Session 2
Biological Control

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INTERCROPPING AND BIOLOGICAL CONTROL OF PESTS AND WEEDS.

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ABSTRACT

The potential and limitations of intercropping and biological control of pests and weeds are discussed. In perhaps most intercropping systems biological control is one of the effects which are responsible for the observed suppression of pest populations. Intercropping also has a direct controlling effect on weed populations in the field. The relationship between weed suppression and intercrop competition is an essential element in the economic viability of intercropping based production systems. Some technical, economical and psychological constraints of intercropping are discussed.

INTRODUCTION

Due to increasing human populations and related environmental problems, discussions on how to achieve sustainable development in socio-economic, industrial and agricultural aspects of society are taking place. The precarious prospects for food production for a growing world population elicits opposing arguments on the correct approach. There are experts who argue that all scientific, technical and chemical means at our disposal should be directed primarily to higher food production to meet the expected demand. According to others this would mean playing "va banque" and would end in disaster because of the eventual destruction of once productive agricultural land. They advocate the development of agricultural production methods which keep the agroecosystems concerned in such a condition that productivity is at least stabilised, if not increased, on a long term basis. Such development would safeguard future, long term production capacity and healthy, good quality products. Sustainable production systems are increasingly demanded by the market and consumers.

Integrated crop protection is gradually being developed to minimize the inputs of pesticides in the production of agricultural commodities. Elements of integrated control such as biological control, microbial control, breeding for resistance, modifying the cropping system have to be combined in managing the crop-pest(s) combinations, and the technical and economical possibilities.

INTERCROPPING

Intercropping is: "the cultivation of two or more species of crop in such a way that they interact agronomically" (Vandermeer, 1989). During the last decade numerous studies have been carried out to evaluate intercropping effects in terms of yield and pest control (Vandermeer, 1989; Andow, 1991). Attention has been focussed on field vegetable crops (Andow et al. 1986; Theunissen, 1994; Theunissen et al., 1995).

A general effect of intercropping in vegetables is suppression of the population of most insect pests. This has been found in cabbage crops (e.g. Ryan et al., 1980; Kenny and Chapman, 1988; Kloen & Altieri, 1990; Hofsvang, 1991; Theunissen & Schelling, 1992), in carrots and onions (Uvah & Coaker, 1984), in fennel (Theunissen, 1994b), in leek (Theunissen & Schelling, 1993), and in field beans (van Rheenen et al., 1981; Tingey & Lamont, 1988).

One of the effects of intercropping is the mutual interference between the plant species concerned. This is expressed as competition between main crop and the undersown intercrop, or between mixed crops, and may involve a degree of weed suppression. We found a considerable degree of weed suppression by taller clovers such as *Trifolium repens* cultivars, used as an undersown intercrop. Lower growing clovers such as many cultivars of *Trifolium subterraneum accordingly* showed less weed suppression. In a sense the total or partial control of weeds by an intercrop may be considered as a form of biological weed control. Competition with the main crop and weed suppressing ability seem to be parallel processes in an intercropping situation. Both will have to be considered in the technical and economical evaluation of the utility of intercropping as a pest management principle.

Loss of harvestable yield and acceptable weed control management are the main technical constraints for practical implementation of intercropping. We found that mowing intercrops between rows of leek minimized the inter-crop competition. This resulted in the same plant size and weight of leek undersown with *T. subterraneum* when compared to a monocrop. An additional effect was the timely removal of most weeds before they seeded. This contributed greatly to a check on weed populations in an ecologically acceptable way. Yield analysis at harvest showed for white cabbage and leek that, in terms of financial results, the total harvestable yield is less important than the marketable yield. In fresh vegetables quality is important and marketable weight determines the economic cropping result. In comparisons between unsprayed monocrops and intercrops, the quality distribution of the harvested plants from intercrops showed a significant shift to the best quality classes in white cabbage (Theunissen *et al.*, 1995) and leek (Theunissen and Schelling, unpublished data) (fig.1). Translated into monetary values this means a better revenue per hectare by application of intercropping. Detailed balances of inputs/output must show the economic consistency of intercropping based cropping systems.

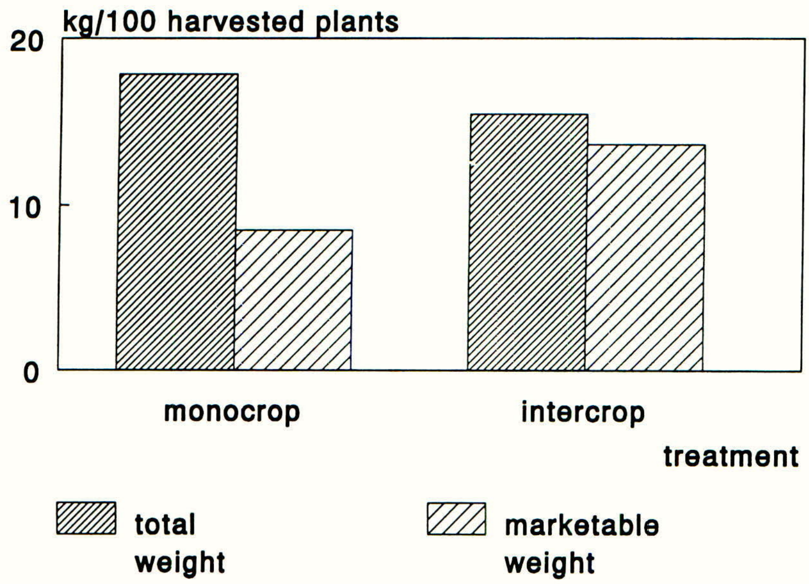

Fig.1. Yield data from an experiment in leek using *T. subterraneum* cv. Geraldton as an undersown intercrop. The total harvestable weight of market trimmed leek plants was larger in the unsprayed monocrop, but the saleable, marketable yield was higher in the intercrop owing to better quality.

BIOLOGICAL CONTROL

Our original reason to try undersowing in vegetable crops was an attempt to create a habitat for natural enemies in intensively cropped field vegetables. In this way we hoped to enhance the chances of natural biological control of pests in these crops. In monocrops, biological control was found to be always too little and too late, partly due to climatic conditions and the biological uniformity in intensive commercial cropping. In a landscape with a very scarce natural vegetation (host plants), and regularly sprayed with pesticides, natural enemies had little chance to survive in large numbers. Field trials with Brussels sprouts, being host plants for many pest species, intercropped with *Spergula arvensis*, showed increased parasitation of cabbage aphids (*Brevicoryne brassicae*) (Theunissen and den Ouden, 1980). Prior and subsequent research on intercropping by other workers and our group showed that stimulation of biological control is one of a number of possible mechanisms which lead to pest population suppressing effects (fig.2).

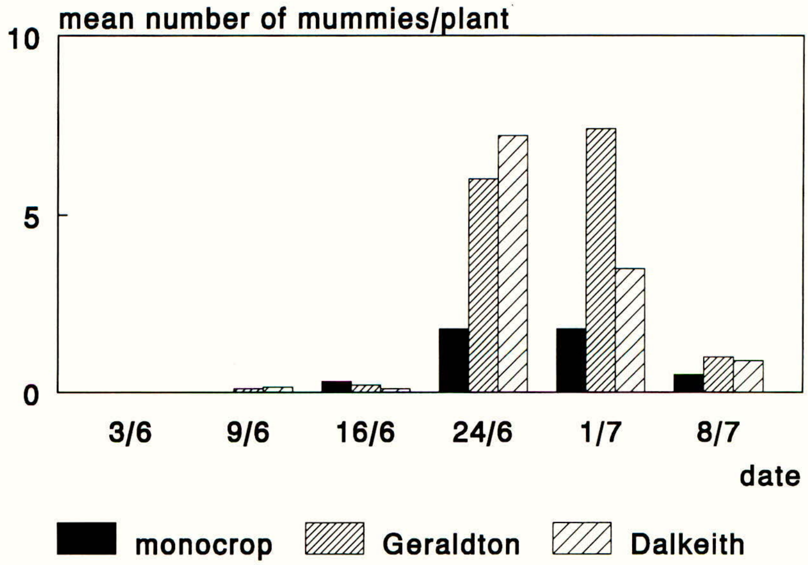

Fig.2. Parasitation of aphids in iceberg lettuce in monocrop and *T. subterraneum* undersown treatments. During the cropping period the numbers of aphid mummies were counted in plants sampled in the treatments. No pesticide applications were used. The weights of harvested plants did not differ between the intercropped and monocropped treatments.

The "enemies hypothesis" (Root, 1973; Risch, 1981) directly refers to biological control as a major intercropping effect. Direct evidence of increased levels of predators was found in clover undersown cabbage (Theunissen *et al.* 1995) and in relay intercropping in cantaloupe (Bugg *et al.*, 1991). While diversification of a cropping ecosystem might stimulate the presence and survival of natural enemies, it is not an automatic process that they indeed interfere with the pests of the main crop. For instance, no effect of natural enemies on *Thrips tabaci* populations was found in intercropped leek, despite drastic reduction of thrips populations (Theunissen, unpublished data). Immigrating pests may react on contrasts between (Smith, 1976) or spectral reflectance intensity (Costello, 1995) of the crop and its background. Caterpillars become more easily infected with virus diseases in the changed microclimate of the crop canopy in intercropped fields. Migrating catepillars of *Mamestra brassicae* get lost in the intercrop and starve, thus causing an extra 50% mortality of these caterpillars in a young cabbage crop where plants do not yet touch (van de Fliert, unpubl. data). Leek plants change in host plant quality for thrips when undersown with clover. These are a few examples of mechanisms apart from direct biological control which can have similar effects on the pest populations concerned.

IMPLEMENTATION

Polycultures are very common all over the tropics and are used for a variety of reasons (Vandermeer, 1989). However, in the intensive temperate regions, cropping practises including mixed cropping is considered economically inefficient, given the ample availability of cheap pesticides to counter the disadvantages of growing monocrops. The costs of human labour and the mechanization of cropping in many countries are also incentives to monocropping. The situation changes drastically when the pesticides become ineffective or the consumer starts to worry about the safety of eating fresh products, or the way in which these products have been grown.

The magnitude of the problems with *Thrips tabaci* in leek crops all over western Europe are a classical example of results obtained by a cropping system which relies totally on the use of pesticides. The quickly decreasing sensitivity of thrips for most, if not all, accepted insecticides resulted in uncontrollable populations, in spite of numerous and still increasing applications. Additional causes may be an increasing area of leek cropping, continuous cropping during the entire year and a very narrow crop rotation scheme. The outcome is a low quality vegetable, producable only with excessive pesticide use and at a high cost.

Undersowing, mixed cropping and other forms of intercropping do not have a place in the present commercial cropping methods and in the considerations of growers. For that reason alone implementation of intercropping based production methods will be difficult. Apart from these psychological hurdles intercropping must be an economically viable approach. Even when the reduction in the pest population (and sometimes disease) is acknowledged, growers have to accept a change from total yield towards marketable yield as criterion for economic success. Increasing consumers' awareness on food safety and methods of production will stimulate, more than scientific arguments can do, a market demand for healthy and clean food products. Integrated production, that is a combination of methods which each influence the agro-ecosystem to realize economically and ecologically acceptable production methods, may provide solutions.

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DEVELOPING STRATEGIES FOR THE NEMATODE, *PHASMARHABDITIS HERMAPHRODITA,* AS A BIOLOGICAL CONTROL AGENT FOR SLUGS IN INTEGRATED CROP MANAGEMENT SYSTEMS

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ABSTRACT

Slugs are likely to increase in importance as pests in integrated crop management systems. The nematode parasite, *Phasmarhabditis hermaphrodita*, has the necessary attributes to be used as a successful biological control agent against slugs and is already on sale in the UK as a molluscicide for use by domestic gardeners. However, for this nematode to be successfully used in arable agriculture, strategies for using the nematode must be optimised so as to provide protection against slugs at low doses, and protection from slug damage must be reliable under a wide range of environmental conditions at and after the time of application. Research to develop strategies for cost-effective use of this nematode is described. Application of the nematode one or two weeks before drilling a susceptible crop (oilseed rape) appeared to have no advantages over application at the time of drilling. However, shallow incorporation of nematodes applied to dry soil was advantageous in protecting a wheat crop from slug damage. In laboratory tests, slugs avoided feeding and resting on soil treated with nematodes, suggesting that it may be feasible to apply nematodes in bands to protect crops from slug damage.

INTRODUCTION

Slugs are important pests of many agricultural and horticultural crops throughout Europe, North and Central America, Asia and Australasia (South, 1992). In the UK, the most important crops damaged by slugs in economic terms are winter wheat, oilseed rape and maincrop potatoes (Port & Port, 1986). These pests are generally controlled using molluscicides formulated as bait pellets. However, the chemicals available are sometimes ineffective and may adversely affect non-target organisms. Furthermore, many of the features associated with sustainable, integrated farming systems are likely to lead to an increase in slug problems (Glen *et al.,* 1994b). These features include more varied crop rotations, non-inversion tillage, incorporation of crop residues and the use of cover crops to reduce nitrogen leaching.

The bacterial feeding nematode, *Phasmarhabditis hermaphrodita*, is a parasite of slugs which kills many pest species (Wilson *et al.*, 1993a). It was discovered parasitising slugs at IACR-Long Ashton and developed as a biological molluscicide in collaboration with the Agricultural Genetics Company Ltd.. *Phasmarhabditis hermaphrodita* forms a developmentally arrested non-feeding larva (dauer larva) which is the infective stage. Dauer larvae infect slugs by entering their shell sac above the mantle, develop into adults and reproduce. Eventually, the slug dies and the nematodes spread over the slug cadaver reproducing until the food supply is depleted. The juveniles then fail to develop into adults and form new dauer larvae which disperse in order to infect new slugs. *Phasmarhabditis hermaphrodita* can be mass-reared *in vitro* in rich media containing specific bacteria (Wilson *et al.*, 1993b; 1995a). It is currently being produced in fermenters by MicroBio Ltd (a subsidiary of the Agricultural Genetics Company), and is sold for use as a molluscicide to domestic gardeners in the UK (Glen *et al.,* 1994a). The ability of this nematode to protect crops from slug damage has been demonstrated in a series of field experiments in different crops (Wilson *et al.*, 1994a,b; 1995c).

Research on the use of *P. hermaphrodita* as a biological molluscicide is complemented by a much larger volume of research on the use of entomopathogenic nematodes (families Heterorhabditidae and Steinernematidae) as biological insecticides. Much of this work gives insight into the potential problems which have been, or might be encountered, with the use of *P. hermaphrodita* for slug control. Nevertheless, the use of entomopathogenic nematodes is largely restricted to high value horticultural crops and the problems of using these nematodes as biocontrol agents in arable crops have, in the main, still to be addressed. The paper describes recent research as part of a project in the LINK Programme "Technologies for Sustainable Farming Systems", which aims to establish the principles for cost-effective use of *P. hermaphrodita* in arable crops, especially in integrated and less intensive crop management systems. Areas where future strategic research may help the exploitation of *P. hermaphrodita* and related species as biocontrol agents for slugs are highlighted.

NEMATODE STRAINS

All research and development on *P. hermaphrodita* as a biological molluscicide was done using a single strain of the nematode (UK1) isolated at IACR-Long Ashton. It is possible that by collecting new strains of this nematode and the related nematode *P. neopapillosa,* which also parasitises slugs (Wilson *et al.,* 1993a), nematode strains may be found that are more virulent, easier to produce in fermenters or able to withstand a greater range of environmental conditions than the current strain. Development of such strains would make the product more cost-effective. Furthermore, it may be possible to select better strains of bacteria for rearing the nematode. Different species of bacteria can have profound effects on yields of nematodes in culture (Wilson *et al.*, 1995a) and on the ability of nematodes to infect and kill slugs (Wilson *et al.,* 1995b). *Phasmarhabditis hermaprhodita* is mass produced for commercial use in fermenters where it is grown in monoxenic culture with the bacterium, *Moraxella osloensis*. *Moraxella osloensis* was found to be the most suitable bacterium out of 13 bacterial isolates tested. However, it is possible that there may be other bacteria which would produce greater yields of more pathogenic nematodes. This approach to improving the efficacy or *P. hermaphrodita* as a biocontrol agent for slugs has considerable potential for future investigation.

REDUCING APPLICATION RATE

The currently recommended application rate of *P. hermaphrodita* as a molluscicide for garden use is 3 x 10^9 dauer larvae ha^{-1}. This rate was recommended following three field trials, one in mini-plots with Chinese cabbage (Wilson *et al.*, 1994a), one in field plots of winter wheat (Wilson *et al.*, 1994b) and one in protected lettuce (Wilson *et al.*, 1995c). In all these trials, a range of nematode doses were applied evenly to the soil surface of replicated experimental plots at the time of crop planting or sowing and slug damage assessed and numbers recorded (Wilson *et al.*, 1994a,b;1995c). In all three trials 3 x 10^9 nematodes ha^{-1} or a lower dose was found to give protection equivalent to methiocarb pellets (Draza) applied at the recommended rate of 5.5 kg ha^{-1}. The relationship between nematode dose and reduction in slug damage differed between trials. In the Chinese cabbage trial, protection improved with increasing nematode dose between 1 x 10^8 and 8 x 10^8 ha^{-1}, but showed little or no further improvement at higher doses of up to 2 x 10^{10} ha^{-1}. In the winter wheat and lettuce trials, there was a linear increase in plant protection with increasing nematode dose for all doses between 1 x 10^8 and 1 x 10^{10} ha^{-1} (the entire range of doses tested). Even though a dose lower than 3 x 10^9 ha^{-1} provided good protection in the Chinese cabbage trial, it is unlikely that application rates could be much reduced without the risk of compromising efficacy using current application strategies. It is interesting to note that the recommended application rate for *P. hermaphrodita* is similar to that recommended for application of entomopathogenic nematodes to the soil surface. These doses are extremely high, bearing in mind typical host densities (e.g. for slugs usually less than 500 m^{-2}) and the low numbers of nematodes needed to kill slugs. It is therefore possible that research into the fate and behaviour of applied nematodes may permit lower doses than those currently recommended to be used in the future.

TIMING OF APPLICATION.

In two field experiments with Chinese cabbage (Wilson *et al.*, 1994a), weekly or bi-weekly assessments of slug damage were made in each plot. This enabled nematode efficacy to be studied in relation to time after application. In both trials, the protection given by the nematode increased over the first two weeks, before stabilising. In the previously described wheat trial (Wilson *et al.*, 1994b), nematodes were added immediately after the crop was drilled. Since the worst damage done by slugs to wheat is the hollowing of the seeds shortly after sowing, and the worst damage to oilseed rape is done by feeding on the seedlings immediately after emergence, it was hypothesised that *P. hermaphrodita* might perform better if the nematodes were applied one or two weeks before drilling, rather than immediately after.

An experiment in August-September 1992 investigated the effect of different timings of nematode application on slug damage to a crop of autumn-drilled oilseed rape. Individual plots (12 x 12 m) were marked out before oilseed rape was drilled and three replicate plots were either left untreated or treated with methiocarb pellets at the recommended rate immediately after drilling, or treated with *P. hermaphrodita* 2 weeks or 1 week before drilling or immediately after drilling. Nematodes were applied at the recommended field rate (3 x 10^9 ha^{-1}) to the soil surface in 555 l ha^{-1} of water using a knapsack sprayer fitted with a coarse anvil nozzle. Numbers of oilseed rape seedlings were assessed three weeks later

(Table 1).

Nematode application significantly reduced slug feeding and, thus, increased numbers of rape plants established, irrespective of the timing of application. There appeared to be no benefit from application up to 2 wks before drilling compared with application immediately after drilling. In this experiment, the protection given by methiocarb pellets was superior to that given by the nematodes. The moisture content of the top 1 cm of soil at all application dates was below 10 % w/w; it is likely that this led to nematode death through desiccation. It may also have reduced the ability of nematodes to move down through the soil to avoid exposure to ultra violet light.

Table 1. Mean square root numbers of oilseed rape seedlings 0.25 m^{-2} in untreated plots, plots treated with methiocarb immediately after drilling and plots treated with 3 x 10^9 *P. hermaphrodita* ha^{-1}. Soil moisture content in the top 1 cm of soil at the time of nematode application is also shown.

	Untreated	Nematodes applied at drilling	Nematodes 1 wk before drilling	Nematodes 2 wk before drilling	Methiocarb applied at drilling	S.E.D. 24 d.f.
Plants m^{-2}	2.393	3.709	3.31	3.905	4.731	0.3675
% soil moisture	*	7.0	7.1	9.1	*	*

INCORPORATION OF NEMATODES INTO SOIL

It is known that the persistence of entomopathogenic nematodes when applied to the soil surface is poor and this is generally considered to result from mortality induced by desiccation and solar radiation (Gaugler, 1988). Certainly, we have obtained the best results in field trials, in both wheat and oilseed rape, when nematodes have been applied to moist soil. In many cases, application of entomopathogenic nematodes is followed by irrigation; Georgis & Gaugler (1991) recommended application of 1-2 cm of water after nematode application. The volumes of water required for this would preclude the use of this method in arable agriculture in the UK, with the exception of the potato crop. Other attempts to increase survival of soil-applied entomopathogenic nematodes have used machinery to inject the nematodes below the soil surface. While this technique has been successful in Australia (Berg *et al.*, 1987), it has proved less effective in the U.S.A (Klein & Georgis, 1994), and also requires specialised machinery not available to most farmers.

A simple technique we have used to reduce nematode mortality on the soil surface is to incorporate the nematodes into the soil immediately after application. This was done in a field experiment in winter wheat in autumn 1994. The experiment was a split-plot design with plots of 12 x 16 m. One half of each plot was treated with *P. hermaphrodita* at the recommended field rate one day after the plots were drilled and the remaining half was left untreated. Nematodes were applied using a knapsack sprayer in 520 l ha^{-1} of water per plot. Plots were either left uncultivated after nematode application or cultivated using tractor-mounted spring tines immediately after application. The depth of cultivation was adjusted so

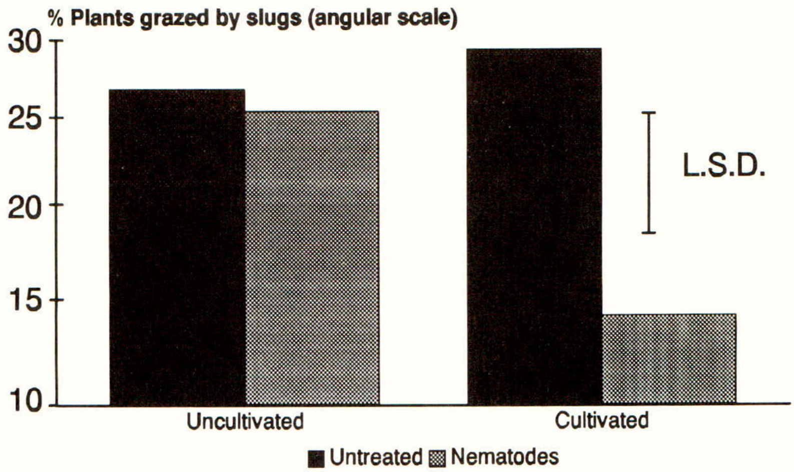

Figure 1 Percent of wheat plants grazed by slugs in untreated sub-plots and sub-plots treated with *P. hermaphrodita* which were cultivated to 2, 5 or 10 cm with spring tines or left uncultivated

that the tines worked the soil to a depth of approximately 2, 5 or 10 cm. Slug damage was assessed when the wheat had reached Zadoks' growth stage 11. There was no evidence of significant differences between the three cultivation depths in their effects on nematode efficacy; therefore, data for all three cultivation depths were combined for the analysis (Fig 1).

The top 1 cm layer of soil contained only 4.9 % (w/w) water at the time nematodes were applied, at approximately mid-day with strong sunlight. Thus, it is not surprising that nematodes left on the soil surface had no significant impact on slug damage. In spite of these harsh conditions, there was a significant, 50% reduction in slug damage on plots that were cultivated after nematode application. This suggests that shallow incorporation of nematodes into soil protected them from desiccation.

PARTIAL TREATMENT OF SOIL

Results from a field trial in wheat in autumn 1991 suggested that slug behaviour was altered in plots where nematodes were applied to soil (Glen *et al.*, 1994a; Wilson *et al.*, 1994b). Laboratory experiments were done to investigate whether slugs can detect the presence of *P. hermaphrodita* in soil and avoid areas of soil treated with the nematode, and whether this response could contribute to a reduction in feeding damage. An experiment was done using five plastic boxes, 26 x 13 x 9 cm high, lined with a 2 cm deep layer of soil aggregates. Half the soil surface of each box was treated with approximately 100 *P. hermaphrodita* larvae cm^{-2} and the remaining half was untreated. Five adult *Deroceras reticulatum* were added to each box. The insides of the boxes were lined with 0.8 mm woven copper mesh, over which slugs do not crawl, thus confining slugs to the soil surface. Ten Chinese cabbage leaf discs, each 3 cm in diameter were placed in each box, five in the nematode-treated half and five in the untreated half, at opposite ends of the box.

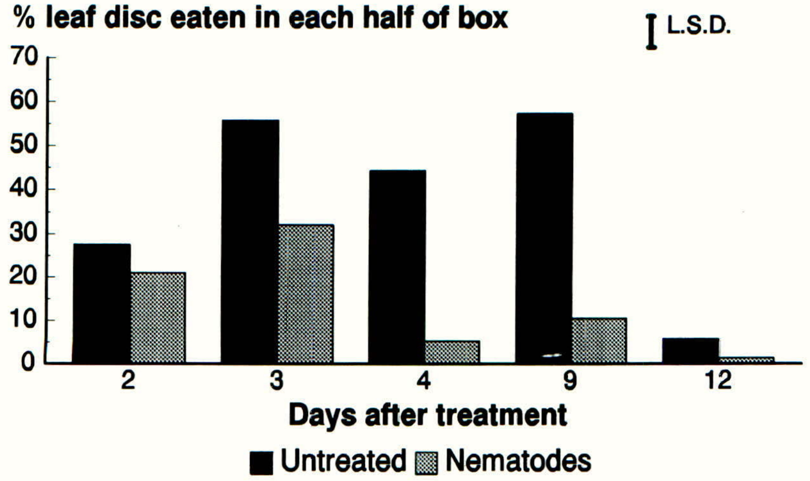

Figure 2. Mean percentage area of Chinese cabbage leaf consumed by slugs in untreated and nematode-treated halves of soil surface in boxes.

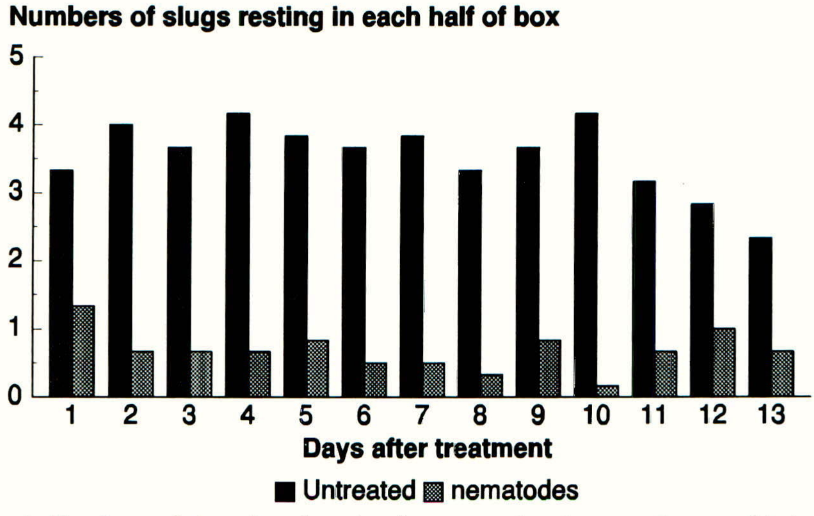

Figure 3. Numbers of slugs found resting in untreated and nematode-treated halves of soil surface in boxes (n=5).

The percentage of each leaf disc eaten by slugs was recorded the next day and the leaf discs were removed. This procedure was repeated at intervals over 12 days (Fig. 2). Every day, numbers of slugs resting on untreated and nematode-treated halves of boxes were recorded (Fig. 3). Slugs consumed significantly more in the untreated half than in the nematode-treated half at all assessments except the first (day 2) and the last (day 12) (Fig. 2), by which time most slugs had stopped feeding as a result of nematode infection. On all days, more slugs were found resting on the untreated than the nematode-treated half. It is hoped that this repellent effect of *P. hermaphrodita* can be exploited, in addition to its effects in killing slugs, to reduce nematode doses by applying the nematodes in narrow bands around susceptible crops. This strategy may be particularly useful in crops grown in distinct rows some distance apart, such as sugar beet and many vegetable crops.

INOCULATIVE RELEASE

Certain cropping systems lead to an increase in slug population size. This is particularly true of oilseed rape, where the dense crop canopy provides conditions ideal for slugs. Winter wheat crops following rape are often severely damaged by slugs. The majority of field trials with *P. hermaphrodita* have used an inundative release of large numbers of nematodes to give a relatively rapid reduction in slug damage to crops. It may be possible to treat oilseed rape crops with a low dose of nematodes in autumn or spring which will stop the slug population building up and, thus, protect a following crop. The damp humid conditions which favour slugs should also favour nematode persistence and activity. However, initial tests of inoculative release have not given encouraging results.

CONCLUSIONS

Phasmarhabditis hermaphrodita is an effective biological molluscicide. It is more selective than available chemical molluscicides and, thus, could form part of integrated crop management systems. There is much scope for strategic and applied research to improve the efficacy of *P. hermaphrodita* and related species as biocontrol agents for slugs, since the genus *Phasmarhabditis* has been studied little. By considering the factors most likely to limit efficacy of *P. hermaphrodita*, and by developing appropriate application techniques, it should be possible to develop cost-effective, reliable strategies for using the nematode in arable crops. Different strategies, which take into account application method, timing, incorporation and selective placement, will probably be needed for the different types of crops damaged by slugs. Research on the biology and ecology of *P. hermaphrodita*, could eventually lead to the ability to manipulate and exploit natural epizootics of this nematode in integrated crop management systems.

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