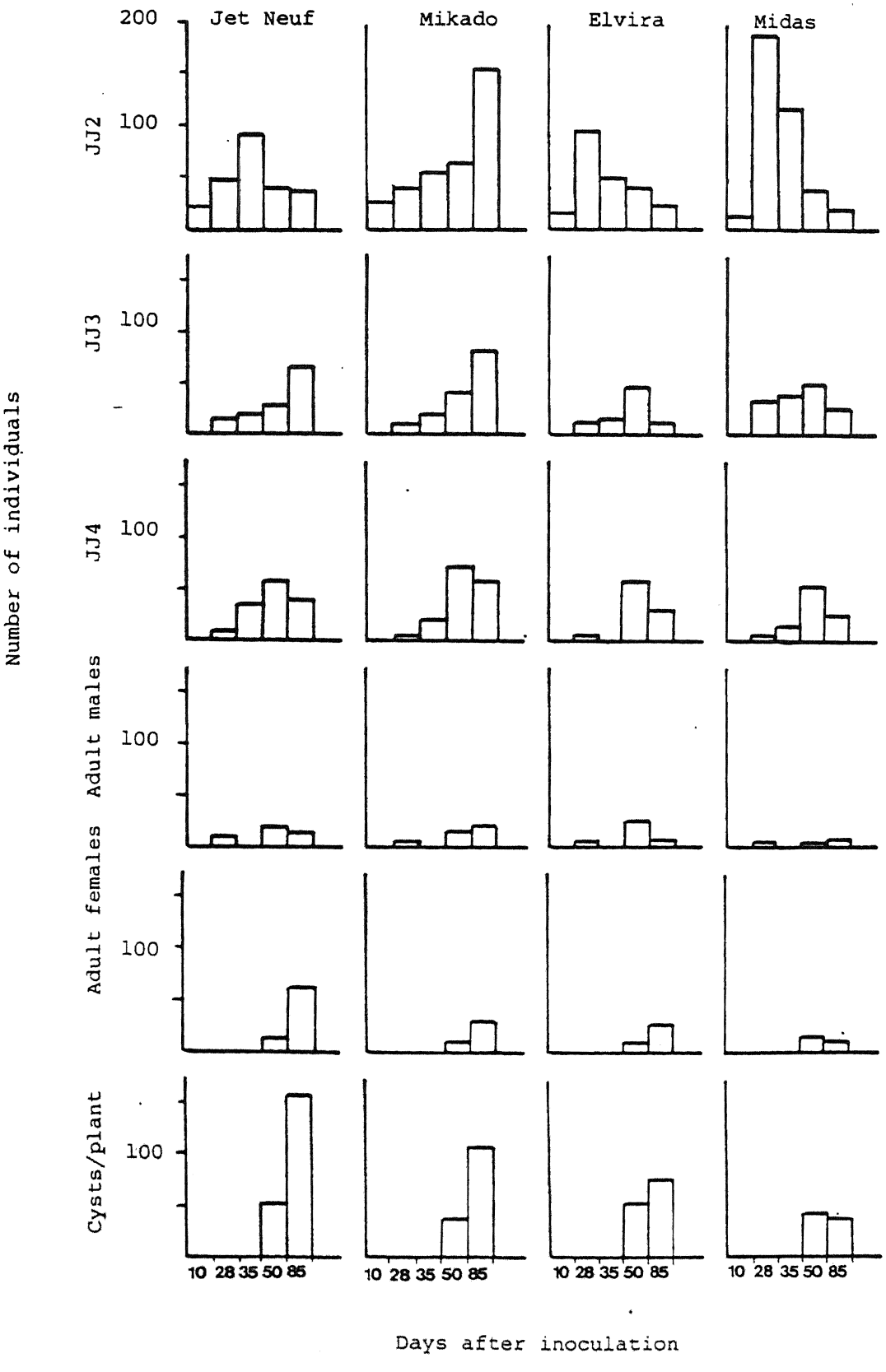


Table 15

Assessment of root growth and multiplication of H.cruciferae and H.schachtii on three cultivars of oilseed rape

	Percentage hatch at 28 days	Juveniles hatched/g root	Root weight at 28 days (g)	Root growth score	Nematodes/ g root at 28 days	Nematodes/ g system at 105 days	Cysts produced/ plant at 105 days	Eggs produced at 105 days /plant	/g root
H.cruciferae									
Ariana ±	21.6	175.0	2.6 3.0	4.2* 5.7	38.4	99.0	84	3432	324.3
Bienvenu ±	16.7	94.8	3.7* 4.4	7.8* 9.3	27.9	45.7	64	2222	146.6
Mikado ±	15.5	125.0	2.4* 3.1	3.6* 4.7	48.3	68.4	96	3942	424.1
H.schachtii									
Ariana ±	32.6	271.6	2.3* 3.0	3.9* 5.7	68.1	184.2	151	6930	761.5
Bienvenu ±	44.1	252.8	2.9* 4.4	7.3* 9.3	62.1	165.0	127	4362	269.8
Mikado ±	39.7	320.1	2.1* 3.1	4.3 4.7	73.3	230.4	173	7221	783.3

* significantly different from uninfested plants, $p<0.05$

\+ infested plants
\- uninfested plants

significantly ($p < 0.05$) less than the roots of uninfected plants and similarly the roots of Ariana, Bienvenu and Mikado infected with H.schachtii weighed significantly ($p < 0.05$) less. Root growth scores (root weight at 105 days/root weight at 28 days) of all cultivars infected with H.cruciferae were smaller than uninfected cultivars whilst the root growth scores of Ariana and Bienvenu infected with H.schachtii were smaller. Bienvenu grew better than other cultivars as indicated by the larger growth scores of infected and uninfected plants. More H.cruciferae and H.schachtii juveniles were found in the roots of Mikado at 28 days than in other cultivars. At 105 days more H.cruciferae juveniles were found in the roots of Ariana and more H.schachtii juveniles in the roots of Mikado. A larger number of H.schachtii juveniles than H.cruciferae were found in the roots at both 28 and 105 days. Most cysts, eggs per plant and eggs per g root were produced by both H.cruciferae and H.schachtii on Mikado by 105 days whilst fewest were produced on Bienvenu.

DISCUSSION

Oilseed rape was more sensitive to nematode damage when sown in soil infested with H.cruciferae than when plants were grown for 28 days in nematode-free soil prior to inoculation. The primary effect of nematodes was to reduce the root weight and root length of plants. Cultivars which were less tolerant of nematode damage also had smaller shoot weights and leaf areas. The addition of a cyst inoculum to the soil just below the seed ensured a locally high nematode density surrounding the young roots and reduced their growth. Differences between the growth of uninfected and infected plants persisted throughout the experiment, presumably because the plants were unable to make complete compensatory growth. Few differences were found between uninfested or infected plants which had been inoculated after 28 days growth in nematode-free soil, indicating that the root systems were extensive enough to tolerate any damage. The different cultivars of oilseed rape which were used in this experiment had different rates of growth and maturation. Jet Neuf which had a low root growth score and so was rated less tolerant (despite an initially large root weight at 28 days) in the preliminary screening experiment in Chapter 5 grew better than other cultivars when inoculated with cysts either at sowing or after 28 days. Midas matured more rapidly than other cultivars and a decrease in its rate of growth became apparent later

on. This was also noted for Mikado and Elvira. Tap roots acounted for a large proportion of the total root weight in young plants but as growth continued more fibrous roots were produced and the tap root did not develop as much as it probably would in the field. Nematode damage reduced the amount of fibrous roots of plants whilst the growth of the tap root was relatively unaffected. The majority of nematodes invade the fibrous roots as most are unable to penetrate the tougher main or tap roots (Kampfe, 1960; McCann, 1979). Although tap roots are produced after the main peak of invasion many will be present for the development of subsequent generations. The relationship between plant growth and nematode multiplication is complex and may be different in pots from the relationship found in the field. However, in the absence of any resistance to either H.cruciferae and H.schachtii differences in the growth characteristics of cultivars may lead to differences in their ability to act as hosts. There is little information concerning the root growth of different cultivars in the field. Bienvenu was found to have a fibrous root length of 17.6 Km/m-2 in unfertilised plots whilst the addition of nitrogen to plots resulted in a root length of 28.3 Km/m-2 (Baraclough et al., 1985). Roots were present to a depth of one metre but 62% of roots were found between 0 and 20cm (Baraclough et al., 1986).

Jet Neuf and Mikado were better hosts of H.cruciferae than Elvira and Midas when either inoculated with cysts at sowing or at 28 days. This confirmed the findings of the preliminary screening experiment. These two cultivars had also developed the most extensive root systems after 113 days of growth. More females developed in the roots of Jet Neuf than other cultivars a result of a larger proportion of juveniles becoming female than in other cultivars. Most eggs were produced on Mikado because individual females were more fecund, a result perhaps of Mikado being a better nutrient source. A large number of second stage juveniles in the soil of pots containing Mikado showed that many F1 juveniles had hatched and a large number had already invaded the roots by 85 days. Elvira and Midas were poor hosts of H.cruciferae again confirming the findings of the preliminary screening. A larger number of juveniles initially invaded the roots of Elvira than Jet Neuf and Mikado but fewer females and eggs were produced. Tap roots accounted for a large proportion of the total root weight of Elvira suggesting that less fibrous root space was available for nematode development. Root length was not markedly reduced indicating that fibrous roots were probably thinner. Evans, et al. (1977) point out

that the thinner roots of the potato cultivar Maris Piper may support less development by providing fewer transfer cell sites for nematode females per unit length. This may also apply to the development of H.cruciferae on Elvira. The largest numbers of juveniles were found initially invading the roots of Midas but fewer females were produced on this cultivar. The physiological changes associated with stem elongation, flowering during the rapid maturity of this cultivar may have provided a less favourable environment for female development. Additionally, few males were found in the soil and indicated a high juvenile mortality in the roots. The amount of development of H.schachtii has also been shown to decrease with the age of plants in oilseed rape and sugar beet (Fedorko, 1961; Steele, 1975).

Fewer juveniles were extracted from the soil of all cultivars at 50 days, indicating that juveniles that had hatched early in the experiment were nearing the end of their infective life and were no longer active. This is similar to the decline in the activity and infectivity of Globodera pallida and G.rostochiensis where few juveniles remained active and were able to invade roots after 50 days (Storey, 1984). The increase in the numbers of juveniles in the soil at 85 days is a result of the hatching of F1 juveniles. The small numbers of juveniles found in pots of Midas at this time are related to the greatly reduced hatching activity of the diffusate produced by older plants of this cultivar.

At the time of these experiments Bienvenu and Rafal were the most commonly grown cultivars whereas the recently developed cultivars Mikado and Ariana are likely to be grown more extensively in the future. In the preliminary screening experiment (Chapter 5) Mikado and Rafal were good hosts of H.cruciferae and H.schachtii whilst Bienvenu was a poor host. In the present experiment Mikado and Ariana were good hosts of both species although Mikado was not as good as it was in the preliminary screening. Although Bienvenu grew better than other cultivars plants were still susceptible to nematode damage. Bienvenu produced fewer eggs per plant and per g root than other cultivars confirming the findings of the preliminary experiment. However, in the field at a large initial nematode density Bienvenu was a better host than Jet Neuf and Mikado, probably because the more extensive root system allowed more nematode development to occur (Evans, et al., 1987). At smaller nematode densities or as in this experiment, where

cysts or juveniles are inoculated into the soil, the localised large nematode densities do not exploit the more extensive root system as much as they would in a natural infestation.

Differences in the multiplication of H.cruciferae and H.schachtii on cultivars of oilseed rape are related both to differences in plant growth characteristics, especially root growth and to inherent differences in the ability of a cultivar to support nematode development (as shown by Mikado and Bienvenu). Whether these differences can be used to control the population growth of either H.cruciferae and H.schachtii can only be satisfactorily assessed in field trials. These results indicate that at small nematode densities rapid maturity and the development of a small fibrous root/tap root ratio may limit the amount of nematode development. However, such cultivars must also give acceptable yields and this will inevitably be the most important characteristic of a cultivar.

7

The effect of initial population density of *Heterodera cruciferae* and *H.schachtii* on the growth of oilseed rape and the multiplication of nematode populations

INTRODUCTION

Recent reports of damage to oilseed rape by H.cruciferae have shown a range of pre-planting nematode population densities (Pi) to have been associated with the poor growth of plants (Evans, 1984; Harris & Winfield, 1984; Southey, 1984; Evans & Spaull, 1985). However, the relationship between Pi of H.cruciferae, yield loss and population growth on oilseed rape is unclear. Symptoms of H.cruciferae damage to oilseed rape are patches of poor growth, appearing in late October and November in the establishing crop. Infested plants have stunted root systems and some may show signs of mineral deficiencies (Harris & Winfield, 1984). Evans (1984) pointed out that root damage probably prevents normal uptake of water and nutrients and suppresses the growth of the root system. The damaged roots may also support less top growth. McCann (1979) found that oilseed rape plants growing in soil infested with H.schachtii had reduced growth rates which resulted in later flowering and pod production than in uninfected plants. Reduced growth rates were attributed to the less efficient uptake of water by plants. The primary effect of nematodes on the growth of potato plants is the reduction of root growth when invaded by juveniles (Evans et al., 1977). The percentage of calcium in the dried hulm of potatoes increased with larger numbers of nematodes in the roots and as the supply of calcium to the roots is accounted for by mass flow in most soils the amount of calcium taken up by plants will be related to the amount of water transpired (Trudgill et al., 1975). Therefore, the calcium content of plants will indicate the extent of nematode damage to the roots.

The nematode population density below which no noticeable damage to plants occurs is termed the 'tolerance limit'. For advisory purposes, however, the tolerance limit is a less important value than the economic threshold, the initial population density above which economic yield loss occurs (Cooke, 1984). Sykes & Winfield (1966) found a linear relationship between the Pi of H.cruciferae and the yield loss of winter cauliflowers and assumed an economic threshold of 25.0 eggs g^{-1} soil. Abawi & Mai (1980) showed that H.schachtii decreased yields of cabbage by 21% at a Pi of 6-9 eggs g^{-1} soil and by 42% at a Pi of 68 eggs g^{-1} soil. McCann (1981) showed that at 40.0 eggs g^{-1} soil H.schachtii caused more damage to cabbage than H.cruciferae. Winter oilseed rape cv. Brink was found to have a

tolerance limit of 3.2 H.schachtii eggs g^{-1} soil and shoot weight was further decreased with increasing Pi (Preste, 1983).

As well as the effect of nematodes on the growth of plants, it is important to know how populations of different initial densities increase on plants. This allows final densities and the potential damage to subsequent crops to be predicted. Sykes and Winfield (1966) found a close correlation between the Pf and Pi of H.cruciferae and showed that winter cauliflowers maintained or slightly increased populations of initial densities from 2.5 to 214 eggs g^{-1} soil. Preste (1983) found that winter rape cv. Brink maintained populations with initial densities up to 1024 H.schachtii eggs g^{-1} soil. A maximum multiplication rate of 21 was found at a Pi of 0.22 eggs g^{-1} soil. The maximum population density that can maintain itself on a food source is proportional to the size of the food source available to developing nematodes (Seinhorst, 1970). Therefore if the latter is reduced by intense intraspecific competition and nematode attack the equilibrium density will be reduced accordingly. The multiplication factor (Pf/Pi) of H.schachtii decreased with an increase in Pi on cabbage (Maas & Heijbroek, 1982) but little is known of the relationship of Pf/Pi and Pi on oilseed rape.

This experiment examines the effect of a range of initial population densities of H.cruciferae and H.schachtii on the growth of oilseed rape and the multiplication of each species at each initial density.

MATERIALS AND METHODS

Nine initial population densities (Pi) of H.cruciferae and H.schachtii (0, 2.5, 5, 10, 25, 50, 100, 150 and 300 eggs g^{-1} soil) were used to evaluate the population growth and pathogenicity of the two species to oilseed rape. Experimental soils were prepared by mixing steam-sterilized soil (loam, sand and gravel in the ratio 3:1:1) with H.cruciferae and H.schachtii infested soil from glasshouse cultures maintained on oilseed rape cv. Jet Neuf. Nematode densities of 100, 150 and 300 eggs g^{-1} soil were prepared by adding and thoroughly mixing cysts with the cultured soil to obtain the greater densities.

Ninety pots (10cm diam.) were filled with 400g of soil containing the desired nematode population densities. Each nematode density was replicated five times. Ten pots were filled with 400g of uninfested soil. Three seeds of oilseed rape cv. Jet Neuf were sown in each pot. Plants were thinned to one seedling pet pot five days after emergence. Plants were kept in two growth cabinets (Fison Fi-toton 600) at 15°C and with a 12 hour photoperiod for 12 weeks. The position of pots was altered every week to randomise any environmental variations. After 12 weeks plants were gently removed from the pots and the roots were rinsed carefully so as not to dislodge any developing females.

i) Plant growth

Shoot and root systems were separated, blotted dry and their fresh weights measured. Relative estimates of leaf area were determined by passing the individual leaves of each plant through a CSP portable area meter. The shoot systems were then oven dried at 60°C for 48 hours and shoot dry weights recorded.

The calcium content of the shoots of each plant grown at each nematode density was prepared for analysis by a dry ashing and acid digestion method. The dry shoot material of each plant was macerated in a blender and then ground into a fine powder using a pestle and mortar. A known weight of material (approximately 0.1g) was weighed into a porcelain crucible and transferred to a muffle furnace at 350-400°C for 3.5 hours. The size of the muffle furnace restricted the number of crucibles that could be used so material was ashed in four batches. After ashing, crucibles were removed from the furnace and allowed to cool. To each crucible of ashed shoot material and to five empty crucibles 1ml of distilled water and 5ml of 1:1 HCl was added. A watch glass was placed over each crucible to contain any effervescence. The crucibles were placed on a hot plate and the material digested for 30 minutes. Any material on the watch glass was washed back into the crucible with distilled water and evaporated to dryness. Ten millilitres of 0.6N HCl was then added to each crucible and heated for 10 minutes on the hot plate. After cooling the contents of each crucible were transferred to a 100ml volumetric flask, diluted to 100ml with distilled water and thoroughly mixed. The solutions were filtered through Whatman No 42 filter paper into numbered polythene bottles.

The analysis of the calcium content of each solution containing digested shoot material and the five blank solutions was carried out using a Baird Alpha 4 flame atomic absorption spectrophotometer with reference to standard calcium solutions. The analysis of the blank solutions determined the calcium content of the reagents which could be distinguished from the calcium content of solutions containing digested shoot material.

ii) Nematode population growth

After 12 weeks the soil surrounding each plant was gently mixed and cysts were extracted from a 100g soil sub-sample by fluidising column. The egg content of cysts were measured by the usual method. Additionally the contribution of egg sacs to the population density was estimated by collecting cysts and egg sacs from a further 100g of soil by the decanting and sieving method described in Chapter 3. Females attached to the roots of each plant were counted, removed and their egg content recorded by opening and releasing the eggs from each female. The numbers of nematodes in the roots and their stage of deveopment were then determined by staining and releasing the nematodes from the roots by the usual method.

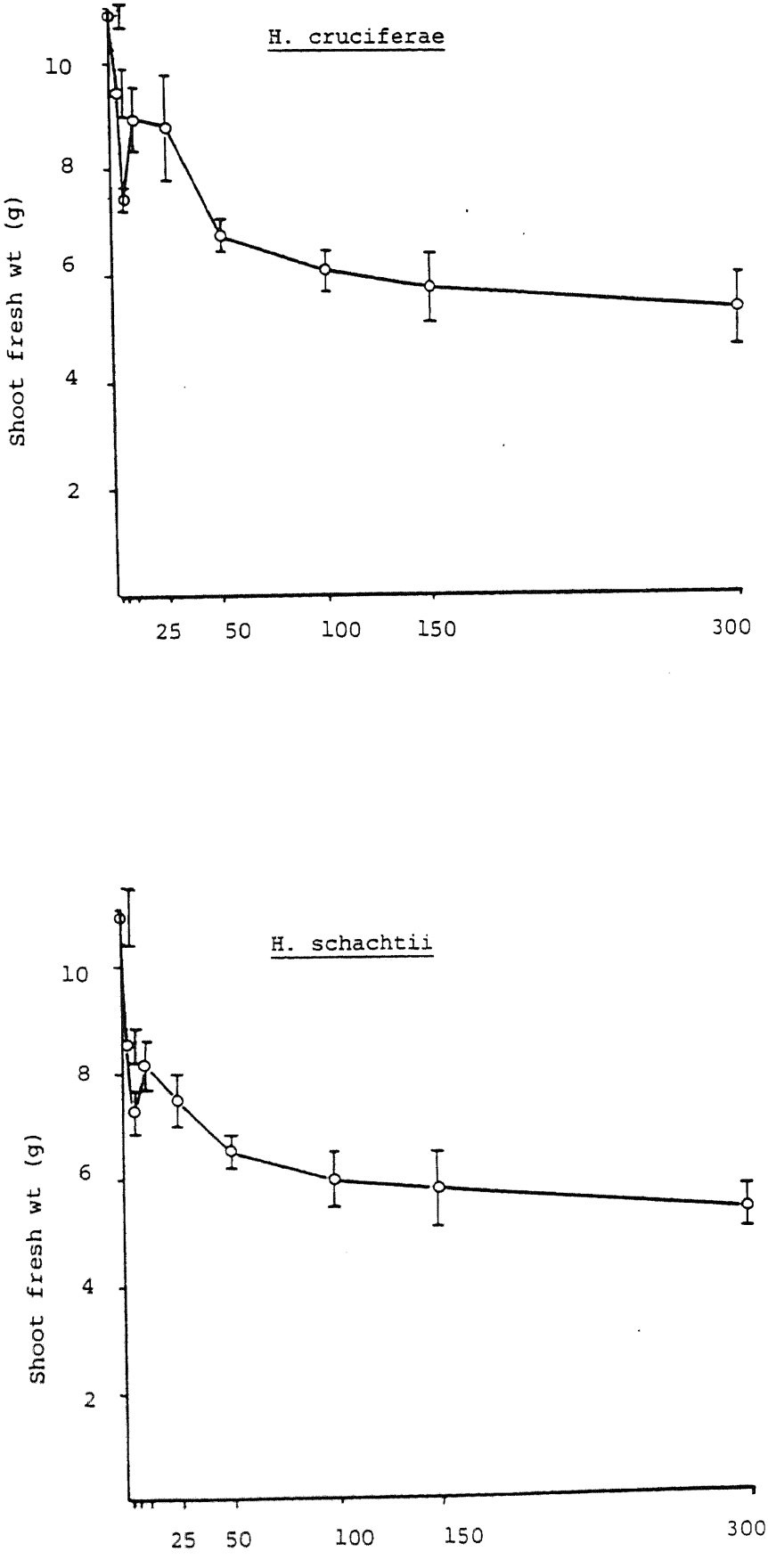
RESULTS

i) Effect of nematodes on plant growth

Plants growing in soil containing large initial nematode population densities (Pi) appeared smaller than plants growing in nematode-free soil shortly after the shoots had emerged. When the plants growing in infested soil were removed from the soil at 12 weeks their root systems appeared less extensive than the root systems of plants growing in nematode-free soil. Plants showed no other visible symptoms of nematode damage. Uninfested and infested plants were still at the vegetative growth phase after 12 weeks and this corresponded to stage one of the growth stage key proposed for oilseed rape by Sylvester-Bradley and Makepeace (1984).

Shoot fresh weights of plants growing in infested soil were significantly ($P > 0.05$) smaller than those of plants grown in

Figure 20 **The effects of initial nematode population density on the shoot fresh weight of plants. Vertical bars represent 95% Con. limits**



nematode-free soil (Fig. 20). Shoot fresh weights of plants exposed to a Pi of 50, 100, 150 or 300 H.cruciferae and H.schachtii eggs g^{-1} soil were significantly (P> 0.05) less than those plants grown in soils of smaller Pi. There were no differences between the shoot fresh weights of plants growing in soil containing these densities. The weight of the dried shoots of plants exposed to a Pi of 5.0 or more eggs g^{-1} soil were significantly (P> 0.05) smaller than the dry weight of plants at 2.5 eggs g^{-1} soil or in nematode-free soil (Fig. 21). Leaf-areas were significantly (P> 0.05) smaller when plants were growing in soil infested by each species (Fig. 22). The leaf-areas of plants growing in soil with a Pi of 25 or more H.schachtii eggs g^{-1} soil and 50 or more H.cruciferae eggs g^{-1} soil were significantly (P> 0.05) less than plants exposed to a smaller Pi. The roots of plants growing in infested soil weighed significantly (P> 0.05) less than those of plants in nematode-free soil (Fig. 23) but there was no further reduction in root weight at Pi's greater than 50 eggs g^{-1} soil. The total fresh weight of plants grown at each nematode density is expressed as a percentage of the weight of uninfested plants (Fig. 23). All nematode densities significantly (P> 0.05) reduced the fresh weights of plants. Initial densities of 2.5 eggs g^{-1} soil of either H.cruciferae or H.schachtii reduced the fresh weight of plants by 13.9% and 22.8% respectively. Fresh weights of plants were further reduced by an increase in Pi with a greatest reduction in plant fresh weight (52.6% and 52.5%) at a Pi of 300 eggs g^{-1} soil. Initial densities of between 2.5 and 25 eggs g^{-1} soil of H.schachtii resulted in a greater reduction in plant fresh weight than that caused by H.cruciferae at these densities.

The effect of Pi on the percentage of calcium in the dried shoot material is shown in Fig. 24. The percentage of calcium in shoots increased with increasing Pi. At a Pi of 5.0 or more eggs g^{-1} soil of H.schachtii and 5.0, 10 or 50 or more eggs g^{-1} soil of H.cruciferae there was a significantly (P> 0.05) greater percentage of calcium in the shoots of infested plants than uninfested plants. Approximately twice as much calcium was accumulated by plants growing in soil containing the largest initial population density of both species than was by plants growing in nematode free soil.

Figure 21 The effect of initial nematode population density on the shoot dry weight of plants. Vertical bars represent 95% con. limits

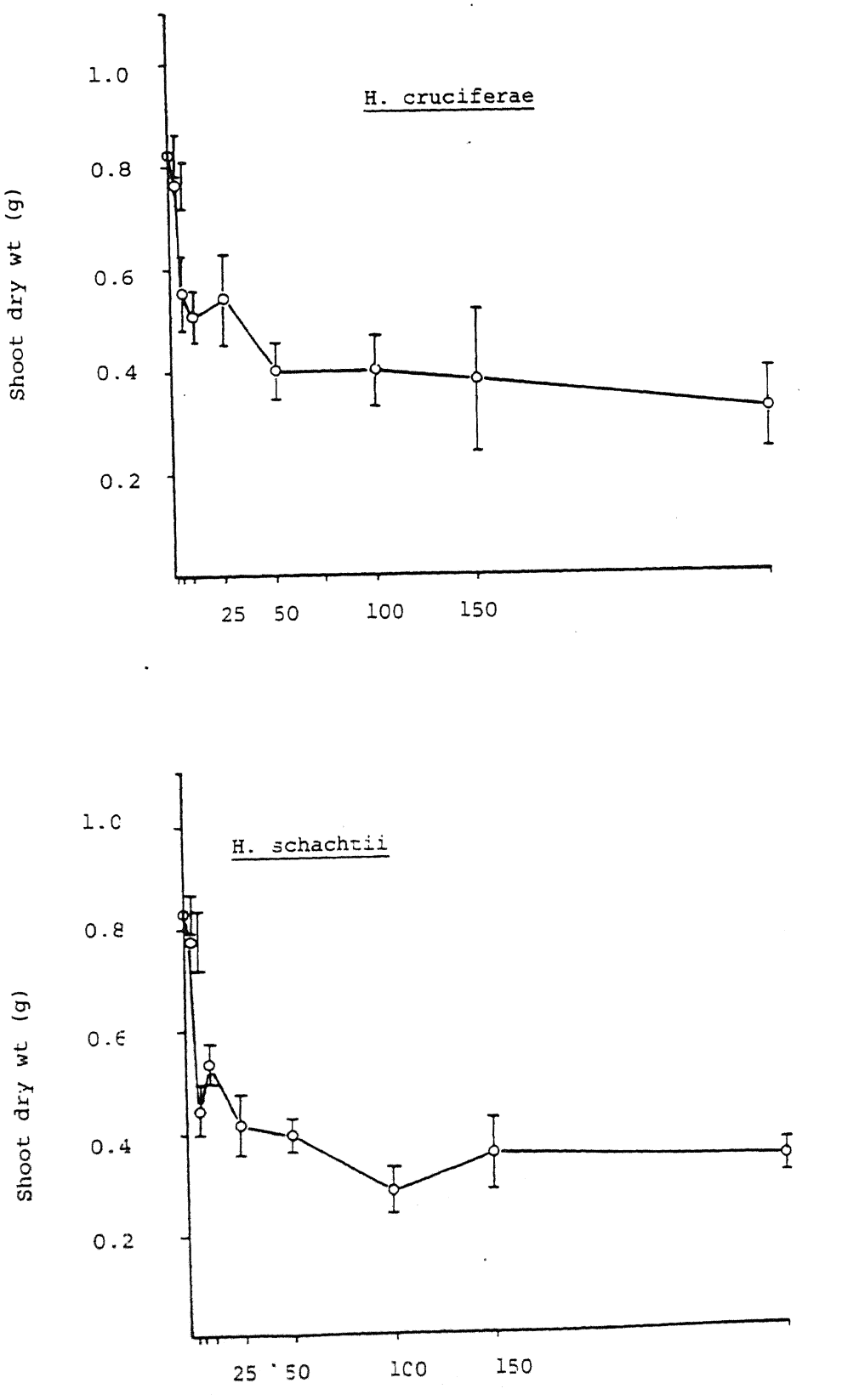


Figure 22 The effect of initial nematode population density on the leaf area of plants. Vertical bars represent 95% con. limits.

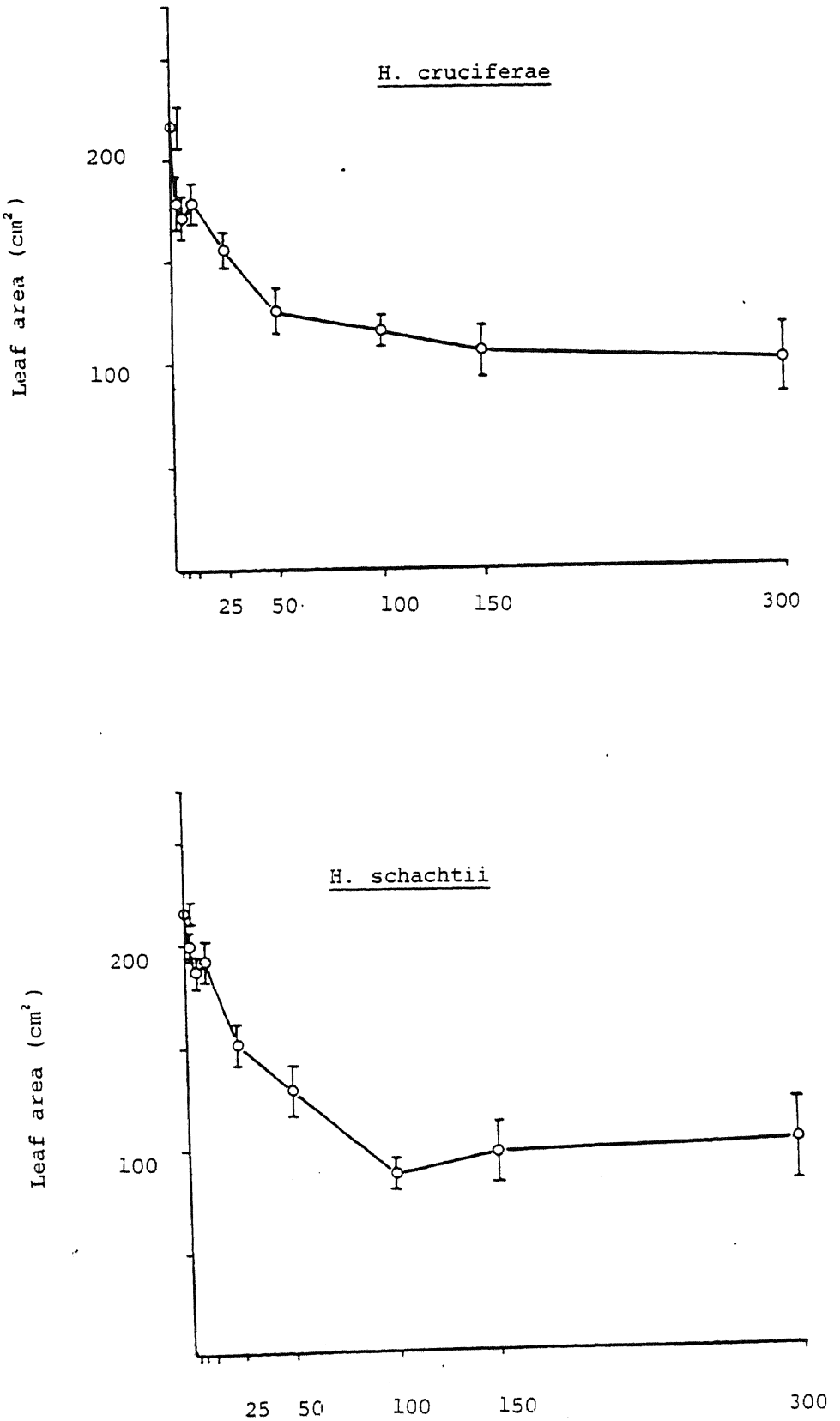
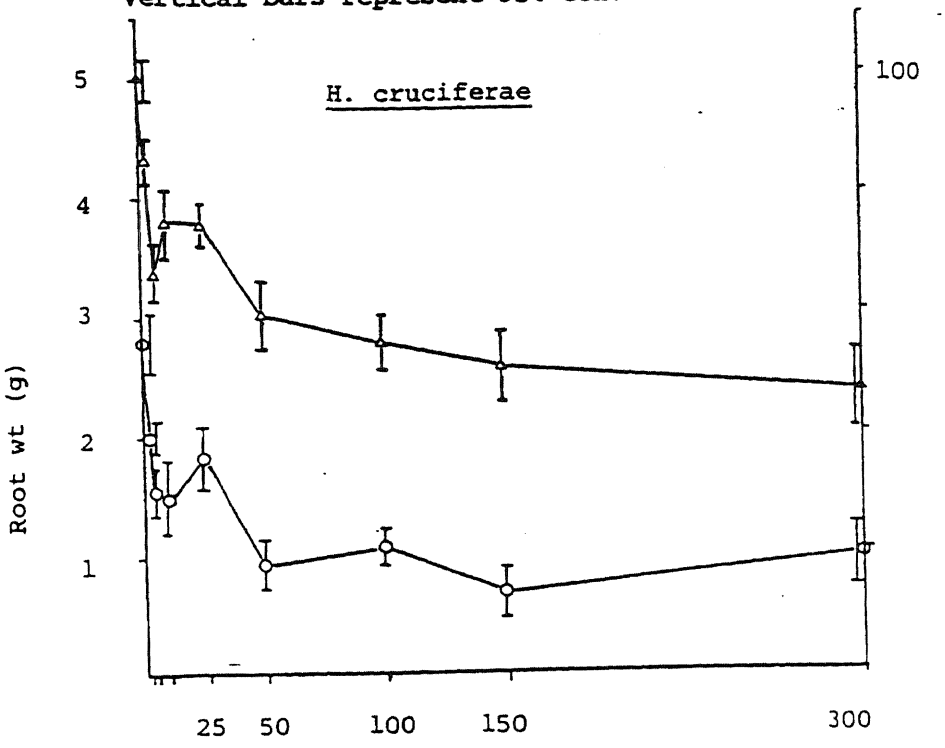


Figure 23 The effect of initial nematode population on root weight (O) and total plant fresh weight (Δ). Vertical bars represent 95% Con. limits.



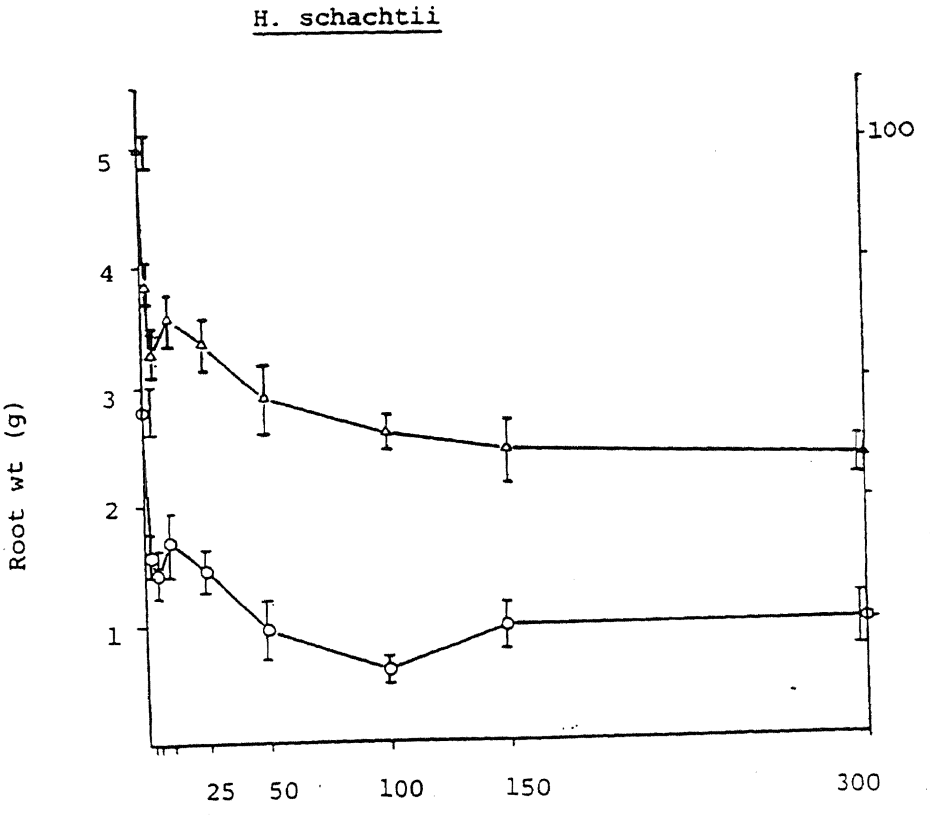
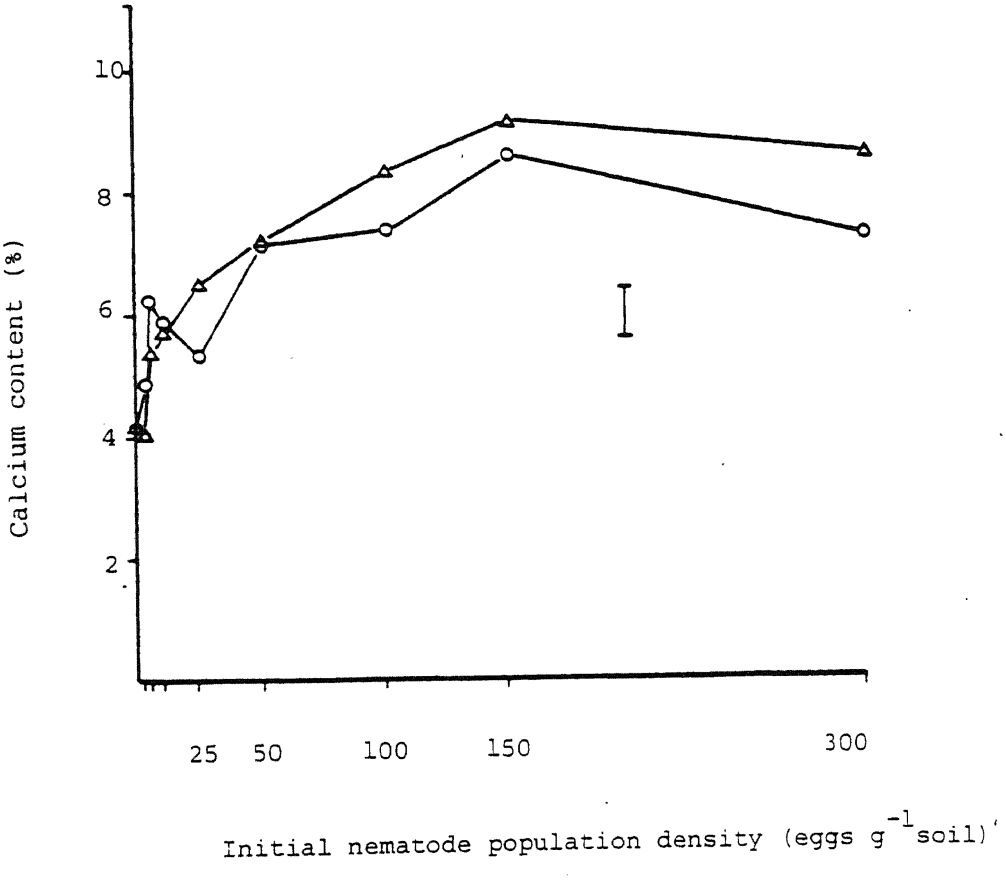


Figure 24 The effect of initial population density of H. cruciferae (○) and H. schachtii (△) on the calcium content of shoots. Vertical bar represents L.S.D. (P = 0.05)



ii) Nematode population growth

Final soil population densities (Pf) of H.cruciferae and H.schachtii increased with increases with initial popultion density (Pi). Fig. 25 shows the regression of log Pf on log Pi for both species for estimates of Pf based on the number of eggs in cysts only and estimates based on eggs in egg sacs and cysts together. Estimates of Pf were larger when based on egg counts in cysts with egg sacs although the differences were not significant. Intersection of the X1 multiplication line by the regression lines gave equilibrium densities of 37.6 and 42.0 eggs g^{-1} soil for H.cruciferae when estimates were based on cysts only and cysts plus egg sacs respectively. The corresponding values for H.schachtii were 61.5 and 68.8 eggs g^{-1} soil.

Table 16: Multiplication of H.cruciferae and H.schachtii expressed as the Pf/Pi ratio relative to initial population density (Pi)

	Initial population density (Pi) eggs g^{-1} soil							
	2.5	5.0	10	25	50	100	150	300
H.cruciferae								
Cysts	5.40	3.74	2.73	1.17	0.74	0.51	0.44	0.32
Cysts & egg sacs	6.54	4.37	3.01	1.38	0.83	0.54	0.46	0.33
H.schachtii								
Cysts	9.87	5.12	3.06	1.35	0.61	0.43	0.49	0.26
Cysts & egg sacs	10.97	5.63	3.39	1.53	0.68	0.46	0.51	0.27

Table 16 shows that the ratio Pf/Pi (the multiplication factor) decreases with increasing Pi. Estimates of final population densities based on the number of eggs in egg sacs plus cysts gave greater multiplication factors although the difference between these two estimates decreased with increasing Pi. Heterodera schachtii achieved a maximum multiplication factor of 10.97 at a Pi of 2.5 eggs g^{-1} soil whereas a maximum multiplication factor for H.cruciferae of only 6.54 was recorded at the same initial density.

Figure 25 **The relationship between initial and final nematode population densities based on numbers of eggs in cysts and egg sacs (●) and eggs in cysts only (○).**

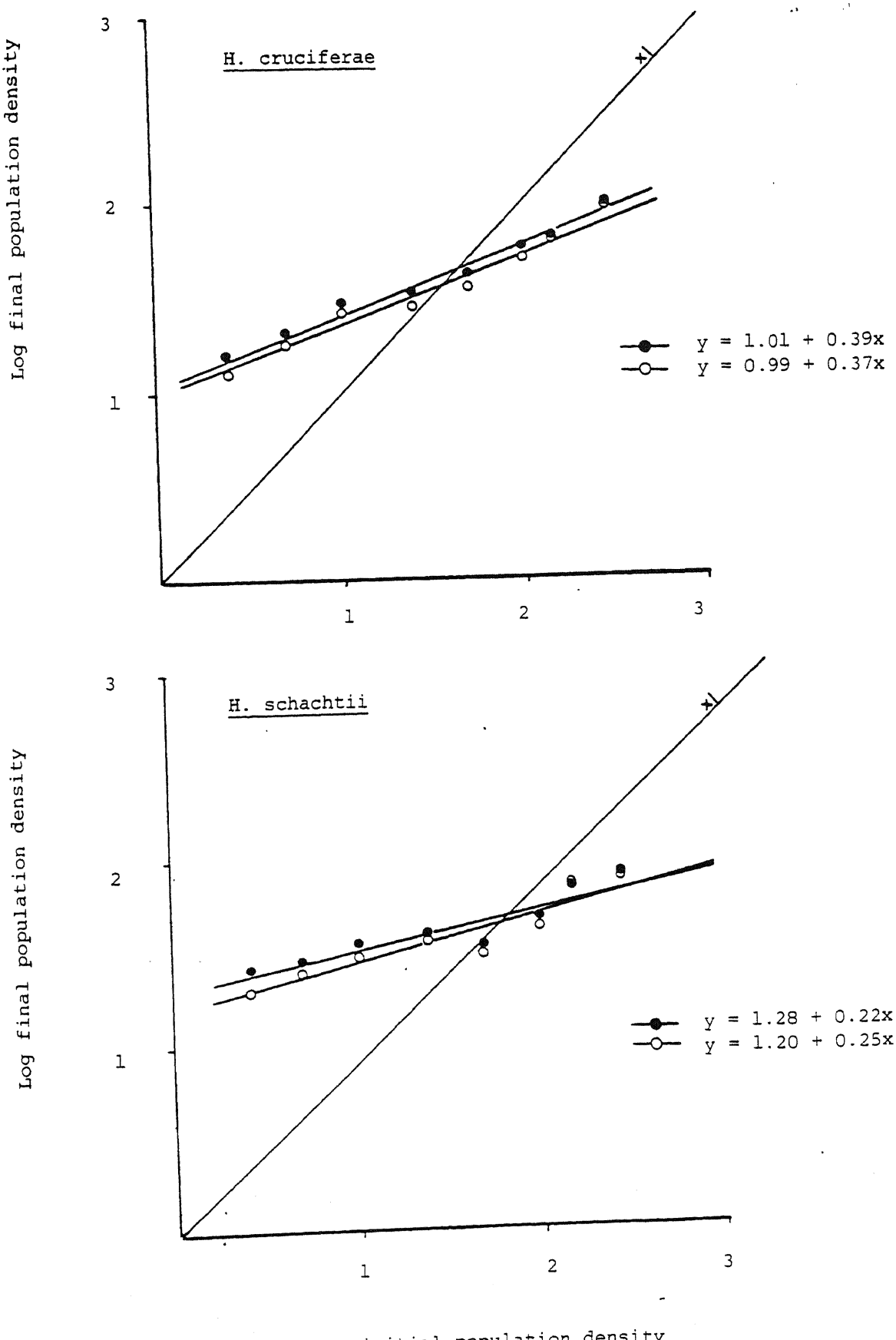


Table 17 gives the percentage of nematodes of both species found in the roots at each development stage after 12 weeks at each initial density. The numbers of juveniles g^{-1} root are also given at each density. The percentage of nematodes of each developmental stage varied considerably at each initial population density. However, few trends were identified between the initial densities of each species. Significantly ($P< 0.05$) fewer second stage juveniles and more adult females were found in the roots of plants growing in soil containing a Pi of 2.5 eggs g^{-1} soil than at other initial densities. The largest numbers of H.cruciferae adult males were found in the roots of plants growing in soil with a Pi of 25 or more eggs g^{-1} soil. Few H.schachtii adult males were found at any nematode density. The largest number of H.cruciferae juveniles g^{-1} root was recorded in the roots of plants growing in soil with a Pi of 150 eggs g^{-1} soil. The numbers of juveniles g^{-1} root of plants growing in soil with a Pi of 25 and 300 eggs g^{-1} soil were the smallest. The numbers of H.schachtii juveniles g^{-1} root increased with increasing Pi up to 100 eggs g^{-1} soil, but decreased again at Pi's greater than this.

Table 17: Percentage of stages of H.cruciferae found in the roots after 12 weeks in relation to different initial population densities

Initial population density eggs g^{-1} soil	2.5	5.0	10	25	50	100	150	300
Development Stage								
J2	8.0	50.0	43.4	5.5	62.7	41.1	47.8	15.5
J3	16.0	15.0	13.0	27.7	6.9	23.5	0.0	10.0
J4	8.0	10.0	13.0	22.2	9.3	6.1	4.5	20.0
Adult male	0.0	0.0	0.0	16.6	2.3	5.8	8.6	15.0
Adult female	68.0	25.0	30.4	27.7	20.9	23.5	39.1	39.5
Juveniles g^{-1} root	480.7	500.0	594.8	375.0	1041.6	607.1	1326.9	394.7

Percentage of stages of H.schachtii found in the roots after 12 weeks in relation to different initial population densities

Initial population density eggs g^{-1} soil	2.5	5.0	10	25	50	100	150	300
Development Stage								
J2	10.0	33.3	50.0	16.6	43.4	40.0	25.0	42.0
J3	6.8	6.6	11.1	27.7	21.7	26.6	22.2	20.0
J4	16.6	26.8	16.6	11.1	14.9	13.4	16.6	12.6
Adult male	0.0	0.0	0.0	5.5	0.0	0.0	5.0	0.0
Adult female	66.6	33.3	22.3	39.1	20.0	20.0	31.2	25.4
Juveniles g^{-1} root	145.2	401.7	397.0	465.5	907.8	937.5	789.4	187.5

DISCUSSION

Both H.cruciferae and H.schachtii significantly reduced the growth of oilseed rape over the 12 week period. However, plants showed no signs of excessive lateral root production, leaf discolouration or chlorotic symptoms as described by Lewis (1971). Total plant fresh weights were reduced by between 48.2 and 52.6% in soils containing initial nematode densities of 150 and 300 eggs g^{-1} soil. At a Pi of 2.5-25 eggs g^{-1} soil H.schachtii caused a greater mean reduction in total plant fresh weight than did H.cruciferae. This confirms the results of McCann (1981) who found that the damage caused by these two species to cabbage varied according to the initial population level but overall H.schachtii was the more damaging of the two species. An experiment described earlier has shown that the initial invasion rate of roots by H.schachtii juveniles at 15°C is greater than that by H.cruciferae juveniles (Chapter 2). As young roots systems are less tolerant of nematode damage they may be expected to suffer more damage from H.schachtii invasion. Shoot dry weights of plants growing in soil with a Pi of 2.5 eggs g^{-1} soil were similar to those of plants growing in nematode-free soil. As the primary effect of nematodes is on root growth a larger number of nematodes might be required before any large effects on the growth of tops become evident. Both nematode species appeared to have a damage threshold of 50 eggs g^{-1} soil beyond which larger initial densities of nematodes cause little further

damage. McCann (1981) found a maximum threshold for damage to cabbage of 80 H.cruciferae eggs g^{-1} soil and 150 H.schachtii eggs g^{-1} soil.

The increase in the amount of calcium per gram of shoot fresh weight of plants growing in soil with increasing Pi reflects the extent of root damage. Calcium uptake is believed to be restricted to the younger part of the root and its rate of uptake affected by transpiration (Evans & Franco, 1979). Calcium is therefore a good indicator of water uptake but Evans (1982c) points out that this is only true if all plants are treated similarly and the calcium content of the whole plant is measured. This will be important in potatoes where much calcium may be contained within the tubers or in the large tap roots or in the rap roots of field grown oilseed rape plants. However, in the present experiment the contribution of roots to the dry matter of plants was small and can probably be ignored. A larger percentage of calcium was found in oilseed rape plants than was found in potato plants by Trudgill et al., (1975) and by Evans (1982a). This may have been a result of watering plants with tap water with a large calcium content. McCann (1979) attributed the reduced growth rates of brassicas infested with either H.cruciferae or H.schachtii to a reduction in the efficiency of water uptake by plants. In an autumn sown oilseed rape crop water stress may be an important factor in reducing the growth of young plants infested with nematodes. In September soils are usually below field capacity and in periodic dry spells following wetter periods in which nematodes are more active water stress may occur. During late autumn and winter sufficient water is probably present in the soils to prevent water stress symptoms. However, damage to root growth also reduces the ability of plants to exploit the soil for plant nutrients and plant growth rates may be limited by nutrient deficiency. The mechanism by which H.cruciferae and H.schachtii reduce the growth of oilseed rape is likely to be a combination of water stress and a reduction in nutrient uptake by plants, both resulting from damaged root systems. Evans (1984) also points out that plants with damaged roots may be more susceptible to infection by root rot organisms and the damage to oilseed rape may be an interaction of these factors. The interaction between H.schachtii and Verticillium dahliae (a wilt fungus) on winter rape cv. Brink has been shown to have a synergistic effect on plant growth over a range of initial nematode population densities (Magnusson, 1986). In order to accurately predict the yield loss resulting from nematode damage the way the plant grows, increases in weight and transforms

assimilates to products that are harvested should be taken into account. Only in crops like sugar beet is yield related directly to root size (Jones & Kempton, 1978). However, in an autumn sown oilseed rape crop good plant establishment and good vegetative growth including the development of an adequate root system are important to ensure good overwintering and rapid spring growth (Bilsborrow & Norton, 1984). Both H.cruciferae and H.schachtii reduce leaf areas and root weights considerably and will determine the extent of vegetative growth. Therefore, the effect of nematodes on the growth of oilseed rape up to flowering determines the yield potential of the crop but in subsequent growth stages the extent to which this potential is achieved may be modified by climatic and agronomic factors (Mendham et al., 1981).

Final estimates of nematode population densities based on the number of eggs in cysts only underestimated the multiplication rate of H.cruciferae and H.schachtii. At 2.5 eggs g^{-1} soil (the maximum multiplication rate) the difference was 1.14 and 1.10 respectively. However, the number of eggs contained within egg sacs represented a smaller proportion of the final population density at large population densities as the ratio of newly provided eggs in egg sacs to initial population density was much less than at smaller initial densities. Heterodera cruciferae and H.schachtii require 71 and 66 days respectively to complete a generation at 15°C; in this experiment therefore one generation would have been completed by each species by 12 weeks and many juveniles may have hatched from F1 egg sacs by this time and the difference between the two estimates of Pf would be less than if estimates were made before juveniles had hatched. The maximum multiplication rates occurred at the smallest values of Pi and decreased with increasing Pi. This decrease in the multiplication rate was consistent with findings for other cyst-nematodes (Jones & Kempton, 1978); however, Sykes & Winfield (1966) reported no decrease in the multiplication rate of H.cruciferae on winter cauliflowers over the range 2.5-214.0 eggs g^{-1} soil. Preste (1983) found the multiplication rate of H.schachtii on winter rape cv. Brink to decrease to unity at approximately 100 eggs g^{-1} soil but at values of Pi up to 1024 eggs g^{-1} soil there was no further decrease in Pf/Pi. At an initial density of 1024 eggs g^{-1} soil and on the basis of the present results, roots would be expected to be severely damaged allowing only a limited amount of nematode multiplication.

The reduction in the multiplication rates of cyst nematodes at large initial densities is a result of a smaller proportion of the juveniles that penetrate the roots becoming adult females, perhaps due to the intense competition for feeding sites. Multiplication rates are further reduced by the damage to plants caused by nematodes (Seinhorst, 1967). In the field, only a proportion of the nematode population is stimulated to hatch by the host plant and, as root damage increases at larger nematode densities, this proportion will decrease. In simple terms this decrease could be considered to be the same as the decrease in plant root weight (Seinhorst, 1970). Both H.cruciferae and H.schachtii were found to reduce root weight considerably. Therefore, at large initial densities, the proportion of the population influenced by the roots will be small and the contribution of newly produced eggs to the final Pf will be less. There was no evidence that nematode development was slowed down by greater population density. Wallace (1969) found that at greater densities Meloidogyne javanica juveniles took longer to become adult in tomato roots. This has not been found in other Heterodera species (Seinhorst, 1970).

There is little information concerning the multiplication of H.cruciferae and H.schachtii on oilseed rape in the field. Evans (1984) recorded a Pf of 94 eggs g^{-1} soil in July after observing a population density of 48 eggs g^{-1} soil in April. The Pi was not recorded but would have been much less than this. In contrast, a population with a Pi of 85 eggs g^{-1} soil had a very small multiplication rate giving a Pf of 29.3 eggs g^{-1} soil (Evans & Spaull, 1986). In this experiment the multiplication of H.cruciferae and H.schachtii populations was most rapid at small initial densities and populations reached equilibrium densities of 37.6 H.cruciferae and 61.5 H.schachtii eggs g^{-1} soil when estimates were based on the contents of cysts only. Further work is needed to establish relationships between the growth of populations and initial nematode densities in the field.

8

The effect of plant age on the hatching of *H.cruciferae*

INTRODUCTION

Winslow (1955) found that H.cruciferae hatched less easily than H.schachtii in root diffusate produced by hosts in the genus Brassica demonstrating that H.cruciferae was more dependent on a chemical stimulus than H.schachtii, which hatches readily in water. Therefore, the effect of the age of plants on the 'potency' of root diffusate had a marked effect on the hatching of H.cruciferae whereas it had less effect on H.schachtii. A method for the determination of the 'potency' or activity of root diffusate is described by Fenwick (1952). This uses log-activity values to characterise the 'strength' of root diffusate and these represent the amount of dilution required to decrease hatching to the level to that in distilled water. Widdowson (1958) found that the log-activity of potato root diffusate increased over the first three weeks of the growth of plants but declined after four weeks with the time of maximum diffusate activity coinciding with the period of rapid root growth. Similar relationships have been established for other cyst nematode species and their host plants (Kerry & Jenkinson, 1976; Beane & Perry, 1983; Tefft & Bone, 1985).

When sown as a winter crop in September, oilseed rape may be in the ground for eleven months. Plants produce leaves over winter but when soils warm up in the following spring stem elongation occurs prior to plants flowering and producing seed pods. Growth can be separated into distinct growth stages (Sylvester-Bradley & Makepeace, 1984) but there is a paucity of information concerning the physiological changes associated with these growth stages and the effects that these may have on root diffusate production. Root growth is reduced after flowering in most plant species and associated with this is a diminished translocation of carbohydrates to the roots (Aung, 1974). A large proportion of oilseed rape roots system undergo secondary growth resulting in a large amount of woody root material. Again there is little information concerning the relationship between root exudation and root system development.

Attempts to isolate and identify the hatching factor of H.cruciferae and other cyst nematodes have been generally unsuccessful (Clarke & Perry, 1977) although in the case of the H.glycines hatching factor, compounds have been recently isolated and characterised (Masamune et al., 1982).

Previous experiments (Chapter 5) have shown that leachate collected from older plants grown in a glasshouse caused fewer H.cruciferae juveniles to hatch than leachate collected from younger plants. However, the percentage hatch of H.cruciferae was small perhaps indicating that H.cruciferae either requires a high concentration of root diffusate if a larger number of juveniles are to hatch or that the techniques for collecting active diffusates are poor. In another experiment (Chapter 6) a much larger number of juveniles hatched after cysts had been added to the soil in which 28 day old plants were growing suggesting that there was a much higher concentration of root diffusate in pots than in leachate washed out of pots with water.

This experiment examines the hatching of H.cruciferae in diffusate collected from plants of different ages grown under either field or glasshouse conditions and derives the log-activity of diffusates. A comparison of the hatching from cysts in either leached diffusates or in soil in which plants were growing is made. Additionally, the persistence of root diffusate is examined in the soil of pots from which either shoots or the whole plant have been removed.

MATERIALS AND METHODS

i) Hatching in leachate collected from field grown plants of different ages.

On 5 September 1985 sufficient pots (13cm diam.) were filled with 1Kg of a mixtue of loam, sand and gravel (3:1:1) and sunk into soil so that their tops were level with the surrounding soil surface. Each pot was then sown with three seeds of oilseed rape, cv. Jet Neuf. Three unplanted pots were also placed in the soil. The area of soil containing the pots was enclosed with one metre high mesh fence sunk to a depth of 20cm and a net was placed over the plot. Two days after emergence plants were thinned to one healthy seedling per pot. A liquid N,P,K fertiliser was added to the pots at this time. Two weeks after sowing and on eight subsequent occasions covering different growth stages of the plants over a period of 45 weeks, three pots were removed from the soil and root diffusates leached from each. Surplus pots allowed diseased and poorly developed plants to be discarded. Four weeks after sowing the unplanted pots were also removed from the plot and leached. The pots removed on each occasion were placed on a

metal tripod above a measuring beaker and sufficient distilled water added to each pot to wash out 25ml of leachate. The leachates collected from each of three pots were then bulked, filtered through Whatman No. 1 filter paper and stored at 5°C. A dilution series of each leachate was prepared by adding distilled water to give strengths of 1:1, 1:16, 1:64 and 1:256. Full strength and diluted leachates were stored at 5°C. The plants from each pot were carefully removed from the soil, washed, blotted dry and the shoots, fibrous roots and woody tap roots separated. The fresh weight of each was measured and the growth of plants assessed with reference to the growth stage key.

The H.cruciferae test population was from a field at Wrangle, Lincolnshire in which oilseed rape had been harvested in July 1985. The cysts were extracted by fluidising column. Three ml of each full-strength and diluted leachate solution were placed in compartments of 25-compartment Repli-dishes which were placed in an incubator at 16°C and kept in the dark. There were three replicates of each treatment. When the temperature of leachate solutions had reached 16°C a batch of fifty cysts was added to each leachate solution and a batch to three distilled water controls. Hatched juveniles were counted and leachate solutions changed at weekly intervals for the next four weeks. At four weeks unhatched juveniles were released by breaking open the cysts and counting the juveniles by the standard method.

ii) Hatching in leachate collected from glasshouse grown plants of different ages

Concurrently with Experiment (i) a sufficient number of pots were sown with oilseed rape seeds as described previously and placed in a glasshouse at a 15°C day and 10°C night temperature and with supplementary lighting over a 12 hour photoperiod. Plants were thinned to one healthy seedling per pot two days after emergence. Enough water was added to pots to keep the soil moist and a liquid N,P,K fertiliser was added weekly. Root leachates were collected two weeks after sowing as described before. However, as plants grew more rapidly under glasshouse conditions leachates were collected at two week intervals on four subsequent occasions and thereafter at 14,16,20 and 24 weeks after sowing. Shoot and root fresh weights were measured and the growth of plants assessed with reference to the growth stage key on

each occasion. Hatching tests using leachate collected at each occasion were carried out as described previously.

iii) Hatching from cysts added to the soil of field grown plants.

Ten weeks after sowing, three pots which had been sown in the first experiment were transferred from the field plot to the glasshouse. Batches of fifty cysts were put into bags of nylon mesh and placed carefully around the root system (5-7cm deep) in each pot. Four bags were placed in each of the three pots. The location of each bag was recorded by pushing a plastic marker into the soil. Enough water was added to pots to keep the soil moist. One bag from each pot was carefully removed weekly for the next four weeks and was gently washed before opening in 5ml of water. The numbers of unhatched juveniles in cysts were counted by the standard method. Thirty-five weeks after sowing a further six pots were transferred from the field plot to the glasshouse. Newly formed cysts were extracted by fluidising column from the soil of ten pots in which oilseed rape plants had been growing for sixteen weeks. Each of these plants had been inoculated with four thousand juveniles four weeks after sowing. Four batches of fifty of these newly formed cysts were put into bags and added to the soil of each of three pots as described previously. Additionally, cysts of the Wrangle population (which had been used in the previous hatching tests) were also extracted from the soil. Four batches of fifty of these older cysts were similarly added to each of the remaining three pots. The location of each bag was recorded by a plastic marker. One bag was removed from each pot weekly for the next four weeks and the numbers of unhatched juveniles in cysts were counted.

iv) The retention of hatching activity by the soil after the removal of plants

At forty-five weeks, twelve oilseed rape plants which were growing in the field plot were cut off at the soil surface and the shoots discarded. Pots were watered to keep the soil moist. After 1,2,4 and 6 weeks three pots were removed and leached by the standard method and a hatching test carried out using the Wrangle test population. There were insufficient pots with healthy plants remaining in the field plot for a comparison to be made between the persistence of diffusates in

pots in which the roots had either been left or removed. At twenty-four weeks, twenty-four plants which were growing in the glasshouse were similarly cut. The soil of each of twelve pots was thoroughly sieved to remove root material and then returned to each pot. Root material was left undisturbed in the soil of the remaining twelve pots to test whether roots continued to produce an active diffusate. After 1,2,4 and 6 weeks the pots were leached and hatching tests carried out using the Wrangle test population.

RESULTS

i) & ii) Hatching in leachate collected from field and glasshouse grown plants of different ages

The activity of root leachates collected on each occasion was assayed by the method described by Fenwick (1952). The hatching curve based on the results in full-strength and diluted leachates was approximately sigmoid when plotted on a logarithmic dilution scale, with little difference in hatch between full-strength and 50% leachate (Fig. 26). The assay of diffusate activity is based on linear regression and therefore only the results from the diluted leachates were used as these formed the more linear part of the curve. An example of the derivation of the log-activity value, based on Fenwick (1952) for the results of the hatching test in which leachate was collected from fourteen week old plants growing outside is given in Fig. 27. As the dilution of root leachate increased fewer H.cruciferae juveniles hatched and the hatch was eventually no greater than that in distilled water. In distilled water the mean percentage hatch (based on values measured in each hatching test) was 2.72 ± 1.14% and in leachates taken from unplanted pots at four weeks 3.91 ± 1.36% of the juveniles hatched. From the point where hatching in diluted leachate equalled the hatch in distilled water in each test a reverse scale of arbitrary units of concentration was drawn, the logarithm to the base 10 of which was designated the 'log-activity value'.

Values were derived for the activity of leachate collected on each occasion and are shown in Fig 28. The shoot and root weight and growth stages of plants on each occasion are also shown. The log-activity of diffusate produced by plants grown in the field plot increased after planting, reaching a maximum value at fourteen weeks. The activity of

Figure 26 **Hatching of H. cruciferae in six concentrations of leachate collected from 14 week old plants. (Vertical bars represent the 95% Con. limits)**

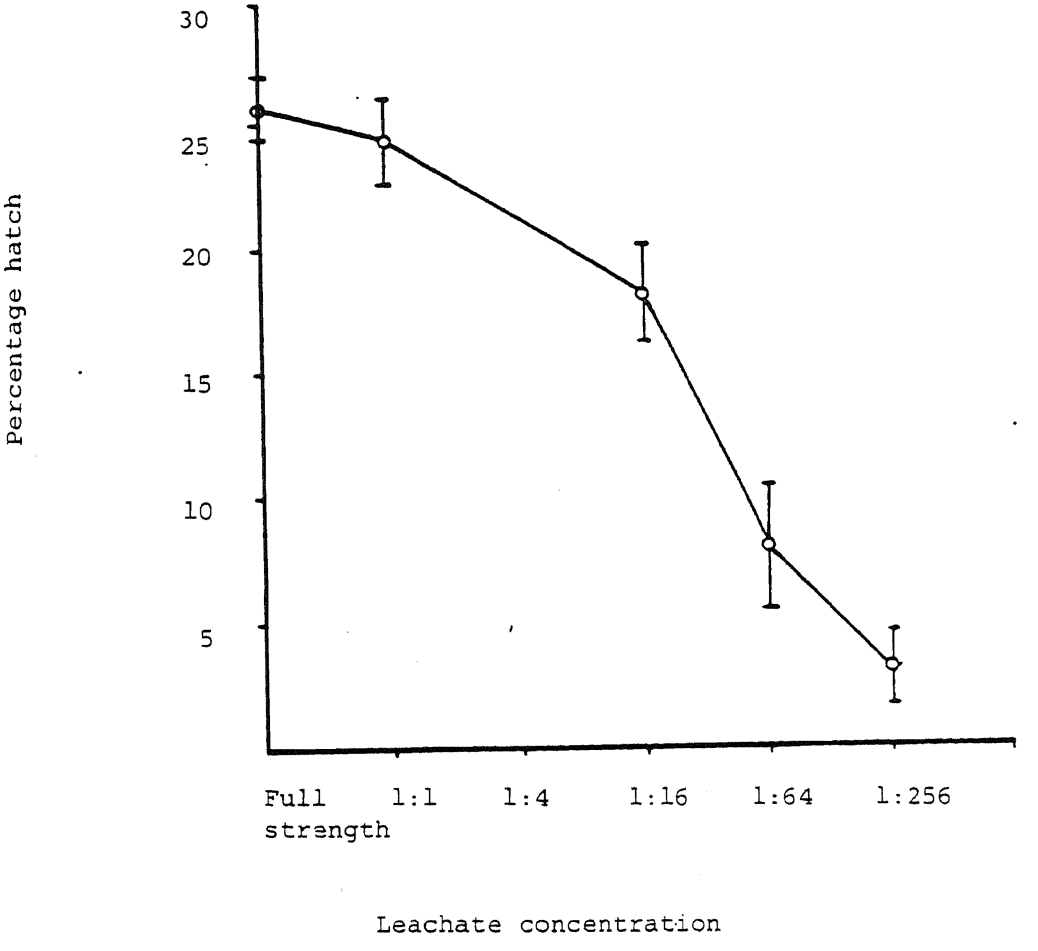


Figure 27 **Derivation of the hatching activity of leachate collected from 14 week old plants**

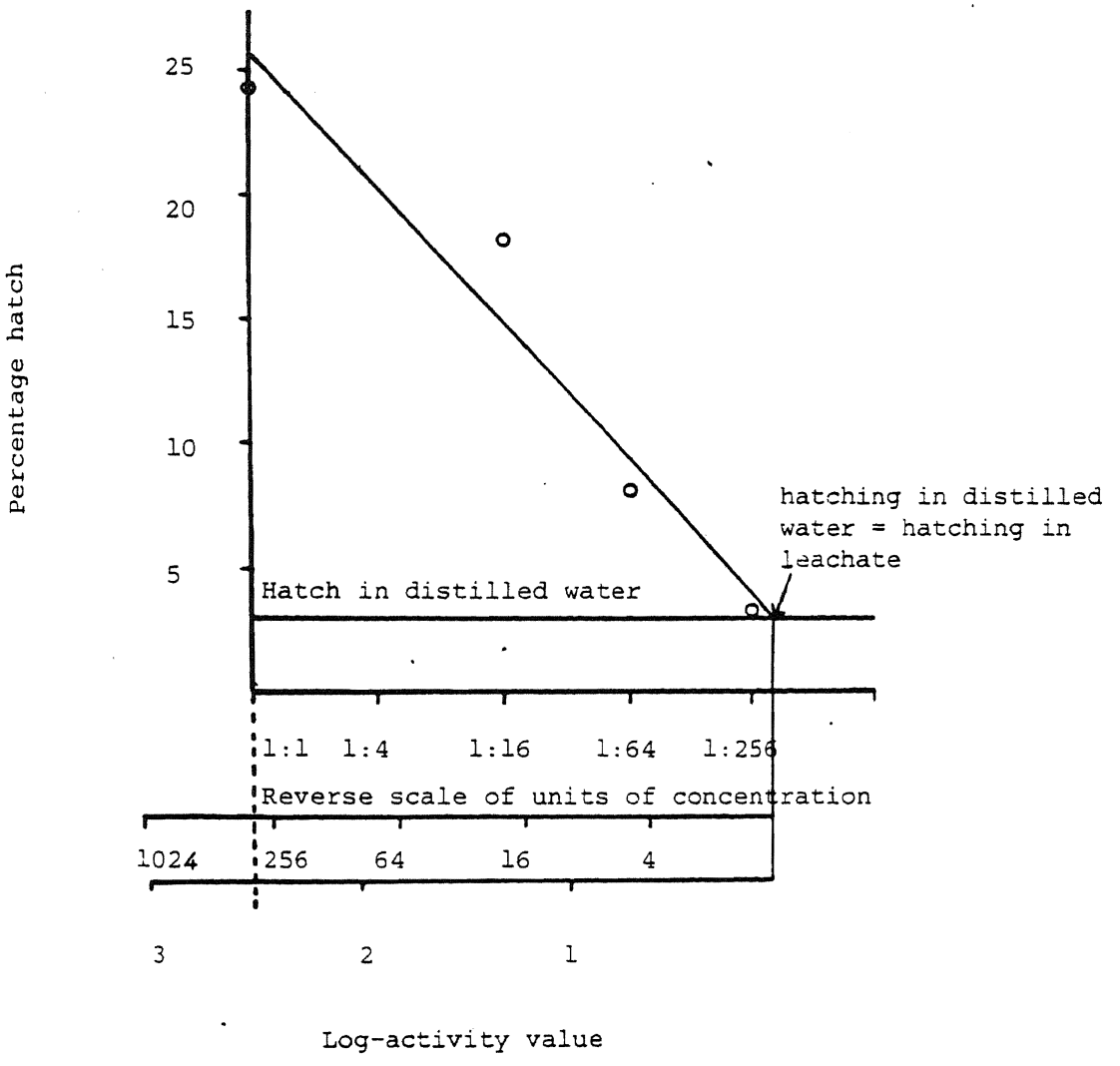
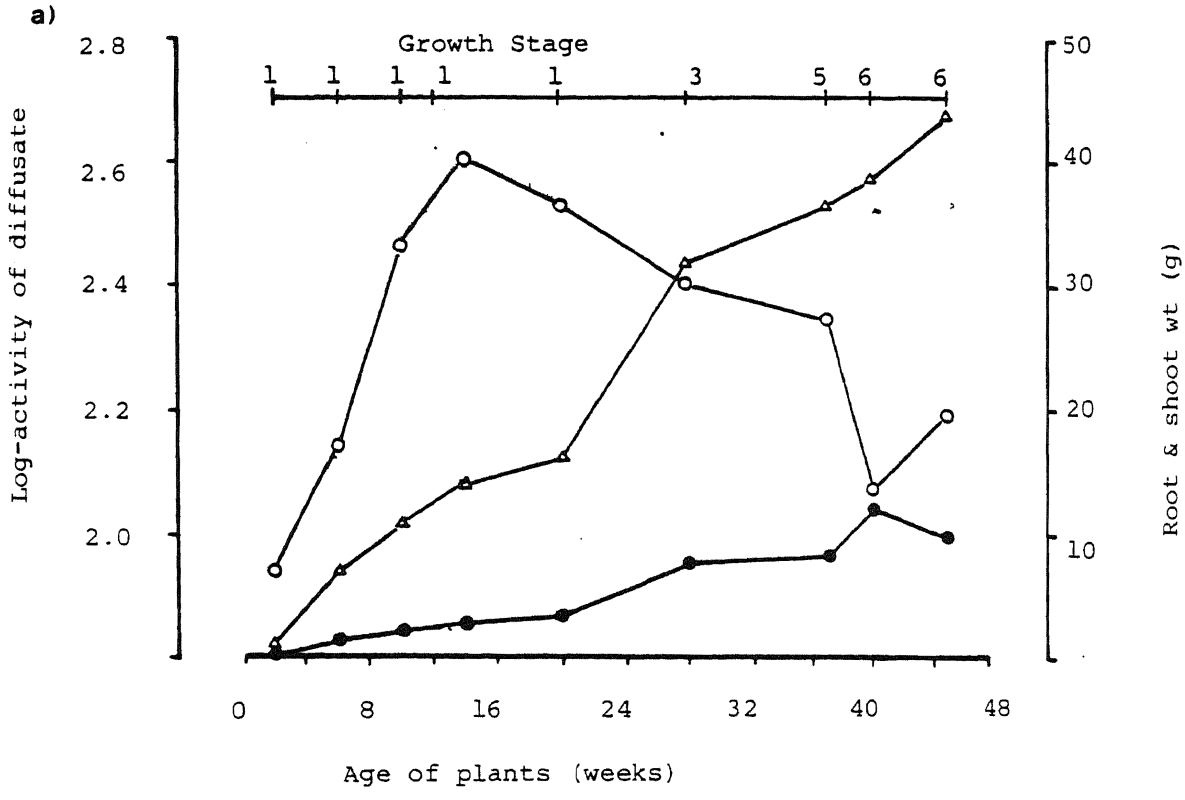
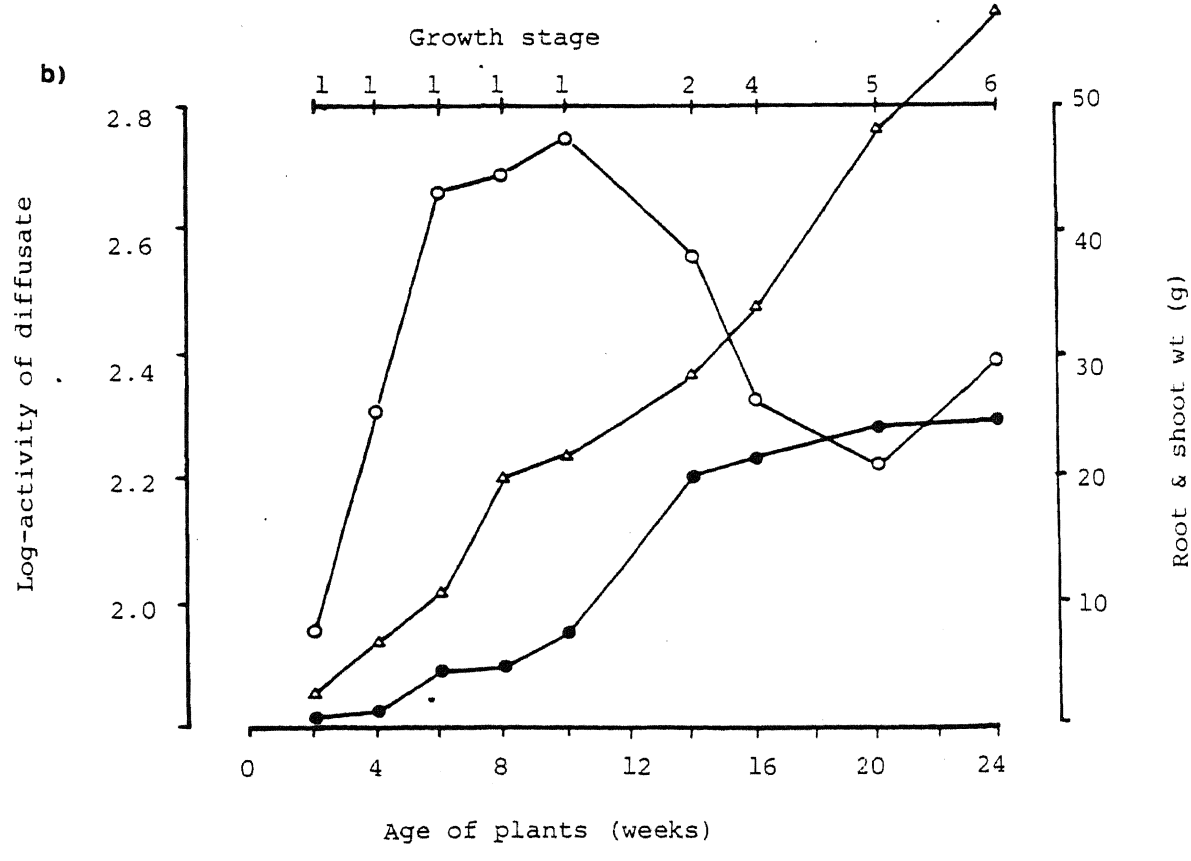


Figure 28 Shoot weight (△) root weight (●) and log-activity (○) of diffusate collected from a) field and b) glasshouse grown plants of different ages





diffusate then decreased with a marked decline in activity after thirty-five weeks. Root weight increased throughout the growing season with a small decrease in the root weight of the oldest plants. Shoot weight increased rapidly in the spring as plants matured and flowered. The log-activity of diffusate produced by plants grown in the glasshouse behaved in a similar manner but over a shorter period. The plants grown in the glasshouse produced a more active diffusate with maximum value occurring at ten weeks. The activity of diffusate decreased after this time and declined more rapidly than that of diffusate produced by plants grown in the field plot. Plants grew approximately twice as fast as those outside reaching a growth stage at twenty-four weeks similar to that of the field grown plants outside at forty-five weeks. Shoots and roots grew better in the glasshouse than in the field plot although root growth was reduced in the later stages of the experiment. Plants grown in the glasshouse produced more fibrous root material than those grown in the field plot (Fig 29). However, a similar amount of woody and tap root material was produced by all plants. Table 18 shows that the percentage of juveniles that hatched in undiluted leachate showed a similar pattern to the log-activity values shown in Fig. 28. A maximum percentage hatch of 25.6% occurred in leachate taken from pots containing field grown plants at fourteen weeks with a maximum hatch of 30.6% in leachate taken from glasshouse grown crops at ten weeks. Regression coefficients are given for the regression of percentage hatch on diffusate dilution at each occasion. Root leachates which had a more active diffusate had greater corresponding regression coefficients suggesting that the form of the hatching curve was not the same for diffusates of different activities.

iii) Hatching from cysts added to the soil of field grown plants

In the cysts added to pots containing ten week old rape plants 30.5% of the juveniles remained unhatched after four weeks (Fig. 30). The percentage hatch was greater than that which occurred in the most active diffusate which had been leached from the fourteen week old plants in experiments i) & ii). A comparison of the hatching from old and newly produced cysts added to the soil of thirty-five week old plants showed that 46.0% of old and 75.1% of new juveniles remained unhatched after four weeks. A smaller proportion of juveniles (15.5%

Figure 29 Weight of fibrous root material provided by field and glasshouse grown plants

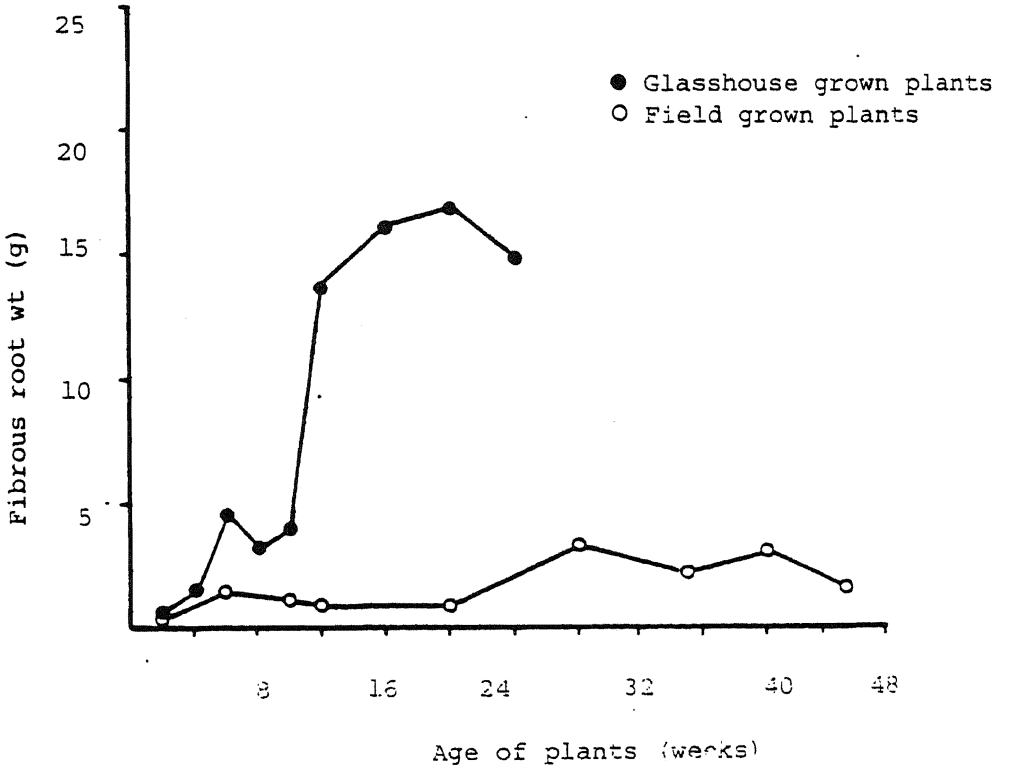


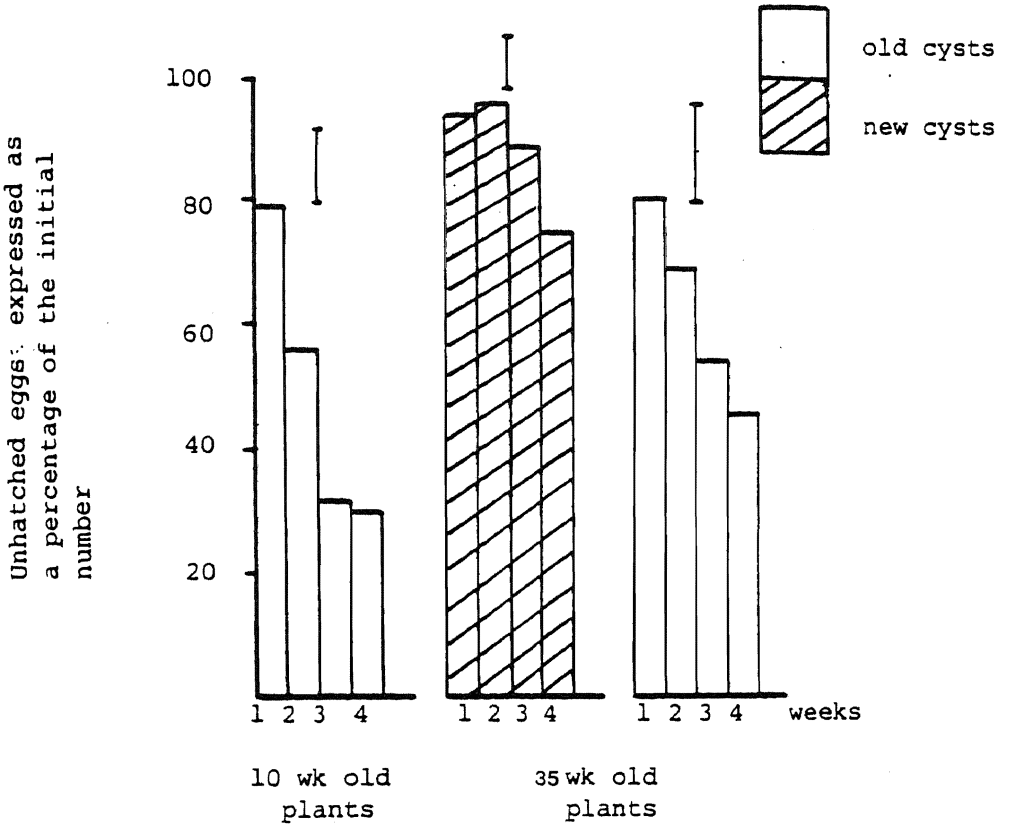
Table 18

The hatching of H.cruciferae in response to root diffusate produced by plants of different ages

Field Grown Plants			Glasshouse Grown Plants		
Time after sowing (wk)	Percentage hatch in full-strength diffusate	Regression coefficient for hatch vs dilution	Time after sowing (wk)	Percentage hatch in full-strength diffusate	Regression coefficient for hatch vs dilution
2	13.1 ± 2.0	1.47 ± 0.80	2	15.1 ± 3.1	2.06 ± 1.13
6	14.6 ± 3.1	1.93 ± 1.00	4	17.6 ± 4.5	2.85 ± 1.45
10	23.1 ± 4.5	5.00 ± 2.57	6	28.1 ± 3.8	5.03 ± 2.78
14	25.6 ± 4.1	5.45 ± 2.76	8	29.5 ± 6.7	4.81 ± 2.38
20	23.5 ± 2.6	4.84 ± 2.42	10	30.6 ± 3.3	5.49 ± 2.76
28	22.1 ± 3.7	3.06 ± 1.51	14	27.2 ± 4.6	4.88 ± 2.45
35	20.1 ± 3.2	3.69 ± 1.88	16	24.3 ± 4.0	3.70 ± 1.84
40	15.3 ± 1.6	1.87 ± 0.96	20	16.5 ± 3.6	2.82 ± 1.41
45	12.4 ± 3.5	1.99 ± 1.01	24	20.3 ± 3.91	3.74 ± 2.03

Means ± S.E.

Figure 30 Hatching over four weeks from old and new H. cruciferae cysts added to the soil of 10 and 35 week old plants. Vertical bars represent L.S.D. (P = 0.05)



less) hatched in diffusate produced by thirty-five week old plants than hatched in diffusate produced by ten week old plants.

iv) The retention of hatching activity by the soil after the removal of plants

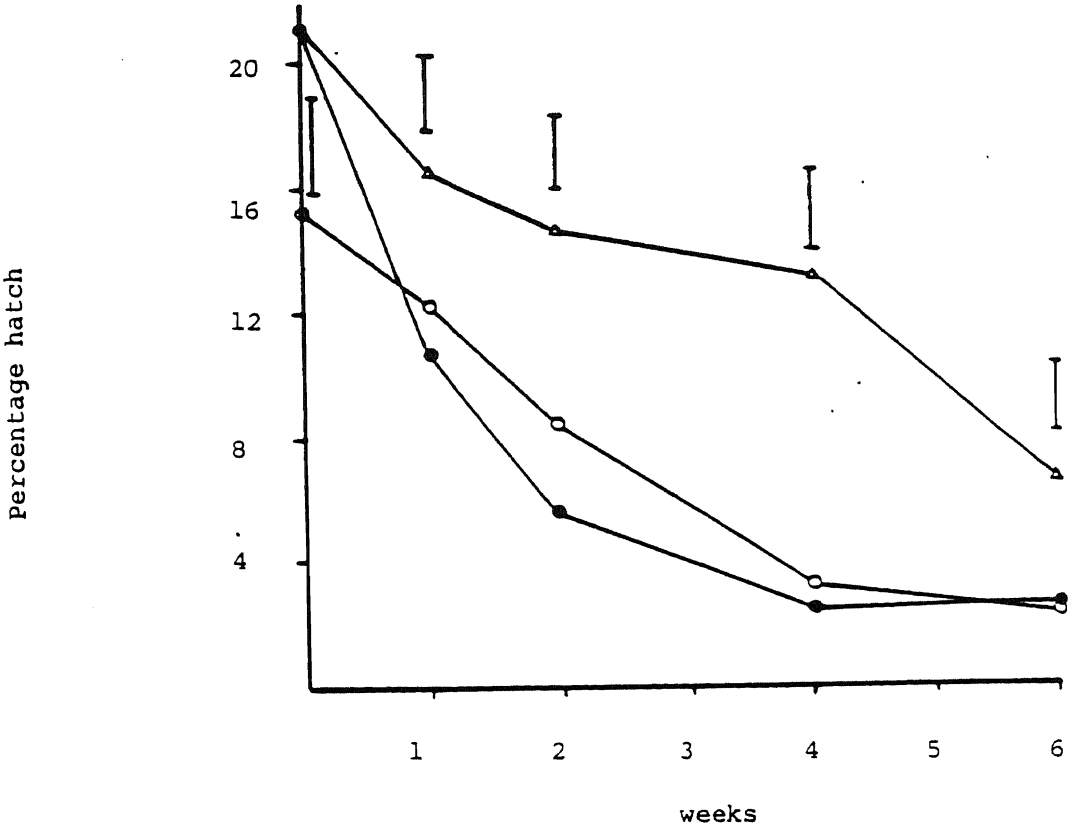
The activity of root diffusate persisting in the soil was not assayed by the hatch versus dilution analysis as linear regressions could not be calculated accurately for diffusates whose hatching activity was similar to distilled water. The percentage hatch in full-strength leachates are given in Fig. 31.

Root leachate produced by plants grown in the field plot from which the shoots were removed at forty-five weeks declined in activity over the following weeks resulting in a similar hatch to that in distilled water six weeks after the shoots were removed. Leachates produced by glasshouse grown plants whose shoots were removed at 24 weeks retained their activity for longer and stimulated a greater hatch than distilled water after six weeks than field grown plants. Hatching in leachate taken from pots from which both the roots and shoots were removed at twenty-four weeks declined more than when the roots were left in the soil and a similar hatch to that in distilled water was observed at four and six weeks.

DISCUSSION

In common with other cyst nematodes the hatching of H.cruciferae in root diffusate was related to the age of rape plants producing the root diffusates. Root diffusates from plants grown in either the field plot or the glasshouse caused greater hatching during the vegetative growth of plants when the root systems were growing rapidly, but hatching activity of diffusates subsequently declined with flowering and seed production. This pattern was similar to the relationship between the hatching of H.glycines and the age of soybean plants (Tefft & Bone, 1985). However, comparisons between different hosts and species are often confounded by an absence of data on root growth, conditions of plant growth and methodology. Such differences will also be reflected in the determination of the log-activity of a diffusate (Evans, 1983). The assay of root diffusate activity showed a mean log-

Figure 31 Retention of hatching activity in soil containing roots of field-grown plants (○), roots of glasshouse grown plants (△) and soil from which glasshouse grown plants had been removed (●). Vertical bars represent L.S.D. (P = 0.05)



activity value of 2.29 for diffusate produced by plants grown in the field plot throughout the experiment and was similar to a value of 2.09 found for H.cruciferae in rape-kale diffusate (Doncaster, 1956). The log-activities of diffusates increased during vegetative growth of the plants at a time which corresponded to large increases in the weight of roots. Widdowson (1958) found that the roots of potato plants produce an active diffusate with a log-activity value greater than 3.0 by the time shoots emerged and found a much greater range of diffusate activity than in this experiment. When a winter crop of oilseed rape is grown plants will be producing most active diffusate in the autumn and over winter. Soil temperature during early autumn is above the threshold for hatching for long periods and much hatching will occur, leading to many juveniles invading the roots at this time. After oilseed rape plants have flowered in late spring the activity of diffusate declines probably as a result of physiological changes in the roots associated with flowering and seed production. Plants grown in the glasshouse produced a more active diffusate probably as a result of greater fibrous root growth than in the field plot. Glasshouse conditions were more favourable for plant growth and the weekly addition of a fertiliser probably encouraged the greater root growth. The addition of extra nitrogen to field crops has also been shown to increase the amount of fibrous root growth (Barraclough et al., 1986). However, the large difference between the root growth of glasshouse and field grown plants may have been expected to result in a greater difference between the log-activities of their diffusates than was actually recorded. There is little information concerning the factors affecting root exudation although the rate of exudation is thought to be related to the nutritional status of the plant, with phosphorus-rich plants having a lower rate of root exudation (Ratnayake, 1978). The less active diffusates of field grown plants may have been a result of the leaching of diffusates from pots by rainwater.

Comparison of the regression coefficients of the hatch versus dilution results demonstrated that there was only a small degree of parallelism between the regression lines of diffusates from different aged plants. The linear relationship between diffusate activity and dilution is only approximate as shown for other cyst nematodes (Calam et al. 1949; Winslow, 1955; Shepherd, 1962). In this experiment, this is shown by a smaller increase in the number of H.cruciferae that hatched in the more concentrated diffusate. As the activity of diffusates of

different aged plants increased this decline occurred at progressively more dilute leachate concentrations. Increasing the dilution at which the hatching curve becomes steep, but without increasing the dilution at which hatch equals that of distilled water gives regression coefficients of greater values for the more active diffusates. Additionally, plants of different ages may produce different root 'secretions' capable of acting as hatching agents but with differing efficacies. Such diffusates may have different regression coefficients. The smaller numbers of juveniles that hatched in the more concentrated diffusate may be due to the presence of inhibitors which have less effect in diluted leachate (Hague, 1958).

The maximum percentage hatch (30.6%) of H.cruciferae in the hatching test using leached diffusates was rather small in comparison to other cyst nematodes but was similar to that found for H.cruciferae from the same population used in experiments by Koshy & Evans (1986). A larger hatch (69.5%) was found to occur when cysts were added to the soil of plants. This implies that leachates collected in this way underestimate the activity of diffusate in the soil. Forrest & Phillips (1984) found that up to 90% of G.pallida juveniles hatched when cysts were added to the soil of potato plants whereas smaller hatches (c.40%) of G.pallida have been recorded in leachates washed from pots (Turner & Stone, 1981; Evans, 1983). The experiments described here suggest that H.cruciferae hatches less readily than other cyst nematodes and therefore requires a much higher concentration of root diffusates before large numbers of juveniles hatch.

In a field infestation of H.cruciferae new cysts will be produced by thirty-five weeks with the eggs containing fully formed second stage juveniles (Chapter 2). Plants are still producing an active diffusate at this time and during the growing season of winter oilseed rape this will correspond to the period when soil temperatures are increasing in the spring. This may allow much hatching to occur and result in an increase in root invasion at this time; as reported for H.cruciferae by Koshy & Evans (1986). However, fewer juveniles hatched from new cysts than from older cysts in these experiments. This confirmed the results of a previous experiment (Chapters 3 & 4) and suggests that there is a diapause of the kind described by Evans & Perry (1976) in eggs. As many F1 juveniles are able to hatch from egg sacs the small hatch from cysts would ensure the longer term persistence of eggs in

the cysts. Work by Ishibashi et al. (1976) showed the existence of a diapause in H.glycines cysts where many juveniles hatched from egg sacs whereas those eggs in brown cysts required a period of low temperature before hatching occurred.

The roots of plants grown in the glasshouse continued to produce root diffusate for a short period after the shoots were removed. This was similar to results obtained by Perry et al. (1981) for potato roots. However, root diffusate did not persist for long in soil from which the roots had been removed and was similar that of potato root diffusate in the soil (Fenwick, 1956). Leachate from soil containing the roots of field grown plants lost its activity more rapidly than the soil containing the roots of glasshouse grown plants. This may be a result of many of the roots of glasshouse grown plants being physiologically more active than the roots of field grown plants, a result of being grown under different conditions. Therefore, after the harvest of the oilseed rape crop diffusate would persist for only a short period in the soil and limit the period over which any subsequent hatching could occur.

9

The multiplication of *Heterodera cruciferae* and *H.schachtii* as co-inhabitants of oilseed rape

INTRODUCTION

In soil which is infested with both H.cruciferae and H.schachtii mixed populations may persist if land is cropped frequently with host crops. Heterodera cruciferae and H.schachtii share many common host crops such as oilseed rape although sugar beet (the main host crop of H.schachtii) is not a host for H.cruciferae. Thomas (1956) found mixed populations of H.cruciferae and H.schachtii in fields cropped with sugarbeet, winter broccoli and mangolds. Mixed populations have also been found in fields in which just one common host crop such as brussels sprouts is grown (Lear et al., 1966; Lear, 1971). In fields infested with both H.cruciferae and H.schachtii, and in which sugarbeet and oilseed rape are grown in the same rotation mixed populations may be expected to persist, probably with H.schachtii present in greater numbers.

When two similar species are competing for the same host, competition is likely to be greatest in the search for root space (Jones & Kempton, 1978). Competition between H.schachtii and Meloidogyne hapla for invasion and feeding sites has been shown to reduce the multiplication of M.hapla (Griffin & Waite, 1982). Pot tests have shown that Globodera pallida is more successful than G.rostochiensis in mixed populations but the cause of this is unknown (Parrot & Berry, 1976). Seinhorst (1970) suggested competition in which competitive exclusion occurs is to be expected between closely related species as it has been previously reported for species of Pratylenchus (Ferris et al., 1967). In an interaction one species may change the quality of the host and in some instances these changes may be beneficial to one species. For example, Jatala & Jensen (1976) found that H.schachtii produced more cysts on sugar beet when M.hapla invaded the roots 10 days prior to invasion by H.schachtii than when only H.schachtii was present in the roots. M.hapla was thought to modify the physiology of the host plant to the advantage of H.schachtii. Conversely, interactions between M.arenaria and M.incognita on soybean resulted in a suppression of M.arenaria when M.incognita was inoculated prior to M.arenaria (Ibrahim & Lewis, 1986). They suggested that chemical reactions already elicited by M.incognita were more detrimental to M.arenaria. Little is known of the interaction between H.cruciferae and H.schachtii in concomitant infestations. A previous experiment (Chapter 2) indicated that H.schachtii invades the roots earlier and

in larger numbers than H.cruciferae when invasion is restricted to a short period. Heterodera schachtii has also been shown to cause more root damage than H.cruciferae (Chapter 7) which therefore may determine the amount of root space available for the development of both species. However, a study of the interaction between these two species is complicated by their cysts being morphologically similar and therefore the relative proportion of each in a mixed population is difficult to assess. Winslow (1955) suggested that the hatching response of each species to different root leachates might be used to estimate the relative proportions of each species. However, Thomas (1956) found that the hatching from cysts of a mixed population of H.cruciferae and H.schachtii in sugarbeet and cabbage leachate did not accurately assess the relative proportions of each species. Fleming & Marks (1982) found that isoelectric focussing combined with densitometry of species specific protein bands accurately assessed the relative proportions of Globodera rostochiensis and G.pallida in mixed-species samples. This method has not yet been tested for mixed-species samples of H.cruciferae and H.schachtii.

This experiment examines the multiplication of H.cruciferae and H.schachtii in mixed populations at a range of initial nematode densities on oilseed rape. A comparison between the multiplication of mixed and single species populations is made.

MATERIALS AND METHODS

The multiplication of single and mixed populations of H.cruciferae and H.schachtii were examined at five initial population densities: 1250, 2500, 5000, 10,000 and 20,000 eggs per pot. Mixed populations contained equal numbers of H.cruciferae and H.schachtii eggs. At 1250 eggs per pot only single species populations were examined. This enabled a comparison between the multiplication of each population with that of each population when mixed (1250 + 1250 eggs per pot).

Sixty pots (10cm diam.) were each filled with 500g of a loam, sand and gravel mixture (3:1:1) and was sown with three seeds of oilseed rape c v. Jet Neuf. The pots were placed in a Fisons Fi-totron 600 growth cabinet at 20°C and with a 12 hour photoperiod. Shortly after emergence plants were thinned to one healthy seedling per pot. Cysts were extracted by fluidising column from infested field soil in which

host crops of the two species had been recently grown. Batches of cysts were prepared to give as accurately as possible to give the desired number of nematode eggs. Four replicate batches were prepared for each treatment (a total of fifty-six batches of cysts) and each batch was then separated into two approximately equal fractions and each put in a nylon mesh bag. The two bags were then added to the soil in each pot at a depth of 4-5cm around the root system of the plant. Plants were inoculated with cysts 7 days after sowing to reduce the effects of root damage on nematode multiplication. Throughout the experiment the plants were watered as required and fed weekly with a liquid N,P,K fertiliser. Four plants were left as uninfected controls.

After fifty-six days, the pots were removed from the growth cabinet and each plant carefully removed from its pot. The roots of each plant were washed, blotted dry and the shoot and root fresh weights measured. The numbers of nematodes in the root systems were counted by staining and releasing nematodes from the roots by the standard method. The stage of development of each nematode was recorded. The nylon mesh bags were removed from the soil of each pot, washed gently in water and the bags from each treatment placed in a Petri-dish containing 5ml of water. The bags were then opened and the number of unhatched eggs in cysts counted. The soil from each pot was thoroughly mixed and 2 x 200g samples removed. From one sample cysts were extracted by fluidising column whilst from the other sample free juveniles were extracted by the tray method (Whitehead & Hemming, 1965).

Cysts from each pot containing a mixed population were placed in 2ml of water in a compartment of a Repli-dish and the cysts of each species identified. It was not possible to individually identify the large number of cysts produced by the usual technique of removing the vulval cone and examining the associated structures, so identification was based on the following differences in cyst morphology. Heterodera schachtii cysts were much larger and had more prominent vulval cones than H.cruciferae cysts. The presence of bullae in the vulval cone of H.schachtii could usually be identified when examined under a very bright light. Bullae were not visible in the cysts of H.cruciferae. As a check on this identification method a small number of cysts identified as either H.cruciferae or H.schachtii were taken and carefully opened in 1ml of water. The eggs were removed from the cysts

and transferred back to the compartment of the Repli-dish from which they were taken. The vulval cones of the empty cysts of each species were prepared for microscopical examination. The vulval cone was separated from each cyst and any debris remaining within it removed. The cyst-cones were then processed through 10,50,75 and 95% ethanol and 100% butanol solutions leaving them for a few minutes in each. Each of the cones was then placed in a drop of Euparal on a microscope slide and carefully orientated so that the vulval cones could be examined. After 2 hours a further drop of Euparal was added and then a cover-slip, supported by pieces of broken cover-slips, put into position. Cysts of each species were identified by the characteristics described by Hesling (1978) and the number of cysts identified correctly by the previous method were counted. The numbers of eggs contained in the remaining cysts of each species were counted by the standard method. Free juveniles extracted by the tray method from the soil of mixed populations were not separated according to species.

In another experiment the hatching and invasion rates of single and mixed populations were examined. One oilseed rape plant cv. Jet Neuf was grown in each of forty-five pots (9cm) in a growth cabinet as described previously. Fifteen batches of 50 and 25 cysts of each species were prepared. Batches of cysts were placed in nylon mesh bags and bags containing 25 cysts of each species were tied together to give mixed batched of 50 cysts each. Bags containing each species in the mixed treatments were marked with a piece of coloured plastic. Twenty-eight days after sowing one batch was added to the soil of each pot at a depth of 4-5cm around the root system of the plants. After 3,6,9,12 and 15 days three pots inoculated with each treatment were removed from the growth cabinet. The roots of each plant were separated from the shoots, washed and the extent of nematode invasion assessed by staining and releasing the nematodes from the roots by the standard method. On each occasion the bags were also removed from the soil and after washing were opened in 5ml of water. The numbers of unhatched eggs in cysts were counted by the usual method.

RESULTS

From each initial egg inoculum a mean of 77.1% of the juveniles hatched but there were no significant differences between each density (Table 19). More juveniles of H.schachtii than H.cruciferae hatched by

Table 19

Assessment of hatching and the growth of plants infested with either single or mixed species populations

Inoculum*	Percentage hatch after 56 days	Shoot weight at 56 days	Root weight at 56 days
HC 1250	66.0 ± 8.5	10.8 ± 2.1	0.45 ± 0.06
HS 1250	75.6 ± 5.4	11.6 ± 4.5	0.31 ± 0.05
HC 2500	69.4 ± 6.7	10.2 ± 0.8	0.70 ± 0.08
HS 2500	76.6 ± 9.6	11.1 ± 0.3	0.29 ± 0.02
HC & HS 2500	76.0 ± 7.5	12.5 ± 1.0	0.58 ± 0.13
HC 5000	78.9 ± 4.7	10.5 ± 1.1	0.46 ± 0.12
HS 5000	84.6 ± 8.6	13.0 ± 0.9	0.34 ± 0.05
HC & HS 5000	79.1 ± 8.5	12.5 ± 2.8	0.57 ± 0.11
HC 10,000	70.2 ± 6.7	10.8 ± 0.5	0.40 ± 0.15
HS 10,000	77.1 ± 16.9	11.7 ± 0.9	0.47 ± 0.09
HC & HS 10,000	85.0 ± 9.6	9.9 ± 0.1	0.42 ± 0.05
HC 20,000	75.5 ± 6.7	14.0 ± 3.1	0.69 ± 0.10
HS 20,000	82.0 ± 7.3	12.5 ± 2.1	0.65 ± 0.09
HC & HS 20,000	83.8 ± 6.3	11.4 ± 0.9	0.65 ± 0.13
Uninfested plants	-	12.4 ± 1.5	0.89 ± 0.07

Means ± S.Error

* HC; H.cruciferae HS; H.schachtii (number of eggs per pot)

fifty-six days but these differences were not significant. The shoot weights of uninfected plants and infected plants at each nematode density were similar but the root weights of all infected plants were significantly smaller ($P < 0.05$) than uninfected plants. At the three smallest initial densities the root weights of plants infected with H.schachtii were smaller than those with H.cruciferae. The root weights of plants infected with both species simultaneously at 2500 and 5000 eggs per pot were larger than those infected with H.schachtii alone at these densities. At 20,000 eggs per pot roots grew better than at other densities but the differences were not significant.

Table 20 shows that in single species populations at the three smallest densities there were more juveniles and adults of H.schachtii than H.cruciferae in the roots but at the two largest densities more H.cruciferae were found. In single species populations at all densities except the largest there were more second stage juveniles of H.cruciferae than H.schachtii in the roots. However, at these densities H.schachtii had more third and fourth stages and at the two smallest densities more adult females in the roots. At the two largest densities of the mixed species population there were more second stage juveniles and fewer adult females in the roots than in the single species populations. Larger numbers of adult males of each species were found in the roots at densities greater than 2500 eggs per pot.

A similar number of cysts were produced by H.cruciferae and H.schachtii at the smallest density and by H.cruciferae and the mixed population at 2500 eggs per pot (Fig. 32). Heterodera schachtii produced more cysts than either H.cruciferae or the mixed population at this density and this was also reflected in the number of cysts produced per 0.1g of root weight (Table 21). More cysts were produced as the initial egg density increased. At 5000 eggs per pot fewer H.schachtii cysts were produced than by either H.cruciferae or the mixed populations although a similar number of cysts were produced per 0.1g root by each population. At 10,000 eggs per pot a similar number of cysts were produced by either single or mixed populations. A larger number of cysts were produced overall at the largest density but similar numbers were produced by H.cruciferae and the mixed population per 0.1g root to those at 10,000 eggs per pot. At the largest density fewer cysts of H.schachtii were produced than were produced by H.cruciferae or the mixed population and the number of H.schachtii

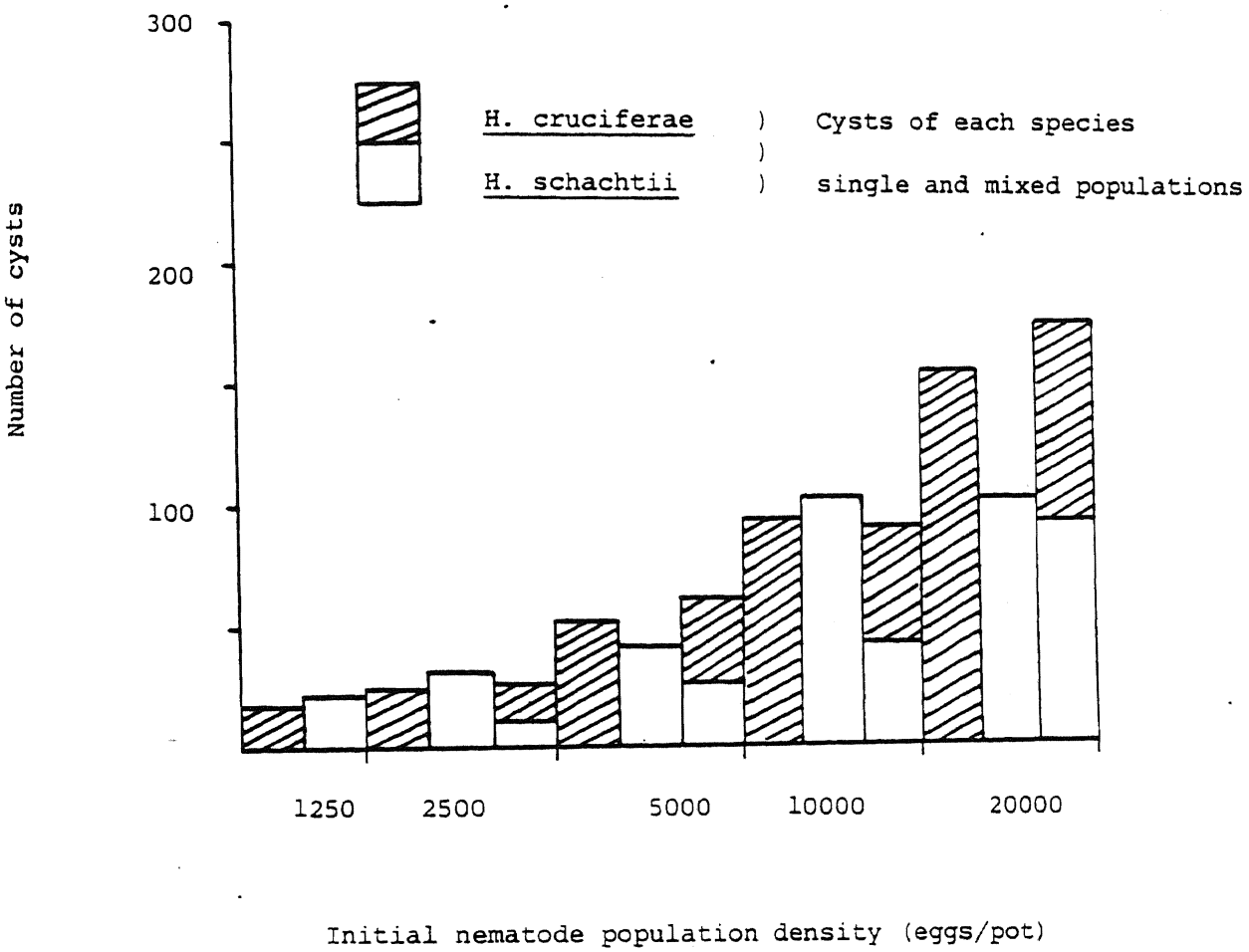
Table 20

The percentage of the total number of nematodes at each stage of development in the roots in either single or mixed species populations

Inoculum*	Development Stage JJ II	JJ III	JJ IV	Adult Male	Adult Female	Total number of nematodes
HC 1250	77.7	10.6	5.2	-	6.5	33.7
HS 1250	22.2	16.3	32.0	-	29.5	42.6
HC 2500	60.0	15.3	11.6	-	13.1	75.0
HS 2500	32.0	15.0	29.0	-	24.0	105.0
HC & HS 2500	50.0	14.9	18.0	-	17.1	65.0
HC 5000	49.9	12.3	8.2	5.0	24.6	82.5
HS 5000	35.5	25.6	21.0	-	17.9	136.9
HC & HS 5000	26.9	26.0	13.6	10.0	23.5	130.0
HC 10,000	24.9	32.6	12.0	6.5	24.0	225.0
HS 10,000	7.7	41.5	26.4	12.0	12.4	195.0
HC & HS 10,000	44.8	26.7	16.9	4.8	6.8	108.7
HC 20,000	5.2	36.3	18.9	8.6	31.0	435.0
HS 20,000	19.3	32.2	9.9	3.2	35.4	232.0
HC & HS 20,000	39.0	28.3	18.3	6.5	7.9	322.5

* HC; H.cruciferae HS; H.schachtii (number of eggs per pot)

Figure 32 **Number of cysts produced by single and mixed populations of H. cruciferae and H. schachtii at five initial nematode population densities after 56 days**



cysts was similar to that at 10,000 eggs per pot. A larger number (174) of cysts were produced by the mixed population at the largest density than by either H.cruciferae (154) or H.schachtii (102).

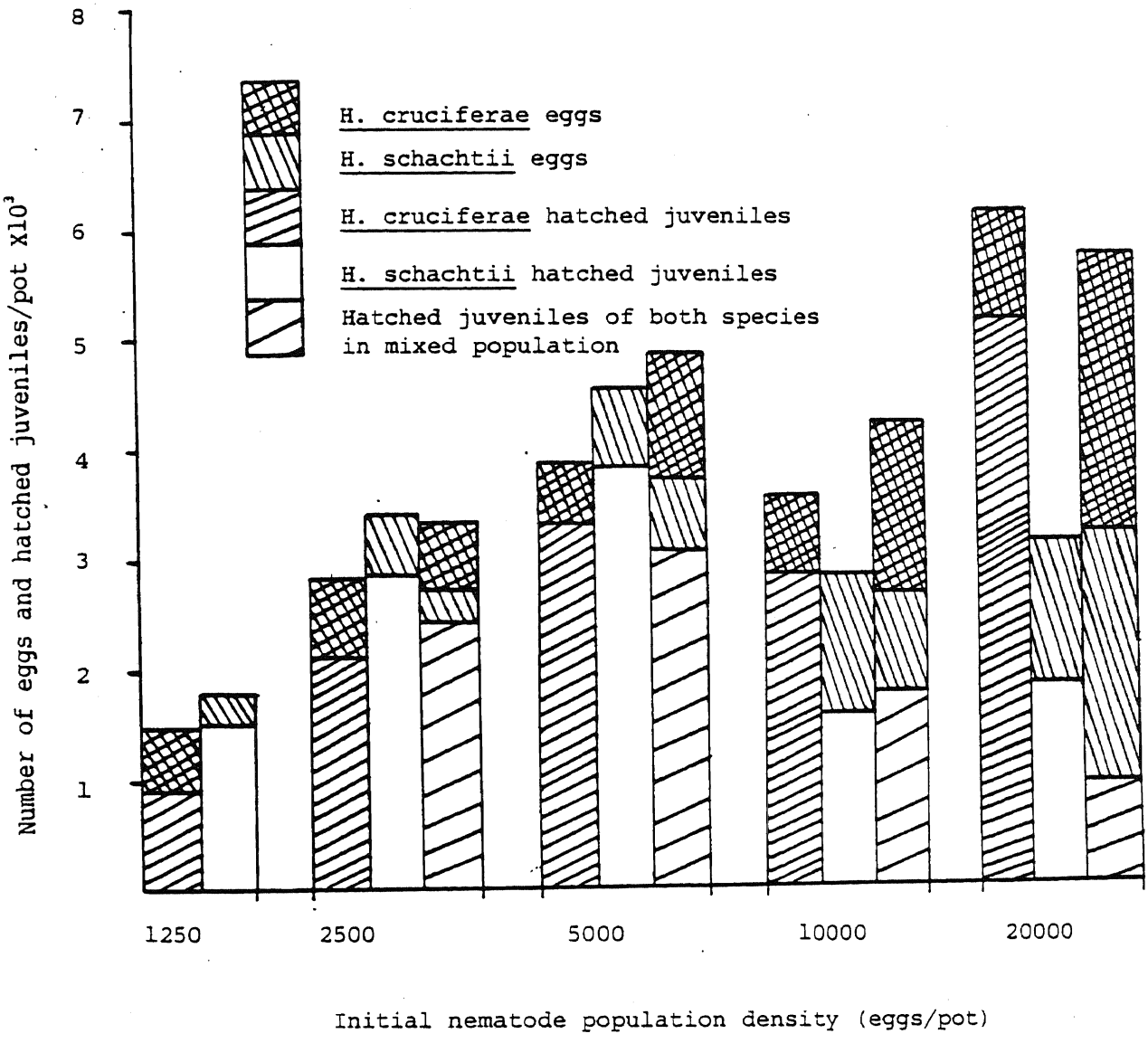
Table 21: The numbers of cysts produced per 0.1g root by single and mixed populations at each density after 56 days

	HC	HS	HC/HS
1250	3.8	7.2	-
2500	3.4	10.5	4.6
5000	11.4	12.8	10.9
10,000	23.6	21.7	21.6
20,000	22.5	15.8	26.7

In mixed populations at all but the largest density more cysts were identified as H.cruciferae whilst approximately equal numbers of cysts were produced by each species at the largest density. Examination of cyst cones showed that 70% of the smaller cysts produced by mixed populations had been correctly identified as H.cruciferae whereas 82.5% were correctly identified as H.schachtii.

The numbers of unhatched F1 eggs and free juveniles in the soil of either single or mixed populations are shown in Figure 33. More eggs and juveniles were produced as the initial density increased up to 5000 eggs per pot. Free juveniles accounted for the largest proportion of populations except in the mixed populations at the two largest densities. More juveniles (3599) remained unhatched in the mixed population than in both single species populations (H.cruciferae, 1109; H.schachtii, 1431) at 5000 eggs per pot. At 10,000 eggs per pot fewer eggs and juveniles were found per pot than at 5000 eggs per pot. A smaller number of H.schachtii eggs and juveniles were found than of either H.cruciferae or the mixed population at either this or the largest density. Significantly ($P > 0.05$) more F1 juveniles remained unhatched in the mixed populations than in the single species populations at the two largest densities and this proportion was greater than at smaller densities. At 20,000 eggs per pot 9668 of the F1 juveniles remained unhatched in the mixed population whereas 1914 and 2579 H.cruciferae and H.schachtii F1 juveniles remained unhatched respectively in single species populations. The large number of

Figure 33 **Number of eggs and hatched juveniles of H. cruciferae and H. schachtii in single and mixed populations at five initial population densities after 56 days**



unhatched F1 juveniles in cysts produced by mixed populations was reflected in a larger number of eggs per cyst (Table 22). Cysts produced by each species in the mixed populations at all densities contained larger numbers of eggs than those produced by single species populations. Of the cysts produced by the mixed population those of H.cruciferae contained more eggs than H.schachtii but this was only significant ($P < 0.05$) at the two largest densities. Fewer eggs per cyst were found in either single or mixed populations at the largest density.

The numbers of eggs produced per pot and the number of eggs and free juveniles in the soil of each single species population at a particular density were averaged to determine the number of eggs and juveniles that would be produced if either H.cruciferae or H.schachtii were developing independently in a mixed population. These values are compared with the numbers of eggs and juveniles produced by mixed populations and are given in Table 23. More eggs were produced by mixed populations than by single species populations; the difference being greater at larger initial egg densities. A comparison of the numbers of unhatched and hatched juveniles in populations showed that the difference between single and mixed populations was not as large as the differences between just the unhatched eggs of each population. Figure 34 gives the results of the hatching and invasion tests and shows that H.schachtii hatched earlier than H.cruciferae. By 9 days 76% of H.schachtii juveniles had hatched whereas only 18% of H.cruciferae juveniles had hatched by this time. Species hatched similarly when in either single or mixed species population. Figure 35 shows that H.schachtii invaded the roots earlier than H.cruciferae; by 9 days 65.2 juveniles of H.schachtii and 27.9 juveniles of H.cruciferae were found in the roots. Examination of root invasion by the mixed population at 15 days showed that fewer juveniles were found in the roots than that expected (half the combined numbers of juveniles which invaded in each single species population at this time).

DISCUSSION

Interactions between H.cruciferae and H.schachtii as co-inhabitants of oilseed rape resulted in differences between both the rates of development and the numbers of eggs produced by females when each

Table 22

The numbers of eggs per cyst produced by single and mixed populations at each density after 56 days

	1250	2500	5000	10,000	20,000
H.cruciferae	23.8 ± 9.6	62.4 ± 8.6	21.0 ± 12.3	15.8 ± 8.4	12.4 ± 4.0
H.schachtii	22.3 ± 9.9	38.7 ± 18.0	32.5 ± 16.1	31.8 ± 12.0	25.0 ± 6.3
H.cruciferae + H.schachtii		61.5 ± 12.0	58.6 ± 15.4	53.0 ± 6.9	46.6 ± 10.5
H.cruciferae*		67.2 ± 15.6	63.7 ± 11.0	63.1 ± 4.9	56.9 ± 12.5
H.schachtii*		55.8 ± 20.3	53.6 ± 6.5	42.9 ± 15.3	36.3 ± 7.4

Means ± S.Error

* Eggs per cyst of each species in mixed populations

Table 23

A comparison of the number of eggs and juveniles in mixed populations at each density and that expected in single populations multiplying independently at the same density

Inoculum level	Eggs produced			Eggs and free juveniles		
	Single	Mixed	(Mixed-single)	Single	Mixed	(Mixed-single)
2500	1314.1	1700.4	386.3	6254.9	6657.2	402.3
5000	1270.0	3598.7	2328.7	8374.2	9691.1	1316.9
10,000	1940.5	4818.3	2877.8	6353.5	840.8	2054.3
20,000	2246.7	9668.1	7421.4	9242.6	11489.7	2247.1

Figure 34 Hatching of *H. cruciferae* and *H. schachtii* in single and mixed species population. Vertical bars represent the L.S.D. (P = 0.05)

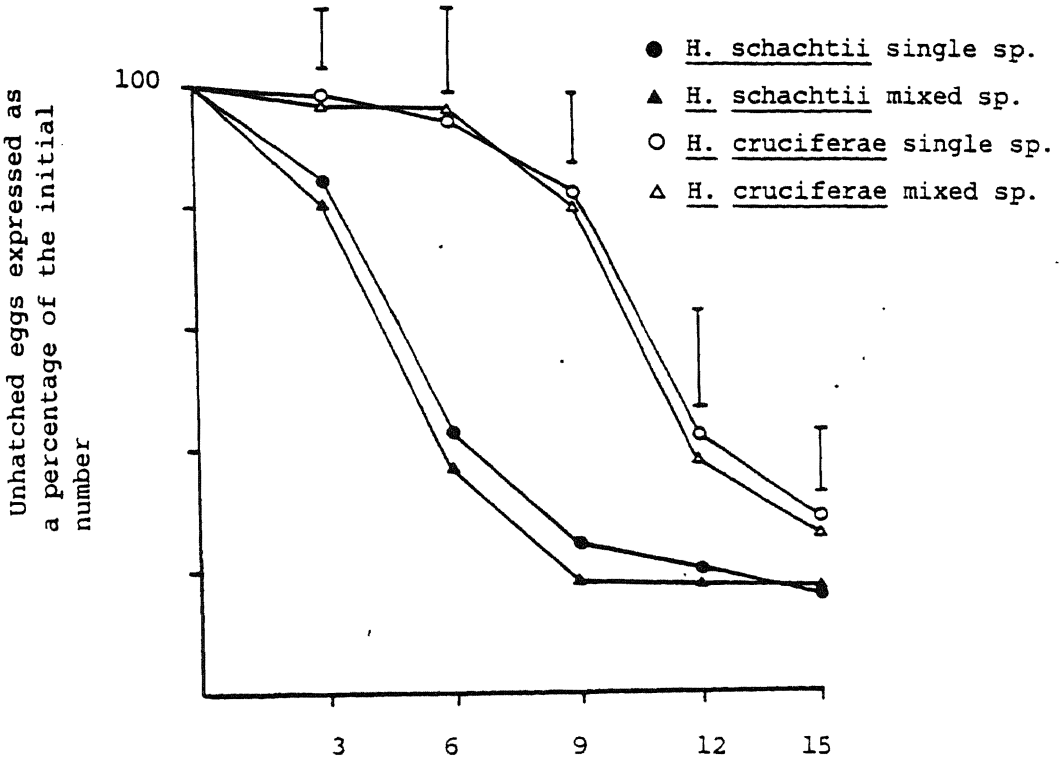
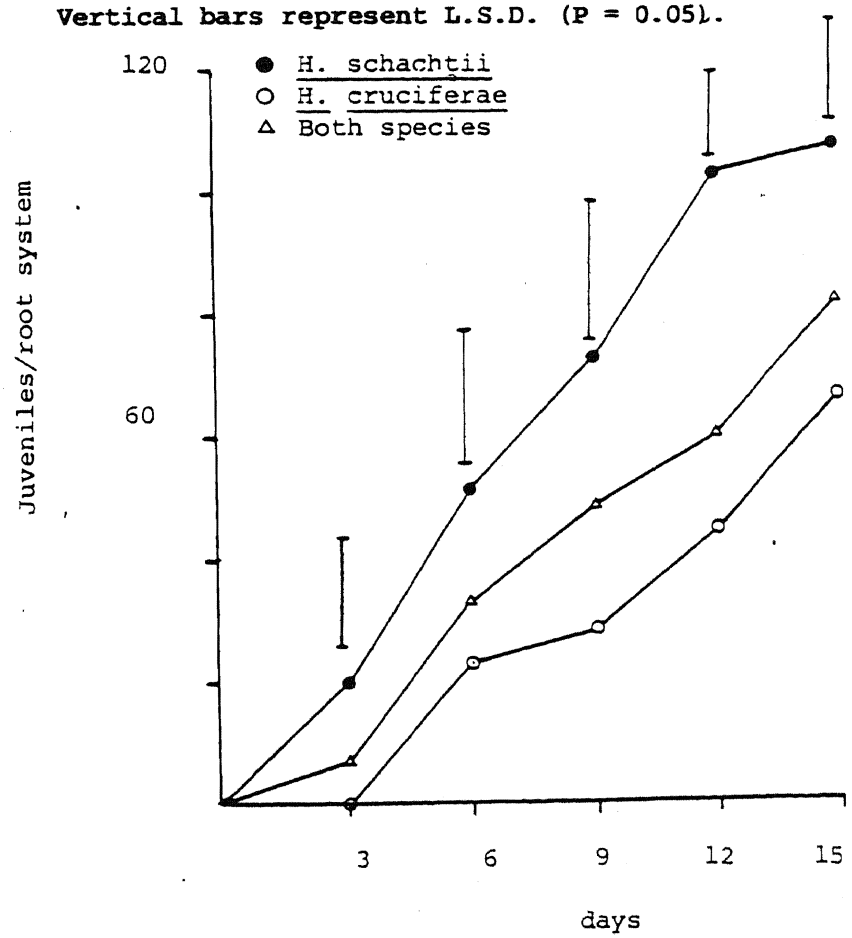


Figure 35 Root invasion from single and mixed species populations Vertical bars represent L.S.D. (P = 0.05).



species was multiplying alone and when mixed populations were developing. Heterodera schachtii hatched earlier and in larger numbers than H.cruciferae and this resulted in the earlier invasion of roots by H.schachtii juveniles. The large percentage hatch of H.schachtii after 9 days was similar to that found after 56 days suggesting that most H.schachtii juveniles hatched early on in the experiment whereas H.cruciferae juveniles hatched more gradually over the experiment. This would allow a significant proportion of H.schachtii juveniles to invade and to begin developing in the roots before a large number of H.cruciferae had either hatched or invaded the roots. Differences in the rates of invasion in the development of each species were shown by the greater proportion of later developmental stages of H.schachtii than of H.cruciferae in the roots after 56 days. The large numbers of H.cruciferae second stage juveniles in the roots at this time suggested either that some later hatching juveniles were still invading or that many juveniles had failed to initiate feeding sites and continue their development. Interaction between species in the mixed populations delayed development, as indicated by the fewer adult females in the roots and by the larger proportion of unhatched eggs than in single species population. The nature of this interaction is not apparent but as the numbers of cyst and egg that were produced were not adversely affected it suggests that development was delayed either at the early stages of development, perhaps as an interaction between juveniles searching for and initiating feeding sites or alternatively, as an interaction after eggs have been produced but before fertilisation had occurred. Green & Plumb (1970) showed that H.cruciferae females secreted chemicals that attracted H.schachtii males although H.cruciferae males were not attracted to H.schachtii females. However, it is unlikely that an interaction at this stage would cause anything but a short delay before the eggs were fertilised and embryonation began. If males of one species were able to fertilise the eggs of the other species females may produce eggs with embryos (Green & Plumb, 1970). Potter & Fox (1965) showed that H.schachtii and H.glycines hybridized but 55% of the F1 progeny were not viable. Pathotypes of G.rostochiensis, G.tabacum, G.virginae, G.mexicana and G.solanacearum have also been crossed but many of the F1 juveniles were also non-viable (Green & Miller, 1969). Fox (1967) failed to cross H.glycines and H.carotae and Yeates (1970) failed to cross H.carotae, H.cruciferae and H.geottingiana. In this experiment H.cruciferae and H.schachtii may have hybridized and the large proportion of unhatched F1 juveniles in the mixed populations may

indicate that non-viable hybrid juveniles had been produced. However, there is no evidence from this experiment to support this hypothesis.

Interaction between the two species did not influence the numbers of cysts produced except at the largest density. A large number of cysts were produced by all populations at the largest density but this was a consequence of greater root growth as the numbers of cysts produced per 0.1g root were similar to those at 10,000 eggs per pot. Multiplication by H.schachtii was reduced at the largest densities, probably due to intense intra-specific competition. Because a large number of H.schachtii juveniles invaded over a short period of time, root damage would be more severe and reduce the amount of space in roots for development. The larger numbers of H.cruciferae in the roots in single species populations indicates that the development was not as temporally compressed as that of H.schachtii. Root damage would be less severe by juveniles invading the roots more gradually from H.cruciferae and mixed populations resulting in less intense competition between juveniles for root space. The numbers of cysts produced in the mixed populations showed that approximately equal numbers of H.cruciferae and H.schachtii were produced. However, as many of the smaller H.schachtii cysts may have been mis-identified as those of H.cruciferae the number of H.schachtii cysts that were produced may have been greater. A larger number of H.schachtii than H.cruciferae were developing in the roots and may have been expected to produce more cysts as found in previous experiments (Chapter 2). Many of the eggs produced by either single or mixed populations would have been deposited in egg sacs although the majority of juveniles would have hatched from them.

More eggs and juveniles were produced by mixed populations than might have been expected than if single populations were multiplying independently of one another. This was more apparent at larger initial egg densities. More cysts were also produced by the mixed population than by each single population at the largest density suggesting that the conditions were more favourable for female development and egg production. The staggered invasion and development of the two species would have reduced the intensity of competition between individuals and would also have reduced the amount of initial root damage caused by nematodes. The invasion and development of H.cruciferae did not seem to be affected by the presence of H.schachtii juveniles already

developing in the roots, perhaps because juveniles were able to exploit new roots produced after the peak of H.schachtii invasion, or alternatively because freshly hatched H.cruciferae juveniles were more active and successful in finding root space than any H.schachtii juveniles which had hatched earlier but had not yet invaded and which would have had lower levels of food reserves. The weights of roots at 56 days do not indicate the relative root weights at the time of invasion although the larger roots of plants infected with both species than those infected with only H.schachtii at the same density suggests that the roots growing in soil containing mixed populations were damaged less. Therefore, the roots may have provided a more favourable environment for nematode multiplication in mixed populations than in single species populations and resulted in females being more fecund. This effect is more pronounced at the two largest initial egg densities when the root damage by mixed populations is less than that by single species populations. Ross (1964) suggested that it was for this reason that H.glycines produced more cysts when in combination with M.incognita on soyabean than when H.glycines only was present. A similar effect may also explain the greater number of cysts produced by H.schachtii in combination with M.hapla (Jatala & Jensen, 1976). In this experiment the addition of cysts to localised parts of the root system may have resulted in much local root damage whilst roots elsewhere in the pots remained relatively unaffected. In mixed field populations at large initial densities plants may be expected to suffer more root damage when grown from seed in infested soil than they did in this experiment where plants were grown in uninfected soil for 7 days. In this respect severe root damage by H.schachtii might limit or exclude any subsequent development of H.cruciferae. At nematode densities at which root damage is less severe, mixed populations will persist but as these populations increase in density more root damage would occur and the logical outcome of such mixed population growth will be the eventual exclusion and reduction of H.cruciferae populations. It is unlikely that in the field only common hosts for the two species would be grown and the inclusion of differential hosts such as sugar beet in the rotation would ensure that H.schachtii would persist as the dominant species.

10

The importance of weeds as hosts of *H.cruciferae*

INTRODUCTION

The persistence of soil populations of H.cruciferae and H.schachtii in the absence of a host crop will be influenced by (i) the ability of eggs to survive in the soil and (ii) the opportunities for nematodes to utilise non-crop hosts to maintain population levels. The host range of H.schachtii has been extensively studied (Jones, 1950; Winslow, 1954; Steele, 1965) and includes hosts in many genera. Jones (1945,1950) showed that cysts produced by weed hosts were a source of infection of subsequently sown sugar beet and implied that populations of H.schachtii may be maintained or even increased by weed species. For this reason Griffin (1982) advised that sugar beet growers should be aware of the reproductive potential of weeds and consider controlling important weed hosts. However, it is only species such as H.schachtii, with a large host range, which are likely to have populations maintained by weeds (Franklin, 1970; Hooper & Stone, 1981). The host range of H.cruciferae is less extensive than that of H.schachtii (Jones, 1950; Winslow, 1954). The effects of weeds associated with oilseed rape cultivation in maintaining populations of H.cruciferae in the absence of a host are unknown but will undoubtedly depend on the types of weeds and their numbers (Evans, 1984). Few host range studies distinguish between good and poor hosts although Winslow (1954) suggested that hosts could be placed in categories based on their ability to either increase or decrease initial nematode population densities. A further classification could be made into those species which do or do not stimulate hatching. Townsend & Davidson (1964) suggested that the susceptibility of weed hosts of Meloidogyne hapla may be more dependent on the chemical nature of the root diffusate than the physical nature of the host. If this is also true for H.cruciferae, then the prime requisite for weed hosts would be that they effectively stimulate hatching.

The importance of a weed host in maintaining population densities of H.cruciferae is also related to its distribution and density. Whilst information on the distribution of weed species is covered to some extent by national and regional surveys there is a scarcity of information concerning the density of specific weeds within individual fields. Such information which would be invaluable to the assessment of the importance of weed hosts is unlikely to be reliable and would have to be very detailed as the weed populations of a field will vary

with the crop and herbicide usage. As oilseed rape usually occurs in rotations with cereals, weeds associated with cereal cultivation are likely to be potentially important hosts. Chancellor & Froud-Williams (1984) list the most frequent dicotyledonous weeds found in a survey of cereal weeds in southern England. Many of these species were similar to those found in another survey of weeds in fields when oilseed rape was grown (Evans, Pers comm.).

This experiment examines selected weed species as potential hosts of H.cruciferae and assesses the importance of weeds in maintaining populations in the absence of a host. To reduce the number of pot tests an initial experiment is described which determines which species produce stimulatory diffusates and those which are invaded by H.cruciferae whilst a second experiment examines the multiplication of H.cruciferae on plants which are invaded by juveniles.

MATERIALS AND METHODS

i) Hatching and invasion tests

Forty three species were selected for testing as potential hosts of H.cruciferae (Table 24). Crucifers and labiates were selected because of their association with cultivated land (Clapham et al., 1981). Common weed species from other families which are associated with arable land were also selected (Fryer & Makepeace, 1977; Chancellor & Froud-Williams, 1984).

One hundred and thirty two pots (9cm) were filled with 300g of a mixture of loam, sand and gravel (3:1:1) and 3 pots were each sown with 10 seeds of each plant species. The pots were placed in a glasshouse with 20°C day and 15°C night temperatures and with supplementary lighting at a 16 hour photoperiod. Plants were thinned to one seedling per pot 7 days after sowing and watered as required. When plants had reached the stage of four true leaves, each pot was leached with two volumes of 50ml distilled water added 30 minutes apart. Three unplanted pots were also leached. The leachates were collected in plastic trays, filtered and stored at 5°C. Cysts were extracted by fluidising column from soil infested with H.cruciferae which had recently been collected from a field in Kent in which damage

Table 24 Weed species selected for screening against *H.cruciferae*

Family	Species	Commonname
Cruciferae	*Brassica oleracea*	Wild Cabbage
	B.nigra	Black Mustard
	B.juncea	Oriental Mustard
	B.campestris	Wild Turnip
	B.rapa	Turnip
	Sinapisalba	White Mustard
	S.arvensis	Charlock
	Sisymbrium officinale	Hedge Mustard
	Isatis tinctoria	Woad
	Thalaspi arvense	Field Penny-cress
	Camelina sativa	Gold of pleasure
	Barbarea vulgaris	Winter cress
	Cheiranthus cheiri	Wallflower
	Capsella bursa-pastoris	Shepherd's Purse
	Alyssum alyssoides	Small Alison
	Cochlearia officinalis	Scurvy Grass
	Raphanus raphanistrum	Wild Radish
	Arabis glabra	Tower Mustard
	Diplotaxis muralis	Stinkweed
	Teesdalia nudicaulis	Shepherd's Cress
Labiatae	*Stachys annua*	Woundwort
	Leonurus cardiaca	Motherwort
	Ballota nigra	Black Horehound
	Calamintha nepeta	Lesser Calamint
	Lamium purpureum	Purple dead nettle
	L.album	White dead nettle
	Nepeta mussinii	-
	Lycopus europaeus	Gipsywort
	Prunella vulgaris	Selfheal
	Galeopsis tetrahit	Common Hemp nettle
	Mentha arvensis	Corn Mint
Polygonaceae	*Polygonum convolvulus*	Black Bindweed
	P.aviculare	Knotgrass
	P.persicaria	Redleg
Compositae	*Matricaria recutita*	Wild Chamomile
Gramineae	*Alopecurus pratensis*	Meadow Foxtail
Papaveraceae	*Papaver rhoeas*	Corn Poppy
Violaceae	*Viola arvensis*	Field Pansy
Caryophyllaceae	*Stellaria media*	Chickweed
	Spergula arvensis	Corn Spurrey
Rubraceae	*Galium aparine*	Goosegrass
Solanaceae	*Solanum persicans*	Black Nightshade
Urticaceae	*Urtica ureas*	Small nettle

to oilseed rape had been attributed to H.cruciferae. Three replicate batches of 50 cysts were prepared for hatching tests in the leachate collected from each plant species. The leachates were warmed to room temperature and 3ml of each placed in each of three wells of a Repli-dish. A batch of cysts was added to each well and three batches also added to wells containing 3ml of soil leachate controls and distilled water controls. The Repli-dishes were maintained at 20°C in the dark and hatched juveniles were counted, removed and replaced with 3ml of fresh leachate weekly. The cumulative number of hatched juveniles were counted after 3 weeks.

Plants which had been used for leachate collection were immediately afterwards inoculated with approx. 2000 eggs and juveniles freshly released from cysts in 3ml of distilled water which were pipetted into each of the pots to a depth of 2-4cm at three locations around the root system. Fourteen days after inoculation the extent of nematode invasion in each plant was examined. Plants were gently removed from the pots and the root systems washed, blotted dry and weighed. The number of nematodes in the roots were counted by staining and releasing the nematodes from the roots by the standard method.

ii) The multiplication of H.cruciferae on weed hosts

Table 25 lists those species which hatched and were invaded by H.cruciferae juveniles. One plant of each of these species was grown in each of 6 pots as before. When plants reached the stage of four true leaves they were inoculated with approx. 2000 juveniles by the method described previously. Five and 12 weeks after inoculation plants were removed from the pots and the soil thoroughly mixed. Cysts were extracted by fluidising column from a 200g sample of the soil and the number of eggs in the cysts counted by the standard method.

RESULTS

i) Hatching and invasion tests

Eighteen of the forty-three species selected for screening against H.cruciferae produced root diffusates that hatched significantly more ($P > 0.05$) juveniles than did either soil leachate or distilled water

Table 25

The hatching of H.cruciferae in leachates collected from weed species and the extent of juvenile invasion 14 days after inoculation

Species	Total number of hatched juveniles	Root weight (g)	Number of juveniles hatched /g root	Number of juveniles /root system	Number of juveniles /g root
Brassica oleracea	401	3.2	125.3	28.2	8.9
B.nigra	351	3.6	97.5	22.2	6.4
B.juncea	309	1.7	181.8	47.9	27.8
B.campestris	288	1.7	169.4	15.7	9.3
B.rapa	221	2.0	110.5	21.2	10.5
Sinapis alba	159	2.4	66.2	10.2	4.3
S.arvensis	79	2.8	28.2	45.5	16.4
Sisymbrium officinale	21	6.7	3.1	42.2	6.3
Isatis tinctoria	66	0.8	82.5	19.9	23.7
Thalaspi arvense	112	1.9	58.9	17.1	8.9
Camelina sativa	70	3.6	19.4	25.0	7.0
Barbarea vulgaris	29	5.4	5.8	26.3	4.9
Cheiranthus cheiri	118	1.6	73.7	13.9	8.4
Capsella bursa-pastoris	135	3.3	40.9	8.5	2.6
Alyssum alyssoides	91	2.4	37.9	1.5	0.6
Cochlearia officinalis	19	0.3	63.3	-	-
Raphanus raphanistrum	155	2.7	57.4	11.7	4.3
Arabis glabra	39	2.1	18.5	4.6	2.1
Diplotaxis muralis	72	1.8	40.0	6.4	3.5
Teesdalia nudicaulis	105	3.1	33.8	12.2	3.9
Stachys annua	47	4.5	10.4	16.6	3.6
Leonurus cardiaca	18	2.1	8.6	-	-
Ballota nigra	26	2.7	9.6	-	-
Calamintha nepeta	5	1.6	3.1	-	-
Lamium purpureum	29	3.4	8.5	-	-
L.album	23	2.7	8.5	-	-

Table 25 (Continued)

The hatching of H.cruciferae in leachates collected from weed species and the extent of juvenile invasion 14 days after inoculation

Species	Total number of hatched juveniles	Root weight (g)	Number of juveniles hatched /g root	Number of juveniles /root system	Number of juveniles /g root
Nepeta mussinii	7	1.7	4.1	-	-
Lycopus europaeus	9	1.3	6.9	-	-
Prunella vulgaris	17	0.6	28.3	-	-
Galeopsis tetrahit	16	1.5	10.6	-	-
Mentha arvensis	27	1.8	15.0	-	-
Polygonum convolvulus	11	0.7	15.7	-	-
P.aviculare	24	1.1	21.8	-	-
P.persicaria	22	0.3	73.3	-	-
Matricaria recutita	7	1.4	5.0	-	-
Alopecurus pratensis	17	2.3	7.4	-	-
Papaver rhoeas	17	1.6	10.6	-	-
Viola arvensis	10	0.8	12.5	-	-
Stellaria media	25	1.2	20.8	-	-
Spergula arvensis	15	0.8	18.7	-	-
Galium aparine	9	0.6	15.0	-	-
Solanum persicaria	22	2.3	9.6	-	-
Urtica ureas	12	1.7	7.0	-	-
Soil water	21	-	-	-	-
Distilled water	18	-	-	-	-
Mean	75.7	2.1	38.2	19.8	8.2
SED	21.6	1.6	12.7	9.3	4.0

(Table 25). Root diffusates produced by Brassica spp. hatched more juveniles than plants of other genera, although more juveniles hatched in diffusate produced by Sinapis alba, Raphanus raphanistrum and Capsella bursa-pastoris than the remaining species tested. Brassica spp. also hatched more juveniles per g root than other genera, with Isatis tinctoria and Cheiranthus cheiri the best of the remaining species. There was no relationship between the root weight of plant species and the numbers of juveniles that they hatched.

Twenty of the forty-three species were invaded by H.cruciferae, including Sisymbrium officinale and Barbarea vulgaris whose root diffusates did not cause H.cruciferae juveniles to hatch. The largest numbers of juveniles were found in the roots of Brassica juncea, Sinapis arvensis and Sisymbrium officinale, whilst relatively few juveniles invaded the roots of Alyssum alyssoides, Arabis glabra and Diplotaxis muralis. The largest numbers of juveniles per g root were found in Brassica juncea and Isatis tinctoria. There was no relationship between either invasion and root weight or invasion and the numbers of juveniles hatched. All the species that were invaded by H.cruciferae were crucifers with the exception of Stachys annua which is a labiate.

ii) The multiplication of H.cruciferae on weed hosts

Cysts were produced on seventeen of the twenty species that were invaded by H.cruciferae (Table 26). No cysts were produced on Alyssum alyssoides, Arabis glabra and Diplotaxis muralis although juveniles had invaded these species in the first experiment. A larger number of cysts were produced on most species by 12 weeks than by 5 weeks and this was reflected in the number of eggs produced at each occasion. At 12 weeks significantly ($P > 0.05$) more cysts were produced on Brassica spp. and on the non-Brassica spp., Sisymbrium officianle and Capsella bursa-pastoris than other species. More eggs were produced on Brassica spp. than other genera by 12 weeks resulting in greater multiplication factors (eggs produced at 12 weeks/juvenile inoculum). Of the non-brassica species, Capsella bursa-pastoris and Sisymbrium officinale had produced more eggs by 12 weeks than other species, with the smallest numbers produced by Barbarea vulgaris, Isatis tinctoria and Cheiranthus cheiri.

Table 26

The multiplication of H.cruciferae on weed species

Species	Cysts produced/plant 5 wks	Cysts produced/plant 12 wks	Eggs produced/plant 5 wks	Eggs produced/plant 12 wks	Eggs produced at 12 weeks juvenile inoculum
Brassica oleracea	20.0	32.1	192.0	536.1	0.28
B.nigra	12.3	26.0	151.3	574.6	0.29
B.juncea	11.0	36.5	82.5	992.8	0.50
B.campestris	9.5	18.3	135.0	472.1	0.24
B.rapa	17.3	21.3	112.5	741.2	0.37
Sinapis alba	7.5	16.0	16.2	92.7	0.05
S.arvensis	5.4	8.2	12.0	41.6	0.02
Sisymbrium officinale	4.6	23.7	22.1	192.2	0.10
Isatis tinctoria	14.3	17.3	84.4	36.5	0.02
Thalaspi arvense	9.3	11.2	80.9	138.8	0.06
Camelina sativa	12.5	15.0	36.2	60.0	0.03
Barbarea vulgaris	9.0	4.1	64.8	24.1	0.01
Cheiranthus cheiri	8.7	8.6	27.0	46.6	0.02
Capsella bursa-pastoris	6.0	22.1	40.1	307.2	0.15
Alyssum alyssoides	-	-	-	-	-
Raphanus raphanistrum	2.1	9.0	42.1	109.8	0.05
Arabis glabra	-	-	-	-	-
Diplotaxis muralis	-	-	-	-	-
Teesdalia nudicaulis	5.6	7.1	17.9	26.3	0.01
Stachys annua	6.0	13.9	19.8	34.7	0.01
Mean	9.5	17.1	66.8	250.4	0.13
SED	3.7	7.5	37.3	81.6	0.03

DISCUSSION

If weed hosts are to play an important role in maintaining populations of H.cruciferae between host crops, two major criteria are that: (i) root diffusates should stimulate a large number of juveniles to hatch and (ii) nematodes can multiply in sufficient numbers in the roots so as to either maintain or increase the initial population density. More juveniles hatched in leachings from plants in the genus Brassica than in other genera of Cruciferae, confirming the findings of Winslow (1955). The hatching tests showed that diffusates produced by Sisymbrium officinale, Barbarea vulgaris and Cochlearia officinalis did not stimulate juveniles to hatch although juveniles could invade and multiply in their roots. As this does not fulfill the first criterion these plants can not be regarded as potentially important hosts. There were no differences in the size of root systems which produced either stimulatory or non-stimulatory diffusates and this suggests that a large number of H.cruciferae juveniles hatch in response to specific chemicals produced by brassicas only.

A host which stimulates a large hatch and which does not support nematode development could be regarded as a trap crop (Cooke, 1985). These will deplete soil populations rapidly between host crops. Three species (Alyssum alyssoides, Arabis glabra and Diplotaxis muralis) produced diffusate that hatched and were invaded by juveniles but failed to support cyst production. Similar results were found by Winslow (1954) who also found that plants of these genera were not hosts of H.cruciferae. However, these species have not been recorded as frequently occurring weeds (Chancellor & Froud-Williams, 1984) and therefore their importance as 'trap-crops' will be insignificant.

Earlier work by Jones (1950) and Winslow (1954) has shown that the majority of the hosts of H.cruciferae occur within the Cruciferae. Heterodera cruciferae has also been found to multiply on the following labiates: Lamium album, L.purpereum, Stachys annua and S.arvensis (Winslow, 1954; Hesling, 1963). The present experiment has identified Stachys annua as the only non-cruciferous host although the numbers of eggs produced were rather small. The development of H.cruciferae on hosts was found to vary considerably. Brassica spp. were better hosts than other genera and supported greater development and greater egg production between 5 and 12 weeks. Brassicas are not common

agricultural weeds but may be locally important on farms where Black mustard and Yellow turnip have been grown for seed (Jones, 1945). However, volunteer oilseed rape which is a frequent weed of cereals (Fryer & Makepeace, 1977) may support the multiplication of H.cruciferae if not controlled. Oilseed rape (Brassica napus) was not compared with other Brassica spp. as a host of H.cruciferae in this experiment. However, in a previous experiment (Chapter 6) where the multiplication of H.cruciferae was examined under similar conditions to those in this experiment, H.cruciferae had a multiplication factor of 0.91 after 85 days. In this experiment, H.cruciferae had a mean multiplication factor of 0.33 after 84 days on Brassica spp. indicating that oilseed rape is a better host of H.cruciferae than other Brassicas.

Den Ouden (1967) showed that volunteer potato plants did not influence the rate of decrease of large potato cyst nematode population densities but smaller nematode densities decreased less when volunteer plants were present. The greater the density of the volunteer plants the larger the proportion of the population that was influenced by them. Volunteer oilseed rape would support nematode multiplication but it is unlikely that sufficiently high densities of volunteer plants would be allowed to grow unchecked.

The largest numbers of eggs produced by H.cruciferae on non-brassica hosts were on Capsella bursa-pastoris and Sisymbrium officinale. Capsella bursa-pastoris is a common weed of arable land and may also support much H.cruciferae multiplication if it is not controlled. Measurements of the multiplication of other cyst-nematodes on non-crop hosts have shown that H.glycines reproduces more rapidly on some weed species than on soybean, its main host crop (Epps & Chambers, 1966). Griffin (1982) showed that the same weed hosts of H.schachtii, collected from differing geographical areas supported different amounts of multiplication at different soil temperatures indicating that there are probably local variations in weed host-parasite relationships.

The importance of weeds as hosts depends not only on their suitability as hosts in pots but will ultimately depend on their root density per hectare. Although many cruciferous hosts of H.cruciferae are commonly associated with cultivated land and are widespread throughout

the UK, it is unlikely that many species will have roots present in sufficient densities to allow nematodes to multiply in numbers which would maintain population levels. Common weeds such as Cleavers, Mayweeds and Chickweed which can form dense mats on the soil surface if uncontrolled, are probably present at densities which could support substantial cyst nematode population densities but they are not hosts of H.cruciferae. However, H.schachtii can multiply on chickweed (Winslow, 1954; Steele, 1965) and this weed species may be an important maintainer host in the population dynamics of this species. The application of pre-sowing herbicides to the soil will preclude the use of many weeds as hosts by nematodes whereas post-emergence herbicides may trap any nematodes that are developing in weed roots.

The lack of information concerning weed densities and the inability to predict the use and type of herbicide makes the assessment of the importance of weed hosts difficult. However, because of the limited host range of H.cruciferae and the small number of plants on which this nematode can achieve substantial multiplication, it is unlikely that weeds are important as hosts and they probably play an insignificant role in the population dynamics of H.cruciferae.

11

The persistence of H.cruciferae in the absence of a host

INTRODUCTION

In the absence of a host unhatched juveniles of H.cruciferae must be able to survive until a host crop is grown again. The ability to survive will determine the period for which non-host crops should be grown or alternatively the amount of damage to a subsequent host crop. Heterodera cruciferae may complete two generations on a crop of oilseed rape and in some years many juveniles may have hatched from second generation cysts and egg sacs by the time the crop is harvested. If juveniles remain infective in the soil for a long period they could act as an additional source of infection for a subsequently sown host crop as well as those juveniles remaining unhatched in cysts. In areas where either vegetable brassicas or oilseed rape are grown in monoculture the survival of hatched juveniles between crops may be important. Additionally, within the growing season of a winter sown rape crop, a proportion of juveniles that hatch in the autumn may not invade roots at this time and might be able to overwinter whilst soil temperatures are below the threshold for invasion and invade the roots when soil temperatures increase in the spring. A small proportion of H.schachtii juveniles were found to remain infective after 7 months in soil in the absence of a host (Golden & Shafer, 1960). However the duration of infectivity depends on the rate at which juveniles deplete their food reserves and this is related to their activity which in turn is dependent on the soil temperature, moisture content and type. Generally, the survival of hatched juveniles in the absence of a host decreases as soil temperature and moisture content increase (Bergeson, 1959; Slack et al., 1972; Davies & Fisher, 1976). Juveniles of H.avenae lost their infectivity rapidly at 20°C but more slowly at lower temperatures; only juveniles stored at 5°C remained as infective after 7 weeks as those at the start of the experiment (Davies & Fisher, 1976). The survival of Globodera rostochiensis and G.pallida was correlated with the use of lipid reserves with a large decline in infectivity associated with a 55% decrease in lipid reserves. Few juveniles invaded the roots after 57 days storage in water (Storey, 1984).

Unhatched juveniles within the cyst of H.cruciferae can, as in other cyst nematodes, remain alive much longer than hatched juveniles in the soil. Unhatched juveniles within egg sacs of H.cruciferae may not be so well protected as those within the cysts and therefore are not so

persistent. The infectivity of juveniles from egg sacs of H.glycines was much reduced when compared to the infectivity of juveniles from mature cysts over a 90 day period of storage at a range of temperatures (Ishibashi et al., 1973). Additionally, the winter survival of eggs of Meloidogyne incognita and M.arenaria was poor with less than 30% of the population surviving (Starr & Jeger, 1985). Such poor survival may be attributed to all the eggs being deposited into a gelatinous matrix by those species, rather than being retained within the female body wall. Although unhatched eggs of Meloidogyne embedded in the glycoproteins of the gelatinous matrix may survive better than hatched juveniles (Vrain, 1978) unhatched eggs within cysts seem better adapted for longer term persistence. This may be a combination of the additional protective properties of the cyst and differences in the physiology of juveniles in cysts and gelatinous matrices.

Viable eggs were found within cysts of H.glycines after 11 years and the eggs of H.rostochiensis were still viable after 7 years although no symptoms of nematode damage appeared in potato plants grown in this soil suggesting that there was a gradual decline in the numbers of juveniles invading plants over this time (Miles & Miles, 1942; Inagaki & Tsutsumi, 1971). Storey (1984) found that the lipid reserves of unhatched juveniles of Globodera had declined by 50% after 7.5 years indicating that low levels of lipid utilisation during dormancy favoured long term persistence. The number of eggs in the soil has been reported to decline by 30-50% per annum (H.schachtii, H.goettingiana and H.rostochiensis) and appears to be density-independent (Jones & Jones, 1974). However, fewer eggs were produced per cyst at high densities of potato cyst nematodes and populations declined more rapidly from high than from low densities as a result (Seinhorst, 1984). Starr & Jeger (1985) suggested that fewer resources were allocated to individual eggs at high population densities of Meloidogyne than at low population densities which reduced the survival potential of each egg. The decrease in the number of H.rostochiensis eggs in the soil has been reported to be a result of spontaneous hatching of juveniles especially in the spring when soil temperatures are rising (Den Ouden, 1960). Preste (1983) found that a H.schachtii population had decreased by 33% after 19 weeks at 16°C, probably reflecting the ease with which H.schachtii hatches in the absence of a specific host stimulus. The persistence of H.cruciferae populations in the absence of a host has not been studied but this species only hatches readily in response to root diffusates and may be

expected to persist longer than H.schachtii in the soil. The decline of populations may be further mediated by damage to eggs by fungal infection. Fungi have been shown to infect a large proportion of H.schachtii cysts (Bursnall & Tribe, 1974) and are important agents in controlling populations of H.avenae (Kerry & Crump, 1977). Little is known of the fungal parasites of H.cruciferae. Predation of H.cruciferae cysts by invertebrates may also deplete soil populations; 25% of H.cruciferae cysts were found to be damaged by Collembola predators (Murphy & Doncaster, 1957) but an accurate assessment of the effect of predators on the numbers of eggs in cysts was not made.

The eggs of H.rostochiensis, H.glycines and H.avenae have been shown to lose viability faster at higher temperatures and in more moist conditions (Lewis & Mai, 1957; Inagaki & Tsutsumi, 1971; Mathur et al., 1974). Yeates & Visser (1979) found that more juveniles of H.trifolii hatched after soil was stored at low temperatures but air drying of cysts was found to reduce hatching. Unhatched juveniles are thought to be tolerant of sub-zero temperatures and avoid freezing by supercooling (Perry & Wharton, 1985). However, the hatching of H.schachtii and H.glycines was reduced when cysts were frozen and hatching decreased in direct proportion to the length of time cysts remained frozen (Viglierchio, 1961; Slack & Hamblen, 1961). Physical conditions within the soil vary with depth and the effects of freezing will be more pronounced nearer the soil surface. Storey (1982) found that the survival of Globodera rostochiensis through the growing season was not correlated with depth below the furrow but Lamondia & Brodie (1986) found that in fallow soil the viability of eggs of G.rostochiensis declined more slowly at 5cm deep than at 15cm depth. All viable eggs in cysts on the soil surface died within 5 months, probably in this case due to high temperatures. Starr & Jeger (1985) showed that the over winter survival of M.incognita and M.arenaria increased with depth and suggested this may be due to the insulating effect of the soil.

This experiment examines the duration of infectivity of hatched juveniles in the soil at different temperatures and the decline in the numbers and infectivity of unhatched juveniles in cysts at different depths in the soil over a period of 96 weeks. Additionally the survival and hatching of juveniles from cysts stored in soil under

controlled conditions is examined and the decline of different populations of H.cruciferae compared.

MATERIALS AND METHODS

i) Duration of infectivity of hatched juveniles in the soil

Approximately 800 cysts were extracted by fluidising column from soil infested with H.cruciferae. Batches of approximately 100 cysts were then added to each of eight compartments of a Repli-dish containing 3ml of root leachate at 15°C. Root leachate was taken from a stock solution stored at 5°C which had been collected from 28 day old oilseed rape plants. After 7 days hatched juveniles were removed from each compartment, discarded and the root leachate replaced with 3ml of fresh leachate. After a further 7 days hatched juveniles were again removed from each compartment and added to a measuring cylinder. Collecting the juveniles that hatched between 7 and 14 days ensured that a reasonably uniform batch of juveniles was used in the experiment. Hatched juveniles from each compartment were bulked and the juvenile suspension made up to 50ml with distilled water. The total number of hatched juveniles was counted by the standard method before aliquots of the suspension, containing approximately 200 juveniles, were added to soil in each compartment of a Repli-dish. Ninety-five Repli-dishes compartments had previously been filled with 2g of a mixture of loam, sand and gravel (3:1:1). Juveniles were pipetted in to each compartment along with 1ml of root leachate taken from the stock solution. Each Repli-dish was then sealed with cling-film. Repli-dishes containing 30 filled compartments were placed in a cooled incubator at either 5 or 10°C and Repli-dishes containing 35 filled compartments in a growth cabinet at 15°C. The infectivity of the juveniles stored under the different conditions was examined by inoculating 28 day old oilseed rape plants (cv. Jet Neuf) growing in 50g of a mixture of loam, sand and gravel (3:1:1) in 5cm diameter pots. Pots had been placed in a growth cabinet at 15°C with a 12 hour photoperiod prior to inoculation. After 21,28,35,42,56 and 70 days the soil from one compartment of a Repli-dish stored at each temperature was added to each of one of the oilseed rape plants. The infectivity tests were replicated five times. The initial level of invasion was checked after one day using soil stored at 15°C. The contents of single compartments of a Repli-dish were added at four locations

around the root system. Each compartment was rinsed with 1ml of water which was also added to the soil surrounding the plant. After inoculation the pots were returned to the growth cabinet and after a further 14 days the plants were removed from the pots and the roots of each plant gently washed from the soil, blotted dry and weighed. The extent of juvenile invasion on each occasion was assessed by staining and counting the nematodes released from the roots by the standard method.

ii) Persistence of unhatched juveniles in the soil

(a) Microplot experiment

Soil was collected 2 weeks after the harvest of an oilseed rape crop from a field infested with H.cruciferae. The soil was well mixed and the population density estimated by the standard method. An outdoor microplot, 1m x 1m. was sited in an open area and was constructed using wooden sides to retain the surrounding soil. The plot was then filled with the infested soil to a depth of 25cm with the soil level with the surrounding soil surface. Initially monthly samples and subsequently bi-monthly samples of the soil were taken over a period of 24 months using a graduated corer of 2.0cm diameter, 25.0cm length. Eight cores were divided into fractions of 0-5, 5-10, 10-15 and 15-20cm below the soil surface. After the samples had been removed the core holes were filed with sand to maintain the soil temperature profile and to ensure these regions were only sampled once. The cysts were extracted from four of each of the fractions and the numbers of cysts and eggs counted by the standard method.

At each sampling period the infectivity of unhatched juveniles was assessed by transferring cysts to three pots (9.0cm) containing 28 day old rape seedlings cv. Jet Neuf. Plants were grown in a glasshouse at 15°C day and 10°C night temperature with supplementary lighting to give a 12 hour photoperiod. Plants were watered as required and given a N,P,K liquid fertiliser weekly. Three replicate batches of 50 cysts were selected initially from bulked soil of all depths but from 8 weeks onwards from the following combined soil fractions, 0-10cm and 10-20cm. Cysts were pipetted in 2ml of water into the soil of each pot, at three locations to a depth of 2-4cm surrounding the root system. Twenty days after inoculation, the roots of each plant were

removed, blotted dry and weighed. The extent of juvenile invasion was then assessed by the standard method.

(b) Persistence of unhatched juveniles under controlled storage conditions

A bulk of H.cruciferae infested soil was divided into two 60Kg amounts. One was spread out on plastic sheets and air dried for one week and the other was kept moist. The moisture content of each fraction was determined by weighing 5 x 10g amounts of soil and then drying them at 80°C in an oven for 48 hours. The soil was weighed again after this period and the weight of water lost on drying expressed as percentage moisture content. Each fraction was then further divided into approximately 2 x 30Kg amounts and placed in large plastic containers with tightly fitting lids and stored at either 4-6°C or 15-20°C. At monthly intervals for 3 months and thereafter at 6,9 and 11 months, cysts were extracted from 5 x 200g soil samples by fluidising column. At each sampling, three replicate batches of 50 randomly cysts were selected from each treatment and placed in 3ml of Flavianic acid (an artificial hatching agent) in Repli-dishes and kept in the dark at 15°C. After 21 days the total numbers of hatched juveniles were counted and the numbers of unhatched juveniles per cyst counted by the standard method.

Additionally, the effect of sub-zero temperatures on the hatching of juveniles was examined by placing 1Kg samples of soil (moisture content 16.2%) at 5°C, -10°C or at fluctuating temperatures of 12 hours at 5°C followed by 12 hours of -10°C for 28 days. After this time cysts were extracted from each soil sample and five batches of 50 cysts from each treatment added to 3ml of Flavianic acid in each of 5 compartments of a Repli-dish. Repli-dishes were placed in the dark at 15°C and the numbers of hatched juveniles counted after 21 days.

(c) The decline of different populations

Soil from fields known to be infested with H.cruciferae and where crops had partially failed was collected from sites near Fakenham (Norfolk), Whitstable (Kent) and Wrangle (Lincolnshire). The soil from each location was collected shortly after the rape crop had been

harvested, well mixed and the cysts from 5 x 100g samples were extracted by fluidising column and their egg content estimated by the standard method. The soil from each location was then placed in plastic sacks which were tied at the top to maintain soil moisture and left undisturbed in an unheated room at 5-15°C. After 12 and 24 months further estimates of the numbers of cysts and numbers of eggs per cyst were made.

RESULTS

i) Duration of infectivity of hatched juveniles

Invasion by hatched juveniles stored in soil in the absence of a host at different temperatures is shown in Figure 36. The results are expressed as a percentage of the level of invasion of 53.7 juveniles/root system after storage for 1 day at 15°C. Temperature had no significant effect on the level of invasion by 21 days although fewer juveniles invaded the root system at this time than after 1 day. At 28,35 and 42 days fewer juveniles invaded the roots when stored at either 10°C or 15°C than at 5°C. By 42 days invasion by juveniles stored at 10 and 15°C had decreased to less than 20% of the initial level of invasion whereas invasion by juveniles stored at 5°C was 66.8% that of the initial level. After 56 days similar numbers of juveniles invaded regardless of their storage temperature and juvenile invasion was low after all treatments. By 70 days the level of invasion was less than 10% of the initial level.

ii) (a) Microplot experiment

The numbers of cysts extracted from each depth fraction did not decrease significantly at any time during the experiment. However, Figure 37 shows that there was an overall decline of 48.0% over the 96 week period in the number of eggs per cyst. After the first 52 weeks the number of eggs per cyst had declined by an overall value of 34.0%. Figure 37 shows that this decline was greatest in the upper 10cm, the number of eggs per cyst decreasing by 61.6% compared to 34.3% in cysts from the 10-20cm depth fractions. The decrease in the number of eggs per cyst was greatest during the first 24 weeks of the experiment at all depths; over the remaining weeks the decline was more gradual

Figure 36 Invasion by H. cruciferae juveniles after being kept in fallow soil at different temperatures: 5°C (○) 10°C (●) 15°C (△). Vertical bars represent L.S.D. (P = 0.05)

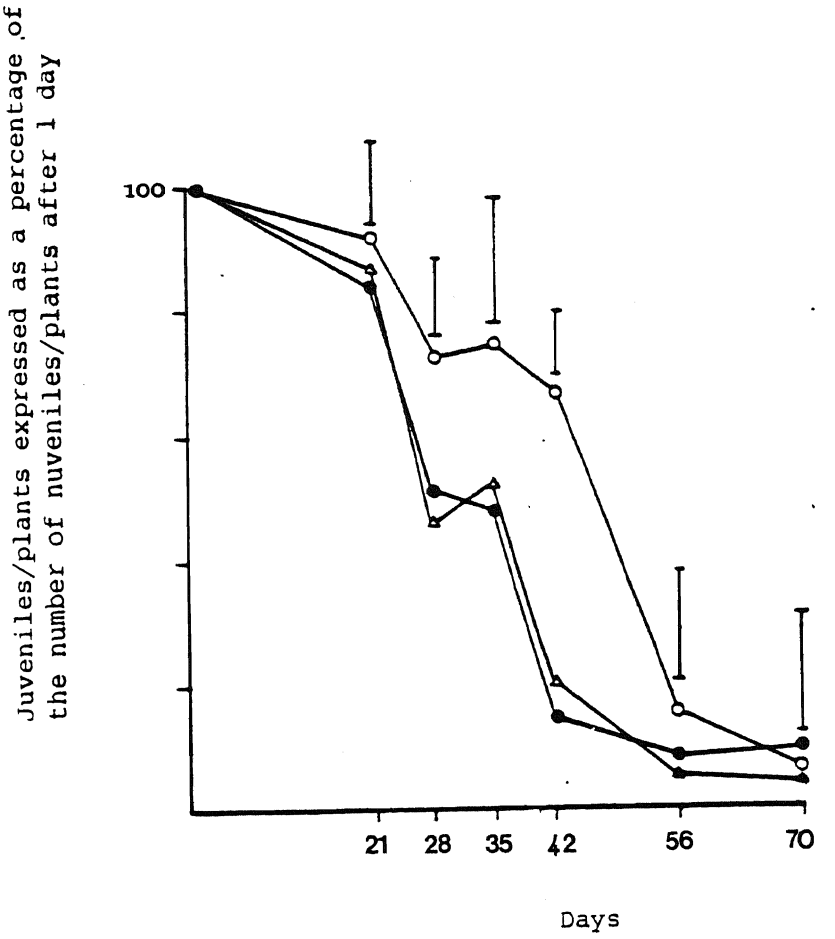
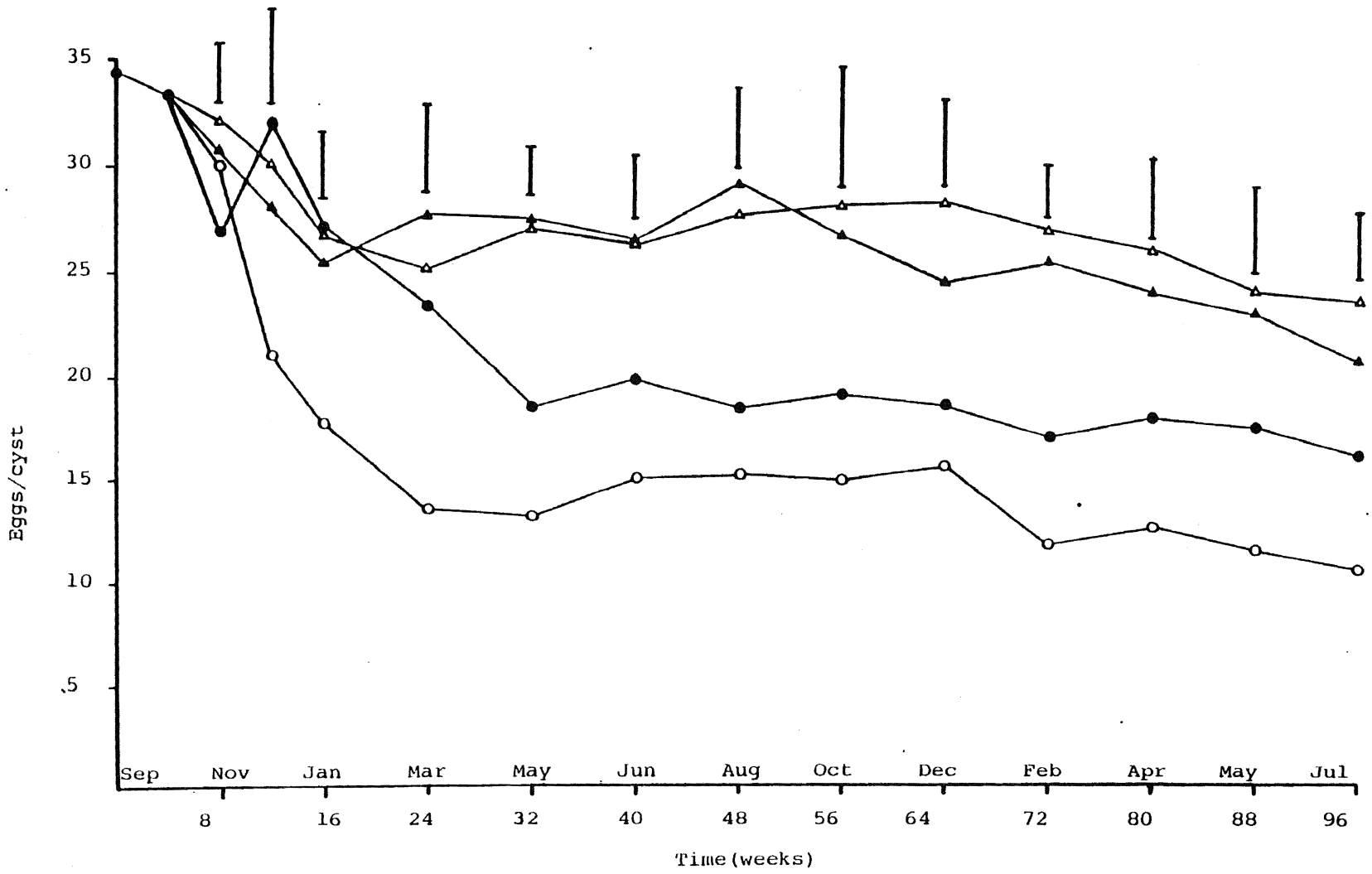


Figure 37 Changes in the number of eggs/cyst over a period of 96 weeks at different depth fractions in the soil : 0 - 5 cm (○) 5 - 10 cm (●) 10 - 15 cm (△) 15 - 20 cm (▲). Vertical bars represent L.S.D. (P = 0.05)



except for a large decrease in the number of eggs per cyst from soil from 0-5cm depth at 72 weeks. During the first year the highest rate of decline coincided with low mean monthly soil temperatures (Fig. 41) but this was not as pronounced in the second year of the experiment. The numbers of juveniles that invaded the roots of plants from cysts from either 0-10cm or 10-20cm depth fractions are shown in Figure 38. Invasion is expressed as a percentage of the level of invasion of 153.5 juveniles/root system at the beginning of the experiment. Juvenile invasion had decreased after 8 weeks from cysts from both depths and had decreased by an overall value of 53.1% after 96 weeks. Fewer juveniles invaded the roots after 16 weeks from cysts at 0-10cm depth and decreased by 62.3% after 96 weeks. Invasion from cysts at 10-20cm depth had decreased by 43.9% after 96 weeks.

(b) Controlled storage conditions

In controlled temperature and moisture conditions the largest decrease in the number of eggs per cyst occurred in soil that had been stored at 15-20°C/18-22% moisture content (Table 27). The smallest decrease was in soil stored at 4-6°C/5-7% moisture content and the numbers of eggs per cyst stored in other combinations of conditions declined to intermediate levels. Hatching decreased by an overall value of 65.3% from cysts stored for 48 weeks (Fig. 39). However, the largest decreases in hatching (82.4%, 66.2%) occurred when cysts were stored in soil at 15-20°C/18-22% and 4-6°C/18-22% moisture content respectively. The large decrease in hatching by juveniles from cysts stored in soil at 15-20°C/18-22% moisture content corresponded to the greatest decrease in the number of eggs per cyst after 48 weeks at these conditions (Table 27). Figure 39 shows that the decline in hatching was initially rapid but after 24 weeks of storage hatching declined more slowly in all soils. Figure 40 shows that when cysts were stored at -10°C hatching still occured when cysts were thawed and placed in root leachate at 15°C. However, a smaller number of juveniles hatched when cysts had been frozen than those that hatched when cysts had been stored at 5°C. There was no significant difference between the numbers of juveniles that hatched when cysts were frozen either continuously for 28 days or when subjected to a 24 hour freezing and thawing cycle for 28 days.

Figure 38 Root invasion from cysts extracted from two depth fractions in the soil: 0 - 10 cm (○) 10 - 20 cm (●) over a period of 96 weeks. Vertical bars represent L.S.D. (P = 0.05)

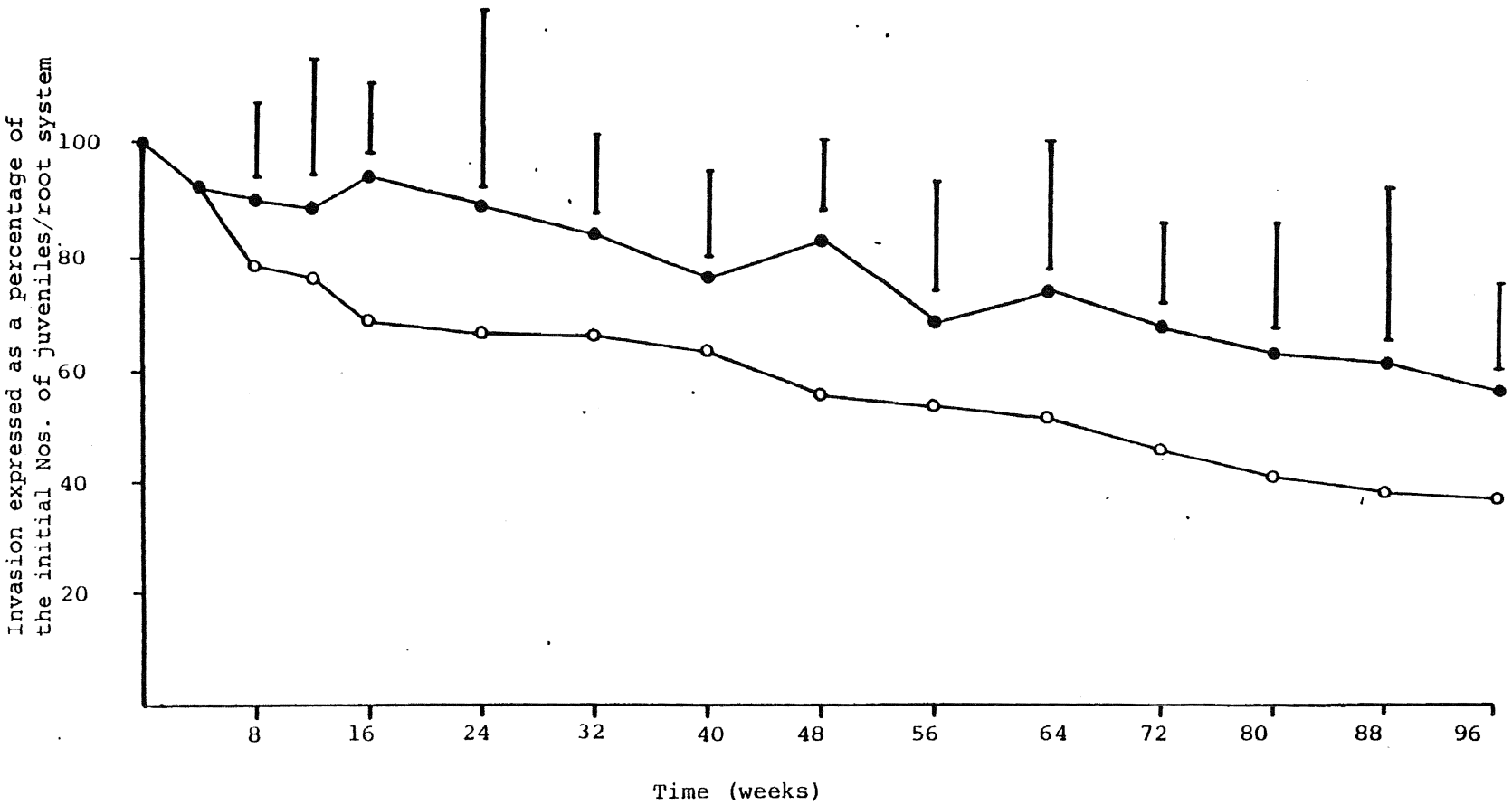


Figure 39 Hatch after 21 days from cysts kept in soil of different moisture content and temperature: (○) 4 - 6°C/5 - 7% (●) 4 - 6°C/18 - 22% (△) 15 - 20°C/5-7%, (▲)15-20°C/18-22%. Vertical bars represent L.S.D. (P = 0.05)

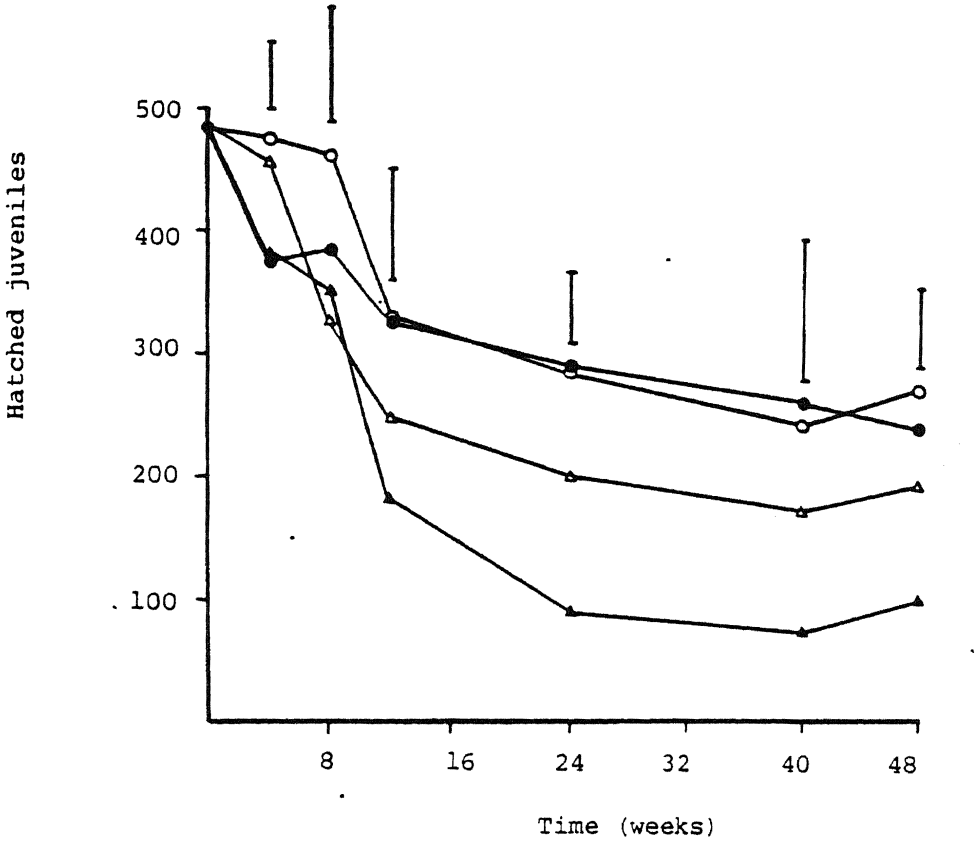


Figure 40 Hatch after 21 days from cysts kept in soil at low temperatures. Vertical bar represents L.S.D. (P = 0.05)

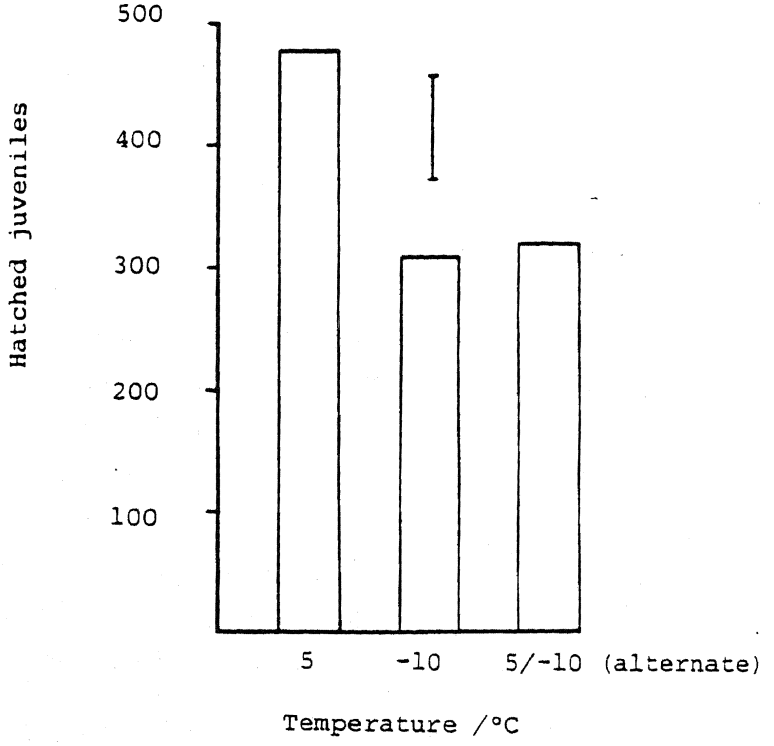


Table 27: The percentage decrease in the number of eggs per cyst after storage for 48 weeks at different soil temperature and moisture regimes.

	Soil temperature	
Soil Moisture	4-6°C	15-20°C
5-67%	26.2 ± 5.1	40.6 ± 3.1
18-22%	39.9 ± 6.7	52.4 ± 5.9

Means ± S.Error

(c) The decline of different populations

Table 28 shows that the soil populations of H.cruciferae collected from Whitfield, Fakenham and Wrangle declined by 46.4%, 29.0% and 76.1% after 12 months and by 55.3%, 88.2% and 80.9% after 24 months respectively. The decline of the Whitfield and Fakenham populations over the first 12 months was due to a decrease in the numbers of eggs per cyst as similar numbers of cysts were extracted on each occasion. However, the large decline of the Wrangle population in the first 12 months was partially a reflection of a decrease in the numbers of cysts extracted but there was no further decrease in the number of cysts extracted after 24 months. Fewer cysts were extracted from the Whitfield and Fakenham populations after 24 months. The decline in the Whitfield population was greater in this experiment than the decline of the same population in the microplot experiment.

DISCUSSION

The duration of infectivity of hatched H.cruciferae juveniles was influenced by soil temperature. A greater proportion of juveniles remained infective for longer at 5°C than at either 10°C or 15°C but after 10 weeks few juveniles were able to invade the roots regardless of the temperature at which they had been stored. Low soil temperatures also prolong the infective life of juveniles of H.glycines and H.avenae (Slack et al., 1972; Davies & Fisher, 1976). At low soil temperatures and moisture content juveniles are less active and lipid reserves will be conserved. This will extend the period in which lipid reserves remain sufficient to meet the energy demands of root penetration (Van Gundy et al., 1967; Storey, 1984). At

Table 28

The changes in the numbers of cysts and eggs of three H.cruciferae populations after storage for 12 and 24 months

Source	Cysts/100g			Eggs /g soil		
	Initial	12 months	24 months	Initial	12 months	24 months
Whitfield (Kent)	48.7 ± 3.6	53.1 ± 2.2	42.6 ± 3.1	16.8 ± 2.7	9.0 ± 2.2	7.5 ± 2.0
Fakenham (Norfolk)	77.6 ± 3.1	84.2 ± 1.3	63.1 ± 2.1	27.2 ± 1.8	19.3 ± 3.5	3.2 ± 4.5
Wrangle (Lincolnshire)	151.0 ± 2.5	101.6 ± 2.9	102.7 ± 1.6	84.2 ± 2.4	20.1 ± 2.3	16.1 ± 3.6

Means ± S.Error

very high soil moisture contents the infective life of juveniles has also been shown to be extended; juveniles of Meloidogyne naasi remained infective for longer in waterlogged soils as a result of a low rate of lipid utilisation due to depleted oxygen levels in the saturated soil (Ogunfowora, 1979). Oilseed rape is usually harvested in either late July or early August when soil temperatures are high (Fig.41) and the soil moisture content may be well below field capacity. In these conditions juveniles would probably lose infectivity relatively quickly and many would become desiccated in very dry soils. Juveniles may remain infective for a subsequent autumn sown host crop but it would be unlikely that many juveniles that hatched just before or after harvest would be able to infect a spring sown host crop. In the presence of a rape crop, few juveniles, except perhaps those that either hatched in late autumn before temperatures fell below the threshold for hatching or those that hatched in response to warm periods during the winter, would be able to overwinter and invade roots in the following spring. Overwintering by juveniles either in the absence or the presence of a host crop would be further reduced by the lethal effects of hatched juveniles becoming frozen (Wharton & Perry, 1985) especially in the upper parts of the soil profile.

Unhatched juveniles of H.cruciferae survived in cysts in the soil for the duration of the microplot experiment and the results indicate that the population would persist in the soil for several more years. The proportion of juveniles that survived was influenced by both time and the position of cysts in the soil profile. A large decline (34.0%) in the population occurred in the first year with a smaller decrease (14.0%) in the second year. The decline was largely a result of decrease in the number of eggs per cyst in the upper 10cm of the soil profile. Populations of G.rostochiensis have also been reported to decrease more in the first year after a potato crop than in subsequent years (Lamondia & Brodie, 1986). Populations left after a host crop consist partly of old eggs and cysts and partly of new eggs and cysts which cannot be distinguished in soil samples. The large initial decline of the H.cruciferae population in the microplot experiment was unlikely to be in response to root diffusates persisting in the soil as hatching activity is retained by the soil for only a short period after the plants have been harvested (Chapter 8). However, many juveniles may have received a hatching stimulus prior to the crop being harvested but emergence may have been inhibited because water

was removed from cysts in the dry summer soils (Cooke, 1985). The increase in the moisture content of soils over winter would allow hatching to occur when soil temperatures exceeded the threshold for hatching in response to either warmer periods during winter or when soil temperature increases in the spring. This would result in the large initial decline in the number of eggs per cyst after the harvest of the crop. A large initial decrease in the content of cysts from the same population also occurred when soil was stored under controlled conditions. In the warmer moist soil a large number of pre-stimulated juveniles would have hatched whereas few juveniles would have been able to hatch in soil stored at temperatures below the threshold for hatching.

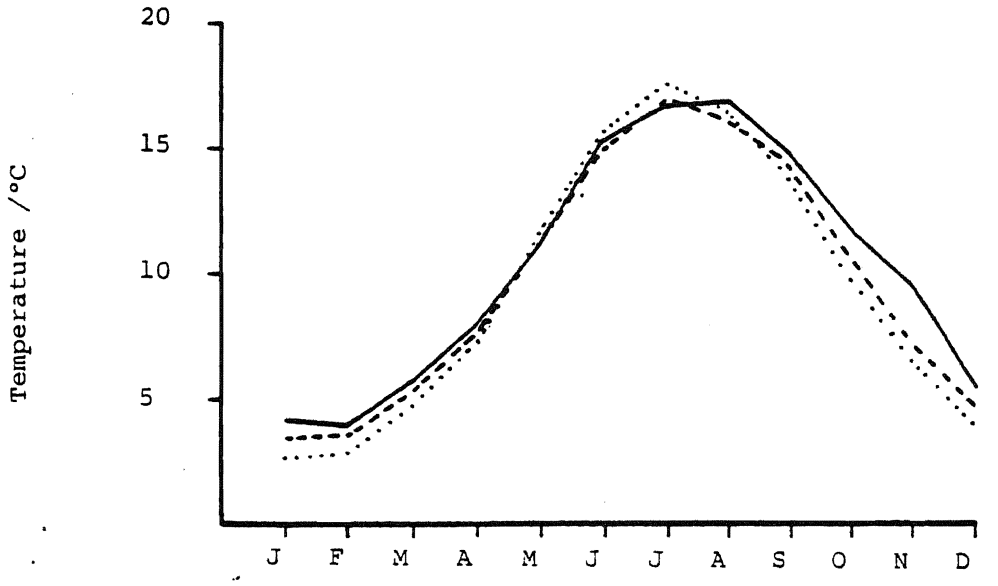
The initial decline in the numbers of eggs per cyst in the microplot experiment did not correspond to a large decline in the numbers of juveniles that were able to invade the roots. Juveniles which had been stimulated by root diffusates before the crop was harvested but which did not emerge at that time may have had greater metabolic rates than unstimulated juveniles and so have reduced their lipid reserves to below levels at which invasion could occur. An increase in the metabolic rate of juveniles has been associated with stimulation (Clarke & Perry, 1977). This would result in many of the juveniles that were initially present in cysts being able to hatch but unable to invade. Consequently, the infectivity assay only showed a decline in the numbers of juveniles invading after further population decline reduced the numbers of initially infective juveniles. This was more apparent as the numbers of eggs per cyst declined more rapidly in the upper 10cm of soil.

In common with other cyst nematodes H.cruciferae survived better at low soil temperatures and moisture. The numbers of juveniles that hatched after being stored in different conditions corresponded to the decline in the numbers of eggs per cyst in each treatment. Although many juveniles probably hatched from cysts stored in warmer and moister soils and accounted for much of the decline in the numbers of eggs per cyst it was difficult to determine the proportion of unhatched juveniles that had died because individual eggs were not examined and counts were made after cysts were ruptured to release eggs and juveniles. A large proportion of juveniles at various stages of decomposition would not be detected by this method. Dormant

juveniles have a very low rate of lipid utilisation and it is difficult to determine to what extent this rate is influenced by external temperature and moisture. However, low soil temperatures and moisture content would ensure a low rate of metabolism and would also suppress any fungal activity or spontaneous hatching. Most unhatched juveniles were able to survive being frozen although a smaller number of juveniles hatched from cysts after thawing than from cysts stored at 5°C. There was no visible difference between juveniles that had been either frozen at -10°C or left at 5°C presumably because very little decomposition would have occurred at sub-zero temperatures. Perry & Wharton (1985) recorded a smaller than expected hatch of G.rostochiensis when cysts were frozen and suggested that exposure to low temperature may delay or alter the response to the hatching stimulus. However, Viglierchio (1961) suggested that the reduction in the hatching of H.schachtii after freezing was due to the death of juveniles within the eggs. The detrimental effects of freezing on a proportion of the unhatched juveniles in a population may be partially responsible for the large decrease in the number of eggs per cyst in the upper 10cm of soil in the microplot experiment. Figure 41 shows that only small differences exist between the mean monthly temperatures at 10,20 and 30cm depth. However, the number of days on which the soil temperature is below freezing is much greater at 10cm depth than at 20cm (Fig. 42). Also, much lower temperatures occur more frequently in the top 10cm of soil. Therefore the insulating effect of the soil will be an important factor influencing the survival of H.cruciferae. The numbers of cysts extracted from each soil fraction were similar on each occasion and suggested that invertebrate predation was probably not responsible for the decline although the effects of fungal activity within cysts may have been greater in the upper soil profile.

Populations of H.cruciferae declined at different rates and suggests that generalised annual decline rates for species may be inaccurate. The Wrangle population which had the largest initial population density of the three populations in this experiment declined more rapidly than the other populations. The large decrease in the number of cysts of this population during the first year was unlikely to be due to differences in extraction technique as a similar number of cysts were extracted after 24 months. As cysts decay slowly and remain in the soil long after they are empty (Jones, 1945) the decrease in the number of cysts suggests that whole cysts had been lost to either

Figure 41 Soil temperatures at three depth throughout the year
(····· 10cm, - - - - - 20cm, —— 30cm)



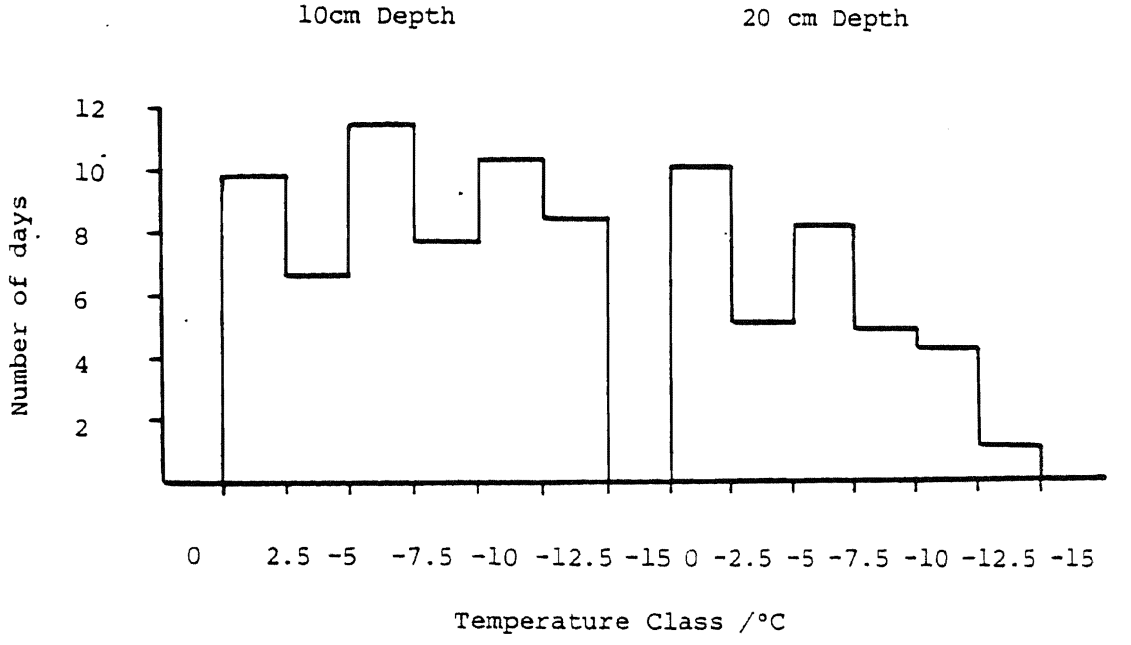


Figure 42 Frequency distribution of sub-zero temperatures at two depths in the soil throughout the year

invertebrate or fungal predation. The large decline in the numbers of eggs per cyst in Wrangle and Whitfield populations in the first year may have been due to a hatching effect, similar to that during the first year in the microplot experiment. The decrease in the numbers of eggs of the Fakenham population in the second year cannot be explained by this and may reflect either spontaneous hatching or a type of predation which resulted in the loss of cyst contents but not whole cysts. The Whitfield population declined more than it did in the microplot experiment, a reflection of the less favourable storage conditions for persistence. Wharton (1982) found that the decline rate of twelve potato cyst nematode populations varied considerably and suggested that this was due to differences in the hatching response of populations as determined by the relationship between the water content of soil and soil type. The large decline of the Wrangle population may be a result of the density-dependent effect proposed by Starr & Jeger (1985) for the decline of high density populations of Meloidogyne. Storey & Atkinson (1985) showed that the initial lipid levels of different populations of G.rostochiensis and G.pallida varied significantly and were influenced by host variety. Factors influencing the initial lipid levels of juveniles will determine the survival of juveniles and the persistence of soil populations and further work is needed to identify these factors. If smaller eggs contain fewer lipid reserves then the persistence of eggs in the soil will be reduced. However, at large initial densities when juveniles are competing for fewer food resources in the roots than are present at lower initial densities a smaller proportion of the population is able to become females and produce eggs (Chapter 7). This perhaps reflects a mechanism which ensures that the eggs produced by these females contain sufficient reserves to allow eggs to survive until another host crop is grown.

12

Application of the Jones and Perry population model for cyst nematodes to the population dynamics of *Heterodera cruciferae* and *H.schachtii* on oilseed rape

INTRODUCTION

Trudgill (1986) has drawn attention to the need of farmers and their advisers to model the effect of different agronomic strategies on cyst nematode population densities and the potential damage to crops. The relationship between pre-planting nematode population density and post-harvest population densities has been described mathematically by Jones et al. (1967), Jones & Kempton (1978) and Jones & Perry (1978). These workers modified the logistic equation for population growth to describe the population dynamics of cyst nematodes on a susceptible host crop during a growing season. Jones & Perry (1978) have also proposed equations to model the effects of non-host and resistant crops, fumigant and carbamoyloxime nematicides, inter specific competition and the effect of weed hosts on the population dynamics of cyst nematodes. The development of a computer programme by these workers enabled the effects of different strategies to be simulated and simplified the fitting of population equations to field data. The equations were developed primarily for Globodera spp. on potatoes and when tested against the results of field trials were found to provide useful qualitative information on the population dynamics of these species. However, the models were considered unreliable for the prediction of nematode numbers in undivided fields which are affected by within field variation in nematode population density, local farm practices and soil-climatic factors. Perry (1983) used the computer model to examine the effects of spatial heterogeniety on the nematode population densities within fields and suggested that if a range of initial densities is sampled for different positions within a field, each initial density should be included separately in the model. The resulting range of final densities may be combined to give a mean value for the whole field; similarly, a more accurate prediction of yield loss can be made.

The population model proposed by Jones & Perry (1978) is based on parameters describing the cyst-nematode life-cycle. In a year in which either a susceptible crop is grown a certain proportion of eggs hatch and the rest are carried over to the next year. If a non-host crop is grown or the land left fallow a different proportion hatch. If a nematicide is used a proportion of the hatched juveniles are killed. A proportion of hatched juveniles locate and invade the roots and root damage occurs. If two species are present interspecific competition is

superimposed upon intraspecific competition to determine the proportion of juveniles able to develop into females. Females give rise to certain numbers of eggs which are carried over to form next year's overwintering eggs.

Jones & Perry (1978) suggest that their model applies best to cyst-nematode species such as Globodera spp. on potatoes and H.avenae on cereals which complete one generation during the growing season. However, H.cruciferae and H.schachtii may complete two generations on a crop of autumn-sown oilseed rape (Chapter 2) and the model has not been tested with such species. This experiment evaluates the application of the Jones & Perry model for the population dynamics of H.cruciferae and H.schachtii on oilseed rape. Different agronomic strategies are simulated to indicate the model's applicability and to provide information on the population characteristics of these species.

MATERIALS AND METHODS

Input to the model consisted of parameters listed in Table 29. Each parameter had a code and was arranged in the format as given in the programme guide. Parameters were introduced to the model via an input channel and were checked before running the model. Some parameters were conditioned on the appearance of previous parameters and were not used in all the runs. The values of parameters were derived from experiments carried out in the current project. The results of more than one experiment were often used in order to determine sensible values. Values for H.schachtii were also obtained from the literature (Jones, 1945, 1956). The parameter values are listed in experiment (i) and remain constant for subsequent runs unless stated otherwise. As the model was designed primarily for Globodera spp. only one nematode generation could be completed during a growing season. In order to allow H.cruciferae and H.schachtii to complete two generations per growing season two years of the host crop were included each time the programme was run to represent one year of the host crop. The assumption that the population characteristics and the host environment remain constant for each generation is discussed later.

Table 29: Parameters used in the Jones & Perry population model for cyst-nematodes

Parameter	Definition
ICROP	Crop type grown
NSP	Number of nematode species
SPNAME	Name of nematode species
CO	Proportion of eggs carried over after non-host crop
CP	Proportion of eggs carried over after susceptible/resistant crop
A	(Eggs laid/female) x (Proportion reaching roots)
E	Equilibrium density of eggs as proportion of logistic equilibrium in absence of other species
EI	Initial population density as proportion of logistic equilibrium
VC1	Effect of competition of species 2 on species 1
R1	Species 1 logistic equilibrium as proportion of the sum of logistic equilibrium for both species
X	Per cent eggs surviving carbamoyloxime type nematicide
C	Compensatory ability of root system of crop to damage
NROT	Number of different crop rotational schemes to be compared
LENSEQ	Length in years of each sequence in each rotational scheme
NUMSEQ	Number of full rotation sequences in each rotational scheme
KROPT	Crop type grown in each year of sequence
NEMUSE	Whether nematicide is used in each year of sequence

i) The effects of different rotations

Oilseed rape is most commonly grown as a break crop between cereal crops and the number of successive cereal crops between the rape crop may vary. Strategies in which a variable number of years in which non-host crops were grown were therefore used.

Rotations were selected in which the most frequent and infrequent cropping of oilseed rape are most likely to occur. The decline of populations in the absence of a host crop over 20 years was also examined. Each rotation was studied at two different initial nematode population densities (EI):

20 years of non-host crop
20 years of 3 years of non-host crop 1 year of oilseed rape
20 years of 4 years of non-host crop 1 year of oilseed rape
20 years of 5 years of non-host crop 1 year of oilseed rape
20 years of 6 years of non-host crop 1 year of oilseed rape

Parameters

	CO	CP	A	E	EI	C
H.cruciferae	0.75	0.30	40.0	0.50	0.50,0.10	1.05
H.schachtii	0.30	0.20	60.0	0.50	0.50,0.10	1.05

ii) The effect of carbamoyloxime nematicide

The effect of a carbamoyloxime nematicide which kills 95% of the hatched juveniles (parameter X = 0.05) was simulated as an application to control a) the first generation b) the second generation c) both generations. In practice, nematicide application would occur as a) an autumn application b) a spring application c) an autumn and spring application respectively. One rotational scheme was used in which oilseed rape was grown in the first year followed by four years of non-host crops. Each strategy was examined at two initial densities (EI = 0.5 and 0.05).

iii) The effect of interspecific competition.

Previous experiment (chapters 2 & 9) have shown that H.schachtii juveniles invade the roots both earlier and in larger numbers than H.cruciferae. For this reason, it is suggested than in concomitant infestations in the field the extensive root damage caused by H.schachtii i.e. the reduction in number of suitable feeding sites in the roots may decrease the amount of subsequent H.cruciferae

development. Heterodera schachtii has a wide host range and additional host crops and/or weed hosts may occur in the rotation with oilseed rape. For this reason, it was assumed that H.schachtii constituted the larger proportion of the mixed population.

The effect of competition between H.cruciferae and H.schachtii was modelled in four situations (a-d). In each, an oilseed rape crop was grown in the first year followed by 4 years of non-host crops. The initial population density (EI) of both species was 0.5 unless stated otherwise.

a) Parameters as for single species populations but with variation in parameter VC.

In this simulation the parameters for H.cruciferae and H.schachtii were as described in (i). Heterodera schachtii had a greater effect on H.cruciferae in the roots than vice versa and competition effects were set at; VC1 = 0.15, 0.50 and 0.75, VC2 = 0.05. The logistic equilibrium of H.cruciferae as a proportion of the sum of the logistic equilibria for both species was set at R1 = 0.25. Therefore, R2 = 0.75.

b) A small amount of H.cruciferae multiplication

The number of H.cruciferae juveniles that invaded the roots, developed to adult females and produced eggs was set at a small value (A=0.05). Such exclusion from development would occur if the root system was extensively damaged by the prior invasion of H.schachtii juveniles. The values of VC1 and VC2 were set at 0.15 and 0.05 respectively.

c) A small initial population density of H.cruciferae

The initial population density of H.cruciferae was set at a small value (EI=0.01). This situation might arise if additional host crops of H.schachtii but not of H.cruciferae (ie sugar beet) were included in the rotation. The model did not allow differential hosts to be included in rotational schemes. The values of VC1 and VC2 were set at 0.15 and 0.05 respectively.

d) Small initial population density and restricted development of H.cruciferae

This simulation combined the parameters of situations b) and c). The values of VC1 and VC2 were set at 0.15 and 0.05 respectively. An additional simulation examined the effect of the same parameters of b) and c) but with the value of VC1=0.75 and VC2=0.05. In this situation H.cruciferae would be expected to be able to achieve negligible multiplication.

RESULTS

The effect of each simulated strategy on each species population is given as the population density at the end of each year to the rotation. The population density is expressed as a proportion of the logistic equilibrium density. If two species are present each population is given as a proportion of the summed joint logistic equilibrium.

i) Different rotational schemes

Figures 43 and 44 show the effect of growing oilseed rape in the last year of a 5 and 7 year rotation of non-host crops. The lower curve in each figure shows the effect of growing non-hosts for the 20 year period. Only the results for EI = 0.05 are shown.

H.cruciferae: The population dynamics of H.cruciferae (Fig. 43) were similar in all the cropping regimes and for each Pi tested. Population densities declined when non-host crops were grown but when oilseed rape was grown Pf values were close to the maximum (ie the equilibrium density). After 10 and 20 years of non-host crops the H.cruciferae population had declined by 94.4% and 99.7% respectively.

H.schachtii: The population dynamics of H.schachtii (Fig. 44) were similar for each Pi tested and in cropping regimes which included oilseed rape more frequently than one year in five years. Populations declined more rapidly than those of H.cruciferae but when oilseed rape was grown H.schachtii achieved larger multiplication rates resulting in Pf values approximately 0.6 of the logistic equilibrium density.

Figure 43 The effect of different rotations on the population dynamics of H. cruciferae over a 20 year period.
(Oilseed rape is grown either after 4 years (1 in 5) and 6 years (1 in 7) of non-hosts or not at all)

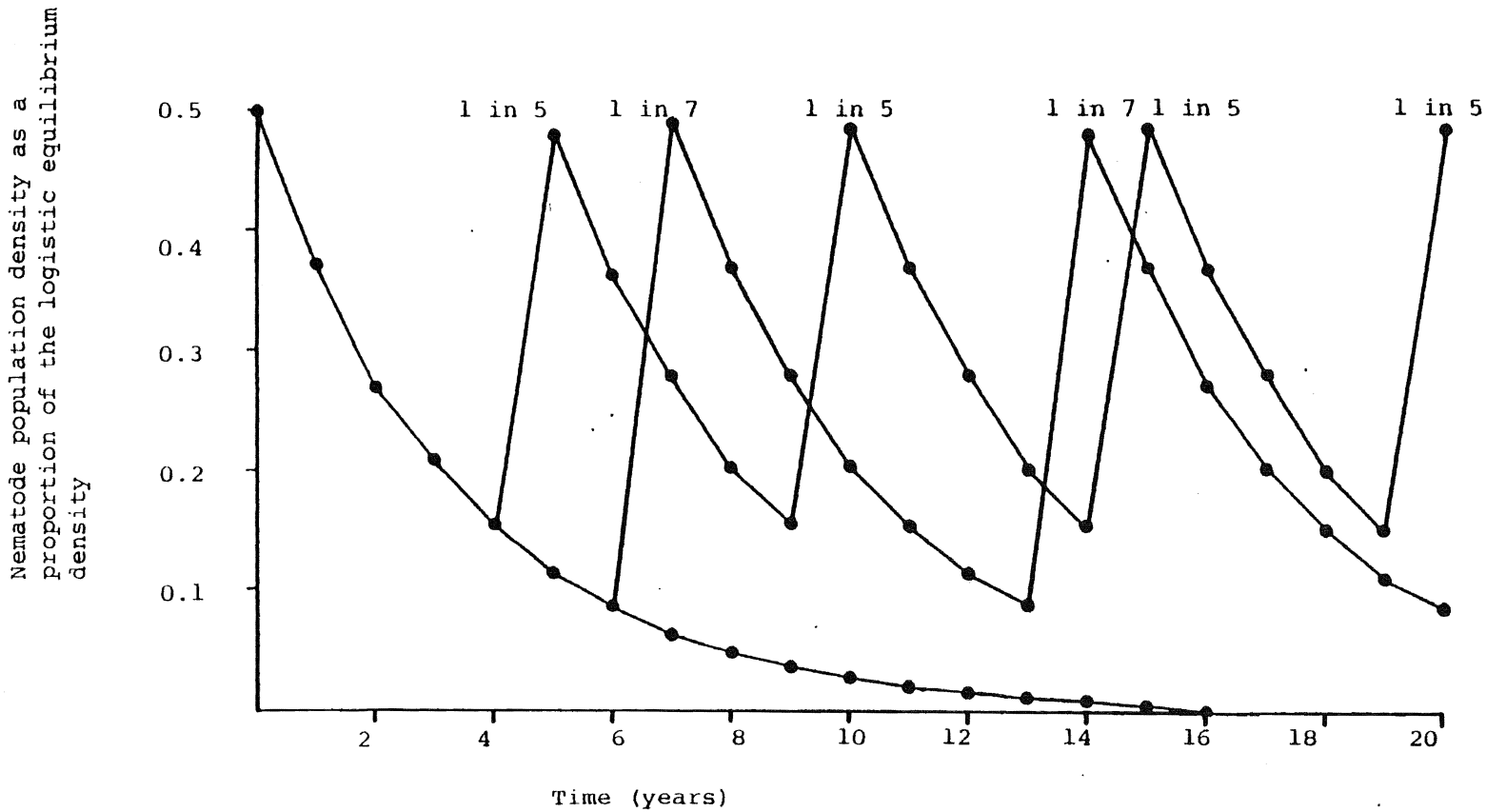
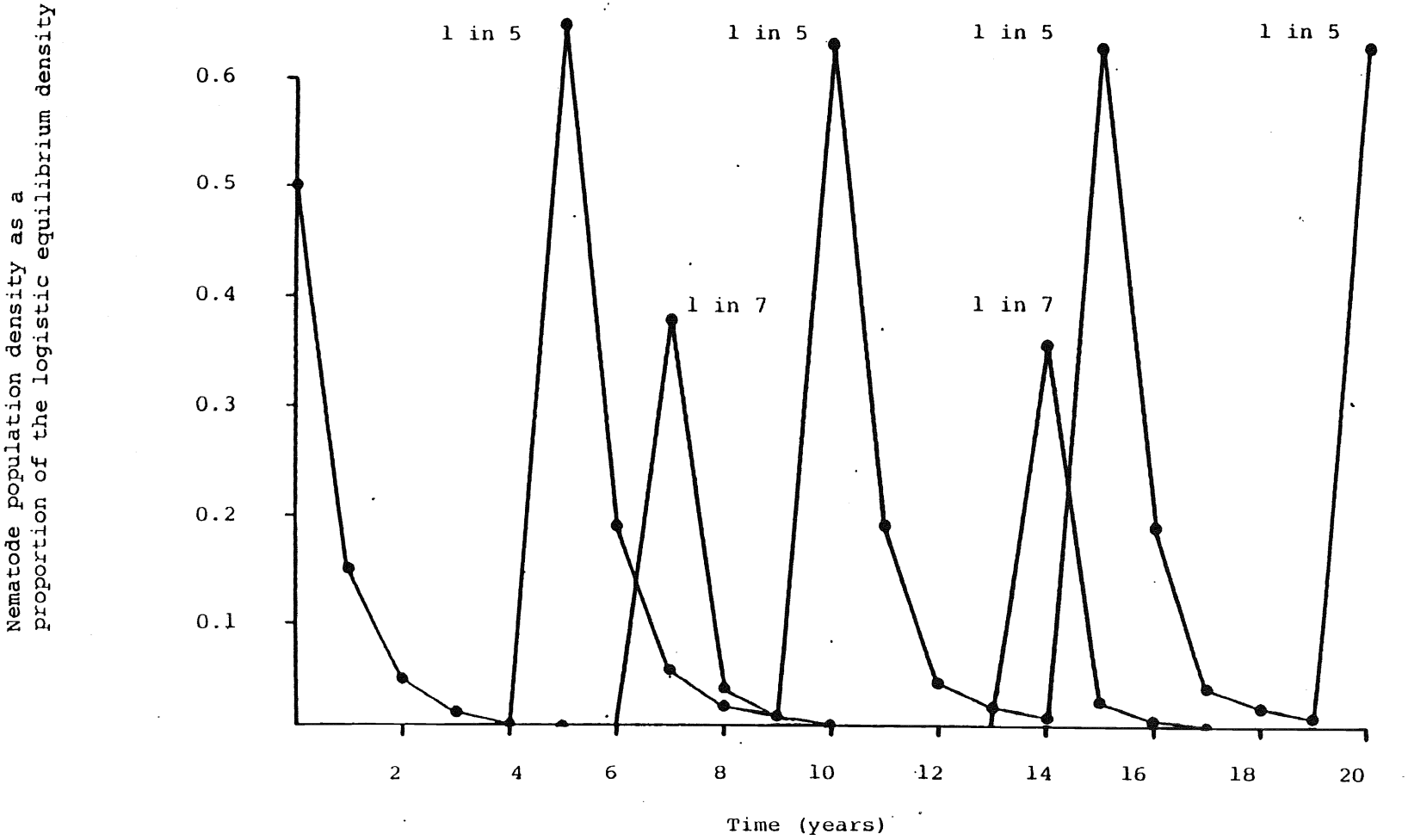


Figure 44 The effect of different rotations on the population dynamics of H.schachtii over a 20 year period (Oilseed rape is grown either after 4 years (1 in 5) and 6 years (1 in 7) of non-hosts or not at all)



When oilseed rape was grown less frequently (1 year in a 7 year rotation) the H.schachtii population increased less on oilseed rape than when it was cropped more frequently resulting in Pf values of between 0.3 and 0.4 x the logistic equilibrium density. After 8 years of non-host crops the H.schachtii population became extinct.

ii) Effect of nematicide

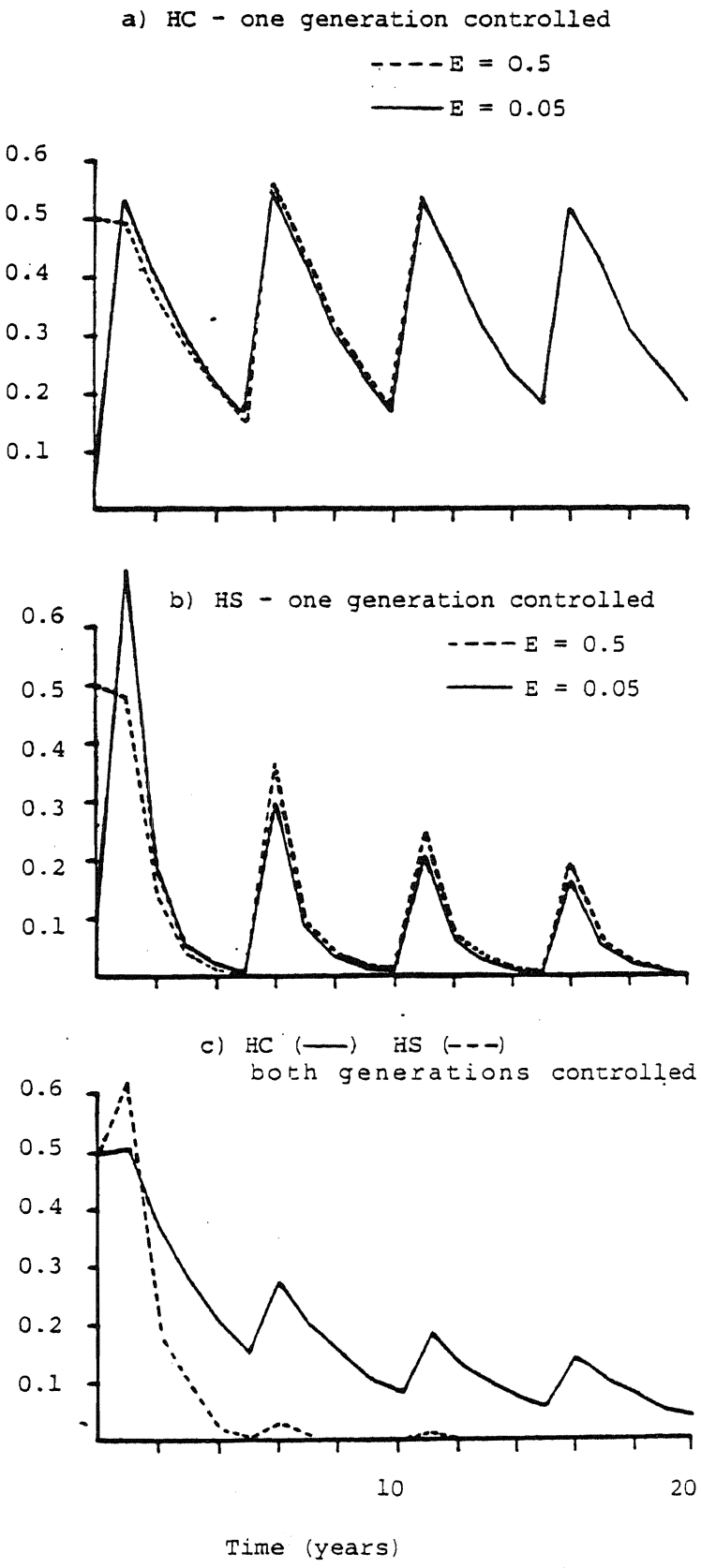
Figures 45a and b show the effect of applying a nematicide to control only one generation of H.cruciferae and H.schachtii during a growing season. Results are shown for each of the Pi's tested.

H.cruciferae (Fig 45a): The nematicide restricted the amount of multiplication that could occur in whichever generation it was targeted at but multiplication was unchecked in the uncontrolled generation. At both Pi's nematode population densities were near the maximum after each oilseed rape crop. Populations increased more than from small initial population densities but overall the difference was slight.

H.schachtii (Fig 45b): On the first oilseed rape crop nematicide applied to control only one generation of H.schachtii had little effect on final nematode densities. The population increased more from the small initial density than from the larger initial density giving Pf's of 0.65 and 0.48 respectively. On subsequent oilseed rape crops, progressively smaller final densities were reached: on the last host crop in the sequence the Pf's for populations with small and large initial densities were 0.19 and 0.17 of the equilibrium density respectively.

Figure 45 c) Shows the effect of applying a nematicide to control both generations during the growing season on a population with a large Pi. Populations of both species achieved little multiplication when both generations were controlled and populations declined over the rotational sequence. The H.schachtii population, which declined more rapidly than the H.cruciferae population, became extinct after 14 years. On the last oilseed rape crop in the sequence the H.cruciferae population multiplied to reach a Pf of 0.14 of the logistic

Figure 45 **The effect of nematicide use on the population dynamics of H. cruciferae (HC) and H. schachtii (HS)**



equilibrium density but the overall trend was to either very small population densities or extinction.

iii) Effect of interspecific competition

The population dynamics of H.cruciferae and H.schachtii in mixed populations on oilseed rape when grown for 1 year after 4 years of non-host crops are shown in Figs 46 a-d.

a) Variable parameter VC

Figure 46a shows the population dynamics of each species when VC1 = 0.50. The proportion of the mixed population density accounted for by each species was stable over the 20 year period with little change in overall population density. When VC 1 = 0.15 (not shown), H.cruciferae achieved more multiplication than at larger values of VC 1 and suppressed the greater multiplication achieved by H.schachtii at larger values of VC 1. When VC 1 = 0.75 H.cruciferae achieved less multiplication than at smaller values of VC 1 but the proportion of the mixed population density accounted for by H.cruciferae remained constant.

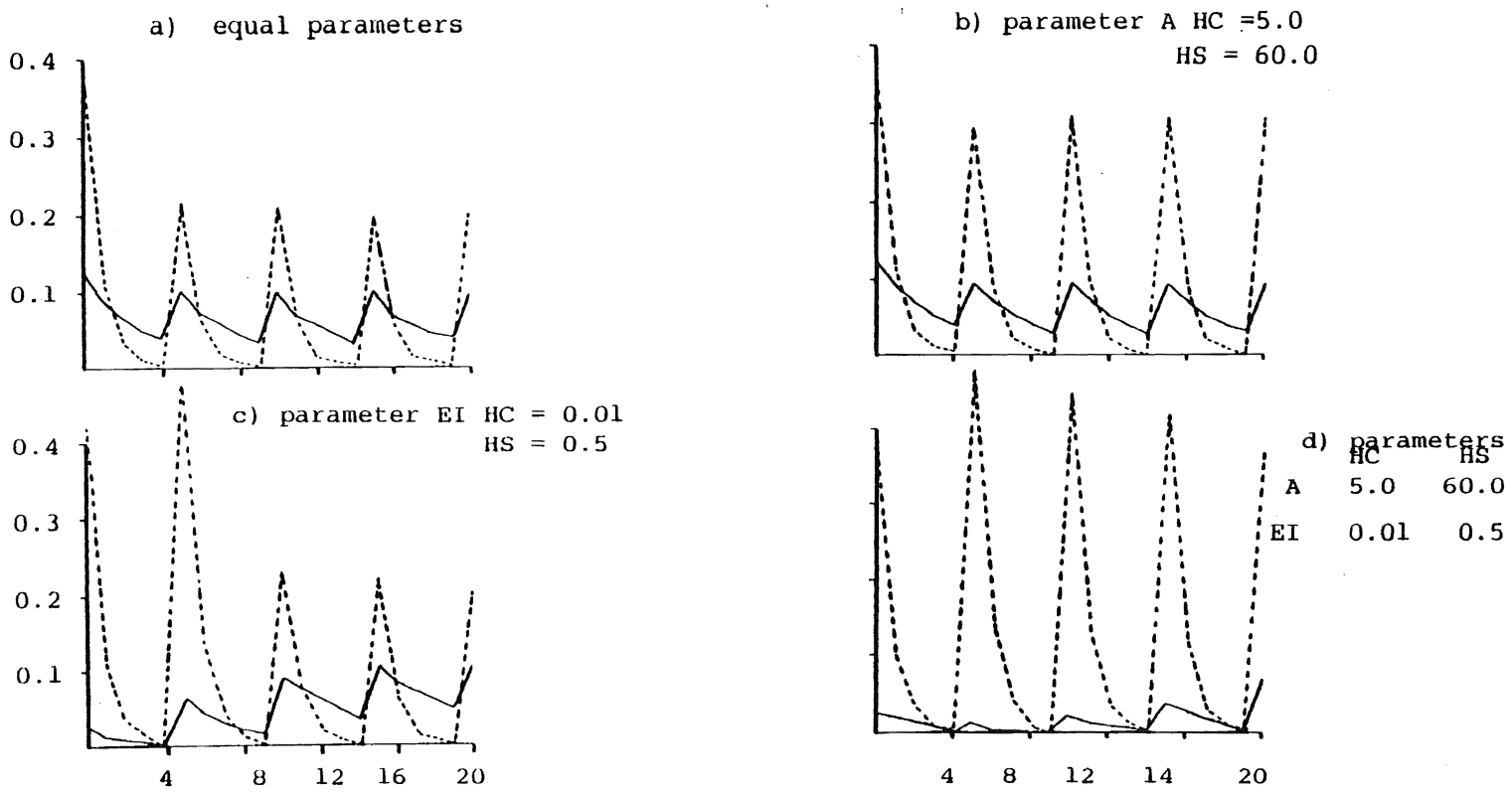
b) Small amount of H.cruciferae multiplication

Figure 46b shows the population dynamics of each species when only a small number of H.cruciferae juveniles were able to invade and develop to females. Multiplication of H.cruciferae was slightly smaller than at larger values of A (Fig. 46a) but the population occupied a constant proportion of the mixed population density. The H.schachtii population achieved greater multiplication than when the value of A for H.cruciferae was larger and occupied a larger proportion of the mixed population density.

c) Small initial population density of H.cruciferae

Figure 46c shows that when the Pi of the H.cruciferae population was small the H.schachtii population was initially able to achieve a much higher multiplication rate but subsequently H.cruciferae achieved

Figure 46 The effect of inter-specific competition between H. cruciferae (HC, ———) and H. schachtii (HS,----) on their population dynamics



progressively higher Pf's when oilseed rape was cropped and thereby progressively suppressed H.schachtii multiplication. On the fourth rape crop in the sequence the H.cruciferae population had a Pf approximately equal to the value of R1: subsequently, therefore, the population dynamics of the two species would follow a similar pattern to that in Fig. 46a.

d) Small initial population density of H.cruciferae and a small amount of H.cruciferae multiplication.

When parameters A and EI for H.cruciferae were set at small values and when VCI was additionally small (0.15), H.cruciferae populations were able initially to achieve little multiplication, so allowing H.schachtii populations to achieve correspondingly greater multiplication (Fig. 46d). The little multiplication achieved by H.cruciferae enabled population densities to increase slowly over the 20 year period, gradually reducing the dominance of H.schachtii. However, when the value of VC 1 was set at 0.75 H.cruciferae became extinct after 7 years. At this time H.schachtii was able to achieve much multiplication and the population increased to give a Pf close to the equilibrium density of this species.

DISCUSSION

Oilseed rape is grown most commonly as a break crop, usually in rotation with cereal crops. Simulating the effect of typical rotations of this kind on populations of H.cruciferae and H.schachtii indicates that oilseed rape is grown frequently enough to maintain populations of both species. In the absence of the host crop, populations of H.cruciferae decline less than those of H.schachtii and so are able to tolerate longer periods of non-hosts. The large decline rate of H.schachtii (in excess of 60% per annum, Jones, 1956) means that populations of this species are more likely to become either underpopulated or extinct under a long run of non-host crops. When oilseed rape was cropped more frequently the final nematode population densities were close to the equilibrium density indicating that considerable multiplication occurs during the growing season. With small nematode densities resulting from several successive years of non-host crops the effects of intraspecific competition and host damage on multiplication will be minimal and, in addition to the

completion of two generations by the nematodes, will contribute to the rapid growth of populations. Data generated by the model for the population growth of Globodera on a potato crop in a four course rotation showed that populations took longer than H.cruciferae and H.schachtii to reach their equilibrium density (Jones & Kempton, 1978). Although the equilibrium density of Globodera may be larger than that of either H.cruciferae or H.schachtii, the slower population growth may reflect the fact that Globodera spp. complete only one full generation within the growing season.

Nematicides that were targeted to control only one generation of H.cruciferae or H.schachtii restricted the amount of multiplication in the treated generation but gave no overall control of H.cruciferae population growth. In combination with the large decline of H.schachtii populations between host crops the nematicidal control of one generation of this species did contribute to the progressive reduction in population growth over the 20 year period. Good control of populations of each species was obtained when both generations were controlled by nematicides. Populations of H.schachtii were controlled more easily than those of H.cruciferae with its population density reduced to negligible levels within 10 years. The decline of H.cruciferae populations using this strategy is slower although after 20 years population growth on oilseed rape is considerably reduced and Pf's would probably not seriously damage any subsequently grown host crop. In practical terms, implementing a strategy which involved the control of both generations would be expensive and nematode development would have to be carefully monitored to determine the most suitable time to apply nematicides to gain the best control.

In competition with H.schachtii the ability of H.cruciferae to persist in larger numbers under non-host crops (than that of H.schachtii) is an important factor influencing the co-existence of the two species. Populations of H.cruciferae became extinct only when the initial density of H.cruciferae was very small and those juveniles which invaded the roots were faced with strong competition from H.schachtii juveniles so that very few H.cruciferae females were able to develop. This would occur when the initial population density of H.schachtii is large causing extensive root damage and so providing few opportunities for the later development of H.cruciferae. When H.cruciferae achieved only a small amount of multiplication, multiplication by H.schachtii

was correspondingly larger as more root space was available for nematode development. As multiplication by H.cruciferae increased H.schachtii multiplication was suppressed, suggesting that intraspecific competition was greater than interspecific as the trend was towards a stable equilibrium (Jones & Kempton, 1978).

The application of the model to the study of the field population dynamics of H.cruciferae and H.schachtii may be criticised in several respects. In the absence of suitable data concerning field populations, parameters derived from pot tests may be inaccurate. In addition, inaccuracies also result from inherent inability of the model to describe the relationship between the initial and final nematode population densities of these species on oilseed rape. The model is designed for species completing only one generation during the growing season. For H.cruciferae and H.schachtii (in order to avoid the problems of deriving a new model) two generations were simulated by using two separate growing seasons to represent the amount of nematode multiplication that would usually occur in one growing season. However, as indicated in previous experiments (Chapter 3,6 and 8) the population characteristics and the host environment are not the same for each generation and the parameters governing nematode development in each generation will differ. The model could be modified in this respect by introducing a separate array of parameters for the second generation and any subsequent generations. This would require much data concerning the population characteristics of each generation but would be ultimately more accurate in describing nematode population growth on oilseed rape.

The ability to introduce differential hosts into the model would also be a desirable modification and would allow other cropping regimes such as those including oilseed rape, sugar beet and other brassica crops to be studied. However, to simulate those rotations would complicate the model as further arrays of parameters would be required for each additional host crop.

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GENERAL CONCLUSIONS

The status of H. cruciferae as a plant parasite in Britain is usually regarded as that of only a locally important pest of brassicas such as cabbage and cauliflower. Heterodera schachtii is a regionally important pest of sugar beet and an occasional pest of brassicas. The results obtained throughout the present study confirm that oilseed rape, which is now grown extensively in Britain, is a good host of both H.cruciferae and H.schachtii. When oilseed rape is grown in pots under conditions favourable to nematode development, populations of H.cruciferae and H.schachtii can increase 6-11 and 11-48 fold respectively. As with other cyst-nematodes, multiplication is density dependent being smaller at larger initial nematode population densities. Farmers can therefore expect a potentially large increase in nematode population density if land infested with either of these species is cropped with oilseed rape. Additionally the risk posed by H.cruciferae and H.schachtii to other host crops grown in the same rotation will be increased and therefore may require the introduction of either rotational or other control strategies.

Winter oilseed rape has a long growing season (September-August) and once juveniles have invaded the roots, their subsequent development is strongly influenced by soil temperature. If juveniles invade the roots shortly after sowing their development may proceed to adulthood before soil temperatures decline over winter. Development will proceed slowly between November and March but will again accelerate when soil temperatures begin to increase in spring. The calculation of the number of day degrees required by H.cruciferae and H.schachtii to complete one generation shows that sufficient day degrees may be accumulated within the oilseed rape growing season for the completion of two generations. This agrees with similar results found by Koshy & Evans (1986) for H.cruciferae on oilseed rape but suggests that H.cruciferae will complete fewer generations than it is thought can occur on other brassica crops (Lewis, 1971; McCann, 1979). Although first generation females may be formed before soil temperatures decline over winter most F1 juveniles probably only mature early in the following spring. The maturation of the second generation will coincide approximately with the end of the growing season. In some years final nematode population densities are unexpectably small (Evans & Spaull, 1986; Evans et al., 1987). This is probably a consequence of a lack of synchrony between the completion of the second generation and harvest so that many nematodes are trapped in either the roots or the soil. Nematodes may be trapped in the roots if

either the harvest is early or nematode development is retarded as a result of cooler than average soil temperatures and/or conditions leading to the delayed invasion of the roots. In warm years when development is advanced or when the harvest is late, many juveniles may have already matured and hatched prior to the harvest and so die either in the soil or in the roots when the crop is harvested soon after. Therefore, final nematode population densities are dependent on the state of nematode development at harvest and therefore difficult to predict.

The multiplication rates of field populations of H.cruciferae (Harris & Winfield, 1986; Evans et al., 1987) do not appear to be as large as in pots. Although the correlation between day degrees and nematode development is independent of temperature, juvenile mobility is dependent on soil temperature and moisture and this will be reflected in the extent of juvenile invasion. Similarly, although host plant development may also be correlated with day degrees the nutrient status of plants developing at a warm temperature regime may be more favourable to female nematode egg production than at a cooler temperature regime. Consequently, in pots where soil temperature and moisture is conducive to greater nematode invasion and female egg production than in the field this will result in correspondingly larger multiplication rates. Additionally, the differences between the root growth of field and pot grown plants may influence the amount of multiplication which occurs. Pot grown plants ensure a large fibrous root density per pot, providing many opportunities for nematode development whilst in the field there may be fewer roots in a similar volume of soil available to the nematodes.

The life-history of H.cruciferae and H.schachtii on oilseed rape is similar to that on other brassica hosts and to that of other cyst-nematode species (Jones, 1950; Lewis, 1971; McCann, 1979). However, the population dynamics of species such as H.cruciferae and H.schachtii which complete more than one generation per crop has received little attention in the past and features of their life-cycle such as the production of egg sacs have often been completely disregarded. The results of the present study show that egg sacs produced by females of H.cruciferae and H.schachtii play an important role in their population dynamics. Hitherto, there has been a paucity of information concerning the functional role of egg sacs and previous

proposals that they provide a source of consecutive progeny (Greco, 1981; Koshy & Evans, 1986) have not been entirely substantiated. These proposals are primarily based on the results of hatching tests that show that a large number of juveniles may hatch readily from egg sacs. Present results show that H.cruciferae and H.schachtii deposit a large proportion of eggs into egg sacs from which many juveniles readily hatch, resulting in the rapid invasion of the roots. Few juveniles hatch and invade from eggs retained within the newly formed cysts. The development of eggs in egg sacs is slightly more advanced than that of eggs in cysts as the eggs that are deposited in sacs are probably those first produced. However, the large difference in the hatching response of cysts and egg sacs is a result of a difference in egg physiology as manifest in the water content of juveniles. Factors that initiate these differences and which probably act through effects on egg shell permeability are unknown and warrant further investigation. The results of hatching tests in which newly produced cysts of H.cruciferae and H.schachtii are exposed to root leachate show that eggs retained within the cyst remain dormant for several weeks before much hatching occurs. Similar results have been reported for H.cruciferae and other cyst-nematode species (Greco, 1981; Beane & Perry, 1984; Koshy & Evans, 1986). This probably represents a mechanism through which cyst-bound eggs are retained for longer term persistence whereas eggs in egg sacs are only short-lived. Therefore, the production and function of egg sacs by H.cruciferae and H.schachtii is a strategically important feature of their population dynamics on a crop such as oilseed rape making sure nematodes are available for continuous multiplication throughout the growing season. As many of the juveniles retained in the cyst-bound eggs may remain dormant this strategy optimises both population growth and survival.

As both H.cruciferae and H.schachtii are capable of parasitising oilseed rape a comparison can be made of their population characteristics:

1) In hatching tests in which field cysts are placed in leached oilseed rape diffusate H.cruciferae hatches much less readily than H.schachtii. In water many H.schachtii juveniles hatch whilst the hatch of H.cruciferae is negligible. When a large hatching stimulus is required by cyst-nematodes for hatching to occur Winslow (1955) argues that it is a logical outcome of

specialization in a species which has become adapted to few hosts. The host range of H.cruciferae is narrow and seems to be largely restricted to crucifers with larger multiplication rates recorded on brassicas than other genera. The ability of H.schachtii to utilize a wide range of hosts has been previously established (Winslow, 1954; Steele, 1965). Hatching is thus much less dependant on a chemical stimulus.

2) In pot tests, where plants are inoculated with juveniles H.schachtii invades the roots both earlier and in larger numbers than does H.cruciferae. Invasion by H.schachtii is concentrated over a short period whilst the invasion by H.cruciferae is prolonged. This earlier and larger invasion by H.schachtii contributes to a shorter generation time and to the development of more females in the roots than for H.cruciferae. Females of H.schachtii are more fecund than those of H.cruciferae (perhaps reflecting differences in the feeding efficiencies of developing nematodes). Heterodera schachtii is more damaging than H.cruciferae to oilseed rape at small initial nematode population densities and this is attributed to the differences in the invasion rates of the two species. At 20°C the multiplication rate of H.schachtii is 7x greater than at 15°C whilst at 20°C the multiplication rate for H.cruciferae is only 3x that at 15°C. Conversely, the results presented here suggest that H.cruciferae is able to develop more readily than H.schachtii at cooler temperatures, indicating that the temperature ranges for the development of the two species are different.

3) The decline of different H.cruciferae populations in the absence of a host crop has been shown to be variable and to be initially faster in the spring following the harvest of the host crop than at other times. In an unplanted microplot a H.cruciferae population declines by 34% and 14% in successive years after the harvest of an oilseed rape crop. In contrast populations of H.schachtii may decline by as much as 60% per annum (Jones, 1956) probably a result of a large water hatch in the spring.

A comparison of the population characteristics of the two species on oilseed rape illustrates their different strategies: that of H.cruciferae is to conserve juveniles until a host crop is grown,

traded against a limited potential for multiplication; the contrasting strategy of H.schachtii enables it to multiply rapidly in large numbers on a wide range of hosts balanced by an inability to persist for long periods in the absence of a host. Modelling the population dynamics of H.cruciferae and H.schachtii in typical rotations in which oilseed rape is included as a break crop shows that the strategy of H.cruciferae is most suitable to the infrequent cropping of oilseed rape and that its populations are relatively stable running fewer risks of becoming underpopulated if there is a long run of cereals between the oilseed rape crops. Underpopulation is likely to occur at very small nematode population densities when males and females are so scattered that a large proportion fail to meet and the multiplication rate is reduced. Populations of H.schachtii (which decline rapidly in the absence of a host) are less stable in a rotation where oilseed rape is grown infrequently as the only host crop and are more sensitive to underpopulation. Populations of H.schachtii are also more amenable to control by nematicide in crop rotations at small population densities.

Heterodera cruciferae may significantly reduce the yield of oilseed rape in the field (Evans, 1984; Harris & Winfield, 1985) but little is known of the relationship between nematode numbers and yield. In pot tests, nematode population densities of 5.0 eggs g^{-1} soil of either H.cruciferae or H.schachtii reduced the weight of oilseed rape plants with a continual reduction in weight up to 50.0 eggs g^{-1} soil at which densities total plant weight was reduced by 50%. Harris & Winfield (1985) reported significant damage to a crop of oilseed rape in soil with a pre-planting H.cruciferae population density of 6.9 eggs g^{-1} soil and similar effects of H.cruciferae and H.schachtii have been reported on other brassica crops (Sykes & Winfield, 1966; Abawi & Mai, 1980; McCann, 1981). However, Evans & Spaull (1986) found that in a recent field trial with oilseed rape on H.cruciferae infested land there was no effect on yield despite an initial population density of 85.0 eggs g^{-1} soil. The relationship between nematode numbers and oilseed rape yield appears therefore to be complex and unpredictable. Extensive nematode damage is probably only likely to occur when many juveniles are able to invade very young poorly established seedlings shortly after sowing. In the pot tests the moist soil conditions would have encouraged much nematode invasion at this stage of growth whereas in September in the field, soils are often dry, impeding the hatching and migration of the invading juveniles. Therefore in the field, young

plants may be able to establish their root systems before many juveniles invade and thereby become more tolerant of subsequent nematode infestation. Results from this study have shown that when oilseed rape plants were grown in soil free from H.cruciferae for 28 days before inoculation few showed symptoms of nematode damage indicating the importance of good plant establishment prior to a challenge by nematodes. This is further reflected in the good yield response of oilseed rape to nematicides in soil infested with H.cruciferae: nematode invasion of the roots is delayed as well as reduced (Evans, 1984; Harris & Winfield, 1985).

The primary effect of H.cruciferae and H.schachtii on oilseed rape is to reduce plant growth. Damage by nematodes will be initiated when invading juveniles destroy root cells and inhibit root growth. Infested plants have smaller root systems that exploit a smaller volume of soil than those of uninfested plants which will decrease the rate of uptake of minerals and water. Lewis (1971) and Harris & Winfield (1985) have attributed conditions (such as leaf discolouration) which are symptomatic of mineral deficiency to be associated with H.cruciferae infestations of oilseed rape and other brassica crops in the field. In pot tests, damage by H.cruciferae and H.schachtii to oilseed rape also reduces the shoot weight and leaf-area of plants. Trudgill (1986) points out that this will decrease the proportion of solar radiation intercepted by the leaves and hence total assimilation. Present results have shown that infested oilseed plants use water less efficiently than uninfested plants as demonstrated by the accumulation of larger amounts of calcium in the shoots of plants grown in infested soil compared to those grown in uninfested soil. These results are similar to those obtained with potato grown in soil infested with Globodera spp (Evans, 1982b). McCann (1979) considered the decreased efficiency of water usage by cabbage plants infested with H.cruciferae to be the main factor responsible for reducing the growth rate of infested plants. Decreased water use efficiency by nematode infested plants may cause oilseed rape plants with small root systems to become water stressed in September when soils are often dry. Poor plant growth at this time will reduce the extent of plant establishment which occurs before plant growth rates decline with the cooler winter soil temperatures and will prejudice the overwintering ability of infested plants.

The ontogeny of oilseed rape involves distinct morphological growth stages, each of which is probably accompanied by physiological changes within the roots. Results presented in this study have shown that these changes may influence nematode development, which continues for the duration of the growing season. Root leachates collected from different aged plants vary in their hatching activity with a marked decrease in activity at the time of flowering and fibrous root senescence. The proportion of woody and tap roots in the root system increases throughout the growing season which decreases the proportion of root available for nematode development. Therefore, development by second generation juveniles occurs in a changing host environment from that of the first generation and the extent of this development is influenced considerably by the maturity of the host. This is reflected in the extent of H.cruciferae multiplication that occurred on different cultivars of oilseed rape with different maturation rates when multiplication was assessed at 50 and 85 days. On all the cultivars except Midas and Westar the number of eggs produced per pot increased between 50 and 85 days whilst on Midas and Westar the number of eggs produced per pot decreased. Midas and Westar matured rapidly and were in the final stages of plant development by 85 days, whilst all other cultivars were still at early growth stages. Therefore, during the final stages of plant growth, the roots provide a less favourable environment for nematode development. As well as a reduction in the hatching stimulus that comes from the roots, juvenile invasion will be made difficult by the maturity of the roots. Additionally, fewer nutrients may be available to nematodes developing in the roots during flowering, seed production and subsequent plant senescence and this will reduce the extent of nematode development.

Whilst no cultivar or line showed resistance to H.cruciferae and H.schachtii there are difference between them in both the amount of multiplication that occurred and in their tolerance of nematode infestation. Differences in nematode multiplication may be the result of two component effects, both of which are inevitably related to rate of maturation of a particular cultivar or line:

1) The effect of differences in the internal root morphology and physiology of cultivars as demonstrated by the number of females and eggs per gram of root. These factors would influence the

ability of nematodes to initiate feeding and their subsequent feeding efficiency in the roots.

2) The effect of differences in the grass root morphology (principally root size) of cultivars on nematode multiplication. These factors would influence the amount of root available for nematode development. In the absence of complete resistance it may be desirable to distinguish between the two effects in order to select cultivars which demonstrate partial resistance to nematode development. However, in practice it is often difficult to distinguish between the two. At the outset of the project Jet Neuf was the most commonly grown cultivar but Bienvenu has since occupied a greater acreage. Mikado and Ariana (a double low cultivar) are likely to be grown more extensively over the next few years. In pots, Mikado is a consistently good host of both H.cruciferae and H.schachtii with more eggs g^{-1} root and eggs g^{-1} soil produced than on other cultivars. Jet Neuf produces more eggs per pot than Bienvenu (a consistently poor host in pot tests) and this is supported by the results of field trials in which Jet Neuf has been shown to increase H.cruciferae population density more than Bienvenu (Evans & Spaull, 1986). Bienvenu develops an extensive root system and is more tolerant of nematodes perhaps indicating that this cultivar may be more suitable to grow on infested land. However, the results of a recent field trial suggest that Bienvenu may increase H.cruciferae populations as much as Mikado (Evans et al., 1987). Although Bienvenu may produce fewer eggs in pot tests the extensive root system may encourage overall more multiplication in the field. No difference was found between currently available single and double low cultivars but as double low cultivars are more desirable and will be grown more widely in the future new cultivars warrant screening against H.cruciferae and H.schachtii as they become available.

The distribution of mixed populations of H.cruciferae and H.schachtii in Britain is unknown but they may occur frequently in areas which have a history of both brassica and sugar beet cultivation. In pot tests, H.cruciferae and H.schachtii interact synergistically as co-inhabitants of oilseed rape to produce larger final nematode population densities than single species population. In concomitant infestations the different invasion rates of the two species reduce

both the intensity of competition between juveniles for root space and the extent of nematode root damage. As a result more females and eggs are produced in mixed populations. The synergistic effect is greater at large initial nematode population densities when the level of competition between juveniles and root damage exert more influence on nematode multiplication. Interaction between H.cruciferae and H.schachtii in concomitant infestations of oilseed rape also results in a greater proportion of F1 juveniles remaining unhatched in mixed populations than in single species population. This is an interesting and unexpected result and has two possible explanations:

1) Interaction between the two species results in delay in the development of nematodes.

2) Cross-fertilisation between H.cruciferae and H.schachtii occurs to produce hybrid juveniles which are either slow in maturing or non-viable.

Modelling the population dynamics of mixed populations shows that when oilseed rape is grown infrequently both species will usually co-exist, each occupying a stable proportion of the mixed equilibrium density. However, the stability of the co-existence is influenced by the relative initial population densities of each species. If the initial population density of H.cruciferae is small and that of H.schachtii is large (as it would be after cropping with sugar beet) H.cruciferae achieves less multiplication in the face of strong competition with H.schachtii and the extensive root damage that would occur at large initial population densities. At such times H.cruciferae populations will be more susceptible to underpopulation and more amenable to controlling by nematicides than H.schachtii populations.

Future cyst-nematode problems on oilseed rape

The effect of the recent widespread cultivation of oilseed rape on the host status of H.cruciferae and H.schachtii in Britain will not be known for some time. Evans & Spaull (1984) point out that the likelihood of significant yield loss occurring in the future depends on whether the crop is grown in areas where those nematodes are already established as field pests. In areas where oilseed rape is the

only host crop grown in the rotation on infested land, nematode population densities will decline to generally safe levels during intervening years of non-host crops. However, nematode damage to oilseed rape is unpredictable and is probably dictated as much by the soil conditions in the short period after sowing when plants are most susceptible to damage as by the pre-planting nematode population density. Therefore, the risk of damage may be large even at small initial population densities in years when soil conditions allow much nematode invasion of young, poorly established plants.

The use of oilseed rape only as a break crop exerts a rotational control on nematode growth although this alone will not prevent the spread of H.cruciferae and H.schachtii to clean fields (Mai & Abawi, 1980). The prediction of post-harvest nematode population densities is complicated by the effect of a complex interaction of soil climatic conditions, cultivar type and agronomic practice. These factors may interact in different ways in any one year to give different final nematode population densities. If oilseed rape is cropped more intensively in the future on infested land the likelihood of yield losses due to H.cruciferae and H.schachtii will undoubtedly be increased. At such times it will be important for advisers to forsee nematode problems and devise suitable control strategies. However, as the population dynamics of H.cruciferae and H.schachtii on oilseed rape have been shown to be complex this may prove difficult.

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