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Second International Workshop on Vine Weevil (*Otiorhynchus sulcatus Fabr.*) (Coleoptera: Curculionidae)

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and
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ADVANCES IN BIOCONTROL

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Preface

For more than 150 years, indisputable evidence is given by published papers and reports of the problems caused by several species of *Otiorhynchus* (Coleoptera: Curculionidae) in many horticultural crops, in viticulture and, partly also, in forestry. Owing to international spread (particularly of vine weevil, *Otiorhynchus sulcatus*) by infested plant material, and to cultivation techniques which favour the propagation of such weevils in several horticultural crops, we have today to deal with a worldwide problem, which can decide the economic success or failure of many nurseries.

The 1st International Workshop on Vine Weevil took place in 1989 in the United Kingdom. This international meeting of scientists led to an intensive exchange of experience and information on the economic importance, biology and epidemiology of several *Otiorhynchus* species, and the discussions resulted in important ideas for further areas of research and development. Since this Workshop, new biological procedures for the control of vine weevil have been developed to a degree where they can now be used commercially. Owing to its particular research activities, the German Federal Biological Research Centre for Agriculture and Forestry (BBA) has had, and still has, a definitive part to play in the development of procedures for biological pest control, including measures against vine weevil.

Regarding biological control, the application of entomopathogenic nematodes of the family Steinernematidae is of particular importance and is now the leading procedure for biological control of vine weevil worldwide. However, owing to the high production costs, it has not been possible to establish the commercial use of entomopathogenic fungi (such as *Metarhizium anisopliae*), even though they have been registered in several countries. Further antagonists, such as heterorhabditid nematodes, are in the final stages of development, and there is evidence that they might solve specific problems encountered by other biological techniques.

With regard to chemical control, there is evidence that by the use of specific application systems and by considering physiological peculiarities of vine weevil, the number of applications against adults may be reduced to a minimum. Also, new technology involving controlled-release formulations is significantly improving control of vine weevil larvae.

The BBA, in its role as the German registration authority for pesticides, is very much involved with questions of testing and registration of plant protection products against vine weevil. Therefore, I greatly appreciate that the 2nd International Workshop on Vine Weevil is to be held at our traditional research centre, which has now been in existence for almost 100 years. Very special thanks are due to the Deutsche Phytomedizinische Gesellschaft and to the British Crop Protection Council for their generous financial and technical support for this Workshop. I wish the experts involved every success.

Braunschweig, 22.04.1996

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Current status of vine weevil as a pest in the UK

Introduction

During the 1980s and 1990s vine weevil (*Otiorhynchus sulcatus*) has become probably the most serious pest of hardy ornamental nursery stock and soft fruit in the UK. Damage to crops is caused primarily by the larvae which feed on the roots and crowns of host plants and, occasionally, stem-girdle susceptible species; 'notching' of foliage by adult weevils can also be of economic importance.

The behaviour, ecology and control of vine weevil has been studied, and continues to be studied, by several workers in the UK and in the rest of Europe. Much work has been carried out on chemical and biological control, but aspects of behaviour and ecology, particularly of the adult weevils, needs further work to help improve current control methods. A detailed review of the biology and control of vine weevil has been published by Moorhouse (1992).

The pest is confined mainly to temperate areas of Europe. Regions with a Mediterranean-type climate, with hot, dry summers, do not favour it (Evenhuis, 1978). However, the conditions on a nursery producing ornamental nursery stock, with most plants grown in containers, irrigated frequently and often shaded, may allow the pest to flourish in areas that would not normally suit it.

Several factors have contributed to the increase in pest status of the vine weevil in the UK, notably:

a) The continued growth of numbers of hardy ornamental plants grown in containers, using peat or peat/bark based growing media, which are ideal for survival and spread of vine weevil larvae.

b) The withdrawal of aldrin, the persistent organochlorine insecticide, in the mid-1980s.

c) The polyphagous nature of the pest, which can survive on a very wide range of cultivated host plants and weed species.

d) Increase in trade of plants between nurseries providing an ideal method of spread in containerised plants.

e) Difficulties in obtaining control of established infestations in the field and in containers.

f) The climate in the UK, with generally mild winters, has allowed considerable adult weevil survival in most years, leading to earlier oviposition and greater root damage by larvae.

Life cycle in the UK

The UK, with its temperate maritime climate, is ideal for the survival and spread of vine weevil. The life cycle in this country is basically no different from that, say, in continental Europe or the USA, and has been well summarised by Moorhouse (1992), but in these areas the winters are much colder and survival of adults in the field correspondingly less. Blackshaw (1992) showed that oviposition by over-wintering adults extended the total period during which eggs were laid (in some years from May to November) and made a significant contribution to the total numbers of eggs produced. This factor affected the efficacy of insecticide treatments as well as their optimal timing.

Hardy ornamental nursery stock (HONS)

Most HONS in the UK are grown in containers, using peat-based or peat/bark-based composts. Work by several authors, including Nielson & Boggs (1985), Blackshaw (1993) and Buxton (1995), have shown that such media are ideal for development and survival of vine weevil larvae. The organic nature of the substrate and high degree of air-filled porosity (normally about 10-14% with HONS subjects) reduces waterlogging and encourages the development of fine, fibrous roots, upon which the larvae feed.

As well as ericaceous plants (such as rhododendrons, azaleas and camellias) vine weevil has also become a serious problem on herbaceous plants such as *Astilbe, Bergenia, Heuchera* and *Sedum*. These plants are very susceptible to weevil damage. Practical experience has shown that subjects such as evergreen azaleas can be killed by just one larva, which girdles the stem at or just above compost level. Standards of control required by the HONS industry are, therefore, extremely high.

The industry relied on incorporation of aldrin 1% dust for control, until the product was withdrawn in 1986 for environmental reasons. Aldrin was inexpensive and very effective, and adequately contained the weevil problem. Once it was withdrawn, however, the weevil population increased, and spread rapidly from nursery to nursery in infested containers.Since the mid-1980s, ADAS entomologists have carried out trials to develop insecticides to replace

aldrin (Buxton *et al.*, 1992; Cross *et al.*, 1996). A range of products was tested, but most gave inadequate control. However, a controlled-release formulation of chlorpyrifos, formulated to release insecticide over a period of up to two years, did show promise. This work culminated in the registration of SuSCon Green in the UK, in 1993. Experience has since confirmed that, when incorporated thoroughly into the growing media, the micro granules of this product give excellent control, but it must be used from the initial potting (liner) stage. Reservoirs of untreated compost lead to poor control of larvae.

This product is now widely used in the HONS industry, particularly in the liner (1 litre pot) stage, and also on susceptible subjects in larger pot sizes, and has definitely helped to reduce the weevil problem. The UK has never had a tradition of applying broad-spectrum insecticide sprays to control adult weevils, mainly because it is felt to be ineffective and environmentally damaging. This is in marked contrast to Holland, where sprays for adults are applied regularly throughout the growing season. There is a need for some spraying for adult weevil control, but mainly on high-risk subjects such as rhododendrons, where leaf notching causes economic damage. Experiments have shown that the pyrethroids (e.g. deltamethrin, fenvalerate) can give good control and also have a repellent/antifeedant effect on treated foliage (Buxton, 1996).

Most of the development work with biological controls in the UK, (insect-parasitic nematodes, and the insect-pathogenic fungus *Metarhizium anisopliae*) has been carried out by HRI entomologists (Moorhouse, 1993). The current state of play is that various nematode species and formulations are now marketed (e.g. *Steinernema carpocapsae*, *Heterorhabditis megadis*) and have proved to be very effective under the right conditions. At present these nematodes do not have to be registered in the UK which has helped their development and introduction. However, microbial agents such as *Metarhizium* need to be registered, which has hindered its introduction, as the process is very expensive. At present, no insect-pathogenic fungi are registered for vine weevil control in the UK.

Practical experience has shown that there are only two windows for effective weevil control with nematodes: March/April and August/September. The compost temperature is the limiting factor, with temperatures of 12-14°C or greater necessary for best results. Physical penetration of large pots by the nematodes in spray solution can also be a problem, but growers are becoming more efficient with such applications.

Protected ornamentals

Traditionally, vine weevil has been a pest of pot plants such as cyclamen, fuchsias and primulas, (Mason, 1960) and, as for HONS, aldrin used to be incorporated routinely into the compost for control. However, over the past 10 years, with development of plug plants by large propagation nurseries, the speed of production and hygiene levels are such that the vine weevil has become a minor pest of these crops. Growers who buy in their plug plants of cyclamen etc. rarely have a vine weevil problem, as the plug plants are clean, i.e. free of eggs or larvae. Occasional outbreaks occur when plants are potted up and placed on the floor of the glasshouse, when adult vine weevil can walk in and oviposit on the plants. Some growers incorporate SuSCon Green into the compost of cyclamen as a preventive measure but, if weevil outbreaks do occur, nematodes applied via a sprayer as a drench, give excellent control in the protected environment.

Soft fruit

In the UK, vine weevil has devastated large areas of strawberries and blackcurrants. The practice of growing strawberries on raised beds with plastic mulches has exacerbated the situation, by providing a protected environment for adults and larvae and making penetration of insecticide sprays more difficult.

Frequent applications of broad-spectrum insecticide sprays, to try and control vine weevil in these crops, has adversely affected populations of weevil predators, such as earwigs, carabids and staphylinids. Work is in progress at HRI East Malling to determine which species of beneficials are important weevil predators in the soft-fruit environment. Mammals such as shrews may also be valuable weevil predators.

Insect-parasitic nematodes have obvious potential for control in these crops but, again, soil type and practical application problems have limited their effectiveness. Experiments have shown that irrigation tapes (e.g. T-tape) can be used successfully to deliver nematodes to the target in strawberries.

In blackcurrants, much work has been done on chemical control by ADAS (Alford, 1985; Umpelby, 1994). Insecticide granules (e.g. aldicarb, carbofuran, phorate) have sometimes given good control of larvae but much depends on placement and timing of the granule applications. The extensive leaf litter around the bushes has meant that penetration of insecticide sprays was often poor, leading to inadequate control of vine weevil in this crop.

Sprays to control adult weevils on strawberries are also often applied, usually in the late evening, using materials such as cypermethrin. Usually some control is achieved but the amount of straw and other organic materials, coupled with the fact that not all adults emerge or feed each night (Moorhouse, 1992) means that much of the insecticide is wasted and ineffective.

Future developments

Research into the development and semiochemical responses of adult weevils has commenced in a joint programme of work between ADAS and IACR-Rothamsted. Better understanding of the behaviour of weevils may lead to improved methods of monitoring or trapping, so that an IPM programme for the pest can be formulated. There may also be progress in developing other, novel insecticides for compost incorporation to control the larvae. Baits for adults also show promise and could be combined with semiochemical responses, to increase the numbers of weevils attracted to bait stations.

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Vine weevil problems on ornamental plants

Vine weevils were and still are economically important pests in ornamental plant productions indoors and outdoors, and a permanent threat for valuable plants in many European and non-European regions. First reports of considerable problems and extensive economic losses due to the feeding activities of vine weevil adults and their larvae date back to the early 19th century. As early as 1833 and 1834, Bouché described symptoms and economic consequences. The increasing importance and spread of vine weevils may be attributed not only to considerations of population dynamics but also to a growing trade of ornamentals, increased use of peat substrates in horticulture and changed production processes (Rasmussen 1978).

Important vine weevil species and host plants

The genus *Otiorhynchus* contains probably the largest number of species of all genera in the Curculionidae found in central Europe (Dosse 1954, Frieser 1981). About 150 different species have been described. However, many of these live endemically in mountainous regions (e.g. see Reiter 1916, Dosse 1954, Schwenke 1974). In other European regions, the number of species with considerable damaging effects on crop plants may, according to Sprick (1989), be no more than 15. In countries outside Europe, *O sulcatus* (i.e. vine weevil) is usually described as the major pest.

According to the world literature, the most important species which cause plant protection problems in nurseries producing ornamental plants, outdoors as well as indoors, are as follows:

– *Otiorhynchus sulcatus*: found almost throughout Europe. Detailed report are available, for example, for France (e.g. Feytaud 1918, Balachowsky & Mesnil 1935), Germany (e.g. Bouché 1834, Umgelter 1978, Bogs & Braasch 1982, von Reibnitz & Backhaus 1993), Greece and Luxembourg (Thiem 1922, 1932), Hungary (e.g. Balachowsky & Mesnil 1935), the Netherlands (e.g. Evenhuis 1978, Simons 1981), Switzerland (e.g. Klinger 1959), Scandinavia (e.g. Fjelddalen 1953, Stenseth 1976), and the United Kingdom (e.g. Smith 1932, Anonymous 1977, Blackshaw 1984, Alford 1991). Also, in the USA and Canada (e.g. Cram 1958, Warner & Negley 1976, Garth & Shanks 1978, Stimmann *et al.* 1985) this species caused massive economic losses, after, as for example in California, it had been introduced artificially on

imported rose plants (Philips 1989). There are even reports of problems with this pest in Australia and adjacent countries (e.g. Hering 1965, Miller 1979).

Smith (1932) listed 77 different host plant species, whilst Klinger (1977) mentioned more than 100 and Warner & Negley (1976) more than 140. These include important ornamentals such as alpines (especially *Saxifraga*, *Sedum* and *Sempervivium*), *Begonia, Calluna, Chrysanthemum, Cissus, Cotoneaster, Cyclamen,* many conifers, *Erica, Fuchsia, Hydrangea, Kalanchoe, Pelargonium, Primula, Rhododendron* (including azaleas) and *Rosa* (e.g. Alford 1991). Von Reibnitz & Backhaus (1993) carried out a representative survey for analysis of the attacks by vine weevil in woody ornamental plant nurseries in northern Germany. This survey covered a production area of 5,450 ha, with an average nursery size of 22.6 ha. Managers of the nurseries named 32 genera of woody ornamentals as having been attacked by *O. sulcatus* during 1988 and 1993. In particular, the genera *Taxus* (79% of all nurseries), *Rhododendron* (77% of all nurseries), *Parthenocissus* (39% of all nurseries), *Gaultheria* (31 % of all nurseries) and *Thuja* (28% of all nurseries) suffered from this pest. It is possible that in certain locations other species of *Otiorhynchus* may have caused the damage, as nurserymen are usually unable to differentiate the species from one another.

– *Otiorhynchus singularis*: found in France, Germany and Great Britain on, for example, Rosaceae, *Magnolia, Rhododendron, Thuja, Buddleia, Clematis, Primula, Tsuga* (Balachowsky & Mesnil 1935, Anonymous 1977, Alford 1991).

– *Otiorhynchus salicola*: recorded in Italy and Switzerland on *Ligustrum* and *Prunus* (Lozzia & Biraghi 1983, Klinger 1988).

– *Otiorhynchus ovatus*: found in the USA as well as in Germany, Great Britain, Scandinavia and in several other European countries on conifers, *Bergenia* and many other herbaceous perennials (Gambrell 1938, Fjelddalen 1953, Dosse 1954, Stein & Küthe 1969, Bogs & Braasch 1988, Alford 1991).

– *Otiorhynchus rugosostriatus*: found in Great Britain (Anonymous 1977, Alford 1991) and in Germany (Balachowsky & Mesnil 1935) on several plant genera.

– *Otiorhynchus raucus*: found (at least in Germany) on Chenopodiaceae, *Saxifraga, Prunus, Bergenia* and many herbaceous perennials (Kaltenbach 1874, Bogs & Braasch 1988).

– Additionally, in certain regions rarer species of *Otiorhynchus* occur. These include: *O. ligustici* in Austria and southern parts of Germany (Kaltenbach 1874, Balachowsky & Mesnil 1935), *O. smreczynskii* on *Syringa* and *Weigela-Hybrids* in the city of Hannover (Sprick 1989), *O. crataegi* on, for example, *Syringa* in southern England, southern Germany, parts of the former Yugoslavia and parts of France (Sprick 1989, Alford 1991) and *O.*

clavipes, especially on light soils on *Syringa, Lonicera, Aucuba, Acer, Viburnum, Weigela* in England, Belgium, France, Germany, the Netherlands and Switzerland (Anonymous 1977, Alford 1991).

Life cycle

All of the above-mentioned weevil species are wingless and are usually spread as larvae or eggs, by means of infested plants or root balls. Nurserymen, however, refer to the weevils' ability to cover long distances by walking, e.g. from hedges to plantations or from one plantation to another. *O. sulcatus* is entirely parthenogenetic, producing unfertilized eggs. These are deposited at intervals between spring and late summer. Bogs & Braasch (1982) described two main oviposition periods under Germany conditions: a first period from April to September and a second one from January to April. Overall, each weevil may deposit more than 800 eggs during its life time (Hering 1956, Klinger 1959, Stein & Küthe 1969).

Generally speaking, there is just one generation per year in outdoor plantations, although individual adults may survive for two or more years (Thiem 1932, also see paper by A. Frers in this volume). In greenhouses, Bogs & Braasch (1982) recorded a lifespan for adults of between 7 and 14 months, while Thiem (1932) stated that adults normally survive for 18 months. Outdoors, vine weevils hibernate as adults, as prepupae or as larvae (Klinger 1959). Goetz (1954) also reported overwintering of *O. sulcatus* in the pupal stage, under German climatic conditions. The specific life cycles of several species of *Otiorhynchus*, which vary according to temperature, food quality, species and soil conditions, have been described and discussed in detail by several authors (e.g. Thiem 1932, Klinger 1959, Stein & Küthe 1969, Umgelter 1978, Bogs & Braasch 1988, von Reibnitz & Backhaus 1993). Owing to the frequent occurrence of vine weevils in protected crops, particularly in regions with intensive production systems for ornamental plants, almost all stages of the pest may be found throughout the year (Evenhuis 1978, Bogs & Braasch 1982, von Reibnitz *et al.* 1993).

Damage to plants

The damage to plants is caused by adults as well as larvae. Adults feed on the green leaves, sometimes also on young, fresh shoots (e.g. *Taxus*). This may not greatly affect the photosynthetic capability of host plants; however, it reduces considerably their market value. Customers fear to introduce this pest into their own plantations. Thus, they will often refuse to buy any plants from nurseries where feeding symptoms on plants are obvious. Plants with even the smallest signs of damage, such as holes eaten out of the leaf margins, are completely unsale-

able. The feeding period for adult vine weevils in greenhouses lasts from 38 to 53 days (Bogs & Braasch 1982). Adults show a typical nocturnal feeding behaviour. During daytime or light periods, the weevils hide in the soil, in soil cracks or in organic mulch material underneath the host plants.

The most important damage, however, to ornamental plants is caused by the larvae. Immediately following egg hatch, the larvae start feeding on young roots or rhizomes, or they bore deeply into corms, roots and tubers (e.g. in *Cyclamen persicum*). An infestation may pass unnoticed for several weeks until the larvae are sufficiently large than main feeding roots are attacked, thus causing yellowing of leaves and visible wilting symptoms. Sometimes, rotting of the attacked plant parts is provoked as secondary disease. The large larvae (L3/L4) also feed on root necks and on the stem base of plants. Even just a few such larvae are capable of killing even large (and accordingly valuable) *Rhododendron* or *Taxus* plants within a few days, by girdling the root necks. This happens, in particular, in the spring, after the larvae have overwintered.

The wide spectrum of host plants, as shown above, frequently leads to a steady state of latent infestation in ornamental plant nurseries.

Control

Attempts to control vine weevils have a long history. Many nurseries take measure against the adults and larvae by repeated, or even regular, applications of insecticides (e.g. chlorpyrifos, cypermethrin, deltamethrin, diflubenzuron, endosulfan, methamidofos, parathion). As chemical control against larvae is often inadequate, many nurseries nowadays successfully use entomopathogenic nematodes for biological control (e.g. see Simons 1981, Albert & Bühl 1987, Klinger 1988, Rovesti *et al.* 1988, Stössel & Ehlers 1990, Backhaus 1990, 1994). Very promising results from experimental work with the fungus *Metarhizium anisopliae*, unfortunately, did not lead to practical applications, owing to the rather high cost of production (Zimmermann 1981, Stenzel 1994, Gilgenberg-Hartung 1994). More detailed accounts of the control of vine weevils are given elsewhere in this vine weevil workshop volume.

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Vine weevil problems in fruit crops

Introduction

Several weevil species of the genus *Otiorhynchus* are recorded as pests in fruit crops, e.g. *O. sulcatus* (Fabricius), *O. rugosostriatus* (Goeze), *O. ovatus* (Linnaeus), *O. clavipes* (Bonsdorff), *O. rugifrons* (Gyllenhal) and *O. singularis* (Linneaus) (Alford 1984). Main damage is caused by the root feeding of the larvae and is most important in strawberry, where it may be caused by *O. sulcatus*, *O. rugosostriatus*, *O. ovatus* (Küthe & Stein 1969), *O. clavipes* and *O. rugifrons*. *O. clavipes* and *O. sulcatus*, in addition, are recorded as pests of other fruit crops: the former from plum, raspberry and, occassionally, apple, currant and gooseberry, the latter from soft fruit (see below). The damage caused by adults of these species feeding on the leaves is usually of no economic consequence. Adult weevils of *O. singularis*, however, can cause damage on apple, pear, raspberry and currant, especially in young plantations, by gnawing grafts, stems, buds, fruitlet stalks, shoots and leaves.

O. sulcatus on strawberry and other soft fruit

Among the *Otiorhynchus* species, the vine weevil, *O. sulcatus*, is the most important pest. It is very polyphagous. About 150 plant species have been described as host plants (Moorhouse et al. 1992). With regard to fruit crops, apart from strawberry, *O. sulcatus* attacks other soft fruit such as black currant, gooseberry, raspberry, blueberry and cranberry, the damage again caused by the larvae feeding on the roots. The vine weevil is recorded as pest on fruit crops from all areas of its distribution: in the temperate areas of Europe, in USA, Canada, mostly in Eastern and Western areas, New Zealand, South Eastern Australia, including Tasmania, Japan and recently Chile.

Most severe damage occurs in strawberries, especially on sandy soils and on strawberry planted through plastic mulch (Stenseth & Vik 1979). The latter cases favour the development of the vine weevil because of elevated soil temperatures. Plants attacked by root-feeding larvae grow poorly, wilt and often die, most often collapsing during the fruiting season. A damage threshold of 2-3 larvae per plant is given (Scherer 1987); however, one larva may be enough to kill the strawberry plant, if it attacks the main roots just below the soil surface. Infestations often result in substantial levels of crop damage and significant economic loss for the grower.

Most reports of outbreaks of *O. sulcatus* on strawberry are several years old. During the few last years, damage in strawberries has diminished and is reported only sporadically. This is mainly due to changes in cultural techniques (see below).

O. sulcatus on *Rubus*

On *Rubus*, in general, relative little damage from root feeding by *O. sulcatus* appears to occur. Plants of red raspberry and blackberry rarely are visibly reduced in vigor by the root feeding. The most serious problem seems to be that adult weevils have become a serious contaminant of mechanically harvested red raspberries. The action of the mechanical harvester causes the weevils to fall off with the ripe berries. They must then be manually removed from the sorting belts. This can be very difficult if large numbers of weevils are present, or if they have had time to crawl inside the hollow berries (Ellis et al. 1991).

O. sulcatus in nurseries

O. sulcatus can cause severe damages in nurseries. According to von Reibnitz & Backhaus (1993), 96 % of the nurseries in the German Länder Schleswig-Holstein and Niedersachsen reported vine weevil infestations and most of them (90 %) had to carry out control measures.

Control Measures

The life cycle of *O. sulcatus* explains why this species is difficult to control. The vine weevil has one generation per year, but there is a considerable overlap between the different stages. Moreover, in many regions, adult weevils are able to overwinter. Thus, egg laying starts rather early in the season, e.g. end of April, and consequently the appearance of new larvae is also advanced. Summer adults begin to lay eggs at the end of July (Scherer 1987a, Garth & Shanks 1978). The species has a high reproduction capacity, with up to 1,500 eggs per female (Stenseth 1979). Oviposition usually ends by the end of August to mid-September. The long oviposition period, together with the fact that the larvae overwinter, explains why larvae can be found more or less all the year round. Only where overwintering of the adults does not occur, is there a short period early in the season when larvae are not present. Moreover, as stated above, cultural practices in strawberry (e. g. using plastic mulch) favour the development of *O. sulcatus*.

Cultural measures are to use healthy plants, to prepare the soil carefully and to use crop rotation. Furthermore, it has to be avoided to establish a new strawberry plantation after or near (50-100 m) a previously infested field. In contrast to former years, when strawberry fields were kept for several years, most fields now are annual or biennial cultures. Thus, the build-up of high weevil population densities is not favoured. Mowing off strawberry fields after harvest also helps to reduce weevil populations. According to Garth & Shanks (1978), 60% of adults

died after the foliage was mowed off and removed as compared with 0-12% mortality in un-mowed plots.
As the utilisation of inherent plant resistance has considerable potential for reducing *O. sulcatus* damage, new selections of varieties might decrease infestations. The tolerance of some strawberry cultivars to the attack of *O. sulcatus*, for example, seems to be related to the ability of the plant to produce and regenerate a large supply of roots (Cram 1978). In the case of highbush blueberry cultivars, some were found to be unacceptable for adult feeding, owing to a feeding deterrent, a lack of necessary nutrients or an imbalance of nutrients (Cram 1970).

In the following biological and chemical control measures are mentioned only briefly as they are covered in detail in other contributions at this workshop. For biological control, nematodes (especially *Heterorhabditis heliothidis*) have shown to be of high efficiency, attaining 80 to 100 % control (e.g. Backhaus 1990, Scherer 1987b) and thus exceeding the efficacy of most chemical treatments. Curran & Patel (1988) describe the use of a trickle irrigation system to distribute entomopathogenic nematodes for the control of vine weevil in strawberries. This technique facilitated the rapid distribution of the nematodes to strawberry plants grown under plastic mulch. Weevil survival (mean no. of live larvae and pupae) was reduced significantly compared with untreated plants. Nematodes are now sold commercially in many countries. Some suppliers sell the nematode *Steinernema feltiae* (=*Neoaplectana carpocapsa*), but this species is up to 30% less effective than *H. heliothidis.*

With regard to entomogenous fungi, high levels of weevil control have been recorded with *Metarhizium anisopliae* (Moorhouse et al. 1992). However, in spite of big efforts for developing a commercial product based on this fungus, it is still not available (Stenzel et al. 1992).

Insecticides can be used as larvicides (by treating the soil) or as sprays against the adult weevils. However, soil treatments, especially as high volume drench applications, are in discussion because of the risks of ground water contamination. The highly effective organo-chlorine insecticides (e.g. aldrin and dieldrin) have been banned in most countries because of their long environmental persistence. Other insecticides, e.g. organophoshates (parathion, methamidophos, acephate, chlorpyrifos) most often are less effective and need several applications (Scherer 1987c). Microencapsulation might offer a solution for extending the persistence of certain insecticides. Adult weevils can be controlled by foliar sprays with broad-spectrum insecticides (pyrethroids, organophosphates, carbamates etc.), targetting the weevils during their preoviposition period. Normally, two applications are needed. For optimum spray timing, careful monitoring of the adult activity is necessary. As the weevils are nocturnal, night spraying can increase the efficiency of the insecticide treatment (Moorhouse et al. 1992).

With regard to an environmentally sound control of the vine weevil, biological control by using nematodes should be spread more into practice. In order to increase the acceptance of this biological control measure, and to achieve high control efficacies, the costs must be maintained on an economically justifiable level and the farmers must be given more advice and instructions.

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Vine weevil problems in viticulture

Introduction

Vine weevil (*Otiorhynchus sulcatus*) is an occasional pest in viticulture. In the past, damage caused by overwintered adults feeding on grape buds in the spring was often overestimated. During the last 20 years, we observed only unimportant feeding on buds by vine weevil. However, larvae of several noctuids and of the geometrid *Peribatodes rhomboidaria* may case serious loss of grape buds (Englert 1979).

The larvae of vine weevil overwinter and pupate in about the second half of June. Young weevils emerge in July and start feeding on vine leaves; such damage, however, is not important. The typical semi-lunar-shaped notches on the leaves allows ready differentiation of vine weevil damage from that by caused by other leaf-feeding insects. Vine weevil larvae can seriously damage the root system of vines. When infestations persist over several years, the vines react with reduced growth and, finally, they die. In attacked vineyards, the vines die-out in patches, similar to attack by phylloxera. Especially in young plantings, infested vines die within a short time.

In Germany, vine weevil occurs mainly in the vine-growing areas of the Mosel and Ahr valleys. However, occasional outbreaks have also been reported in Frankonia and Baden-Württemberg.

Control

From 1953 to 1980, aldrin was used as a soil insecticide. It was spread around the vine stems in spring and provided good control of vine weevil (Hering 1959). From 1979 to 1990, carbofuran was used as an effective insecticide, killing the adult weevils (Englert 1981). Up to 1990, lindane was also registered for application in young plantings.

No insecticide is currently available to control vine weevil in viticulture in Germany, owing to the evident problems of the use of soil insecticides.

Heavy attacks by vine weevil have not been reported in recent years. However, any suddent outbreaks would require chemical control measures, although the latter are currently not

available. Therefore, a working groups of specialists is trying to find suitable insecticides, and a testing programme is planned for the next few years.

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The role of *Otiorhynchus* weevils in forests

Relevant species

The weevil genus *Otiorhynchus* received the common German name 'Dickmaulrüßler'(= 'thick mouth weevil') because of the conspicuous broadening of the short, thick proboscis. Fourteen species of the genus play a role in forests (Schwenke 1974); three of them cause damage to some extent in Central Europe and, therefore, will be presented here.

Of the three species described here, all are unable to fly but possess strong tarsi well suited for climbing, even on smooth surfaces. The red legs of the adults contrast strongly with the shiny black body. In spite of their similar appearance, all three species differ in size, and this is reflected in the German common names: 'Großer Schwarzer Rüßler' (= 'large black weevil', for *O. sensitivus*), adults 12-15 mm long; 'Mittelgroßer Schwarzer Rüßler' (= 'middle-sized black weevil', for *O. niger),* adults 9-11 mm long; 'Kleiner Schwarzer Rüßler' (= 'small black weevil', for *O. ovatus*), adults up to 5 mm long. *O. niger* is most common of these three species and is regarded as the *Otiorhynchus*-weevil causing the most damage in forest trees (Schwenke 1974).

Otiorhynchus niger

A detailed study on *O. niger* was published by Schindler (1958). The weevil is found in the hill country of Central and Eastern Europe and occurs as a typical montane to alpine species at altitudes from 300 m upwards, finding optimum conditions above 500 m. Its occurrence shows a remarkable correspondence with the original distribution area of its main host plant, Norway Spruce *Picea abies,* except for the Adriatic and Nordic-Baltic spruce regions. However, the pest did not accompany Norway Spruce into its artificial planting area in the Northwest German Plain. During the second half of the 19th century, root damage by the larvae was, in some cases, so strong that nurseries had to be abandoned. During the reforestation period

following World War II, there were also many reports on damage by larvae of *O. niger* in forest nurseries.

If the larvae have completed their development in their first summer, the immature weevils overwinter in soil or, like older adult weevils, in litter. They leave the soil or litter in spring, usually beginning in mid-April at a temperature of 8°C. The weevils feed primarily on spruce needles and buds; in spring, they also feed on fresh shoots and, occasionally, on the bark of young plants. In addition to spruce, Douglas Fir and alder are also good host plants. However, compared with damage by larvae, that caused by adult weevils is only slight.

Oviposition occurs in the uppermost soil layer and takes place at the earliest from mid-May onward by two-year-old females and even later by young adults. Maximum oviposition activity occurs in July. Unlike the common cockchafer, the weevils are unable to dig into the soil but instead must rely on natural openings. This behaviour accounts for the fact that freshly dug soil in nurseries is particularly well frequented for oviposition. Eggs are deposited in groups of 05-60 at 05-08 cm depth. In their first year, females produce approximately 40, in their second year c. 240 eggs. The eggs hatch two to four weeks after oviposition, depending on temperature. The first larval stage relies on the feeder roots of grasses and woody plants, whereas older roots are fed upon from the second larval stage onwards. In the third and last stage, even main roots are eaten. The damage is similar to that caused by cockchafer larvae, and an exact diagnosis is possible only after finding the insect. In many cases, however, larvae will have already left the vicinity by the time heavily damaged plants exhibit wilting due to root damage.

The larvae live at a soil depth of c. 02-15 cm, which corresponds with the root zone of young conifers. Most of the larvae, especially those appearing after mid-June, do not pupate but overwinter in the larval stage. Therefore, the heaviest damage is generally caused by the second-year larvae in the year following oviposition. These can kill one-to-four-year-old spruce plants. Owing to this damage, *O. niger* was long regarded as the most important enemy of forest nurseries at higher altitudes, assuming the same role there as cockchafer larvae at lower elevations.

Otiorhynchus sensitivus

The biology and developmental cycle of *O. sensitivus* are similar to those of *O. niger*. Further, this species is long-lived and reproduces over the course of several years. Mating and oviposition also occur during the whole vegetation period. However, this species is adapted to even higher elevations and has some importance in the Alpine region, especially in southern parts (Escherich 1923). Its range of distribution extends from the southern Alpine region to the Balkan Peninsula, where it does not, however, become a pest (Kovacevic 1970). As with *O. niger*, adult feeding is restricted mainly to Norway Spruce, but damage is of no importance. Larval feeding, however, can cause severe loss of plants (Schwenke 1974).

Otiorhynchus ovatus

Unlike the two preceding species, *O. ovatus* occurs exclusively at low elevations. It possesses a single-year developmental cycle and is reportedly able to reproduce parthenogenetically. Larval feeding causes damage, but less in forests than in agricultural and horticultural crops. Reports of severe damage are known, especially from strawberry plantations (Mutz 1950). However, death of young Norway spruce following basal encirclement by adult beetles has been observed (Escherich 1923). This polyphagous species occurs in many parts of Europe, Central Asia and North America (Dosse 1954).

Present role in forests and control measures

A poll carried out in the German Federal States in 1995 showed that feeding damage by *O. niger* was recorded only once in the past 10 years, on two hectares in a middle-aged spruce stand in Thüringen. The present effect of this insect on forests may, therefore, be judged as minimal. One of the reasons is that the number of nurseries belonging to the state forest service has declined, and most of the plants for reforestation are now being purchased from large, privately owned forest nurseries at lower altitudes.

Adult weevils generally enter nurseries in spring, for oviposition and feeding; in the past they were controlled using insecticides with a long persistence. Consequently, nursery plants were treated with substances based on DDT, lindane, endosulfan and organic phosporus compounds. Application rates up to the five-fold dose were necessary, since weevils are known to be difficult to control. Damage from larval feeding was prevented by mixing a high dose of

insecticide with the soil at the planting site. If damage by larvae was found after planting, soil insecticides were sprayed, watered in or injected into soil.

In 1977, the use of DDT was completely prohibited in Germany. Since then, the loss of registration for forest protection substances based on lindane, endosulfan and organic phosphorus compounds has led to a lack of insecticides for the control of many forest pests. Consequently, there are presently no registered substances available for control of *Otiorhynchus* weevils or most other more important coleopterous pests in forests.

One, admittedly outdated, solution to this dilemma was offered by Dosse in 1954, who stated that deeply turning the soil can help control the weevils. Rohde (1966) established that adults of *O. niger* prefer alder to spruce and suggested planting *Alnus glutinosa* in pure young conifer stands to divert the weevils. If we follow such advice, maybe the modern approach to control of weevils in nurseries could be the method described in 1927 by Borgmann: ".... gathering the beetles by picking or knocking them off their host plants. Together with three young helpers, District Forester EIMER gathered 88,000 weevils in a single stand in the course of three days by shaking them off the plants into a pan filled with water to a depth of appr. 30 cm ". We are not quite sure that this is how "integrated forest pest management" is meant to be!

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Adult vine weevil activity in black currant plantations in South West England

Introduction

In South West England, larvae of vine weevil (*Otiorhynchus sulcatus* (Fabricius)) often occur in large numbers on the roots of black currant bushes and they can cause significant damage, particularly when bushes are under drought stress. Adults graze on the leaves, forming notches around the edges, but such attacks are not of economic importance; the weevils, however, often hide on bushes during the daytime and may subsequently become a serious contaminant in trays of mechanically harvested fruits. This paper reviews some observations made in the 1980s, during the decline from use in the UK of persistent organochlorine insecticides, when vine weevil was a particularly significant and escalating pest.

Methods

Observations included pitfall trapping for adults and were made largely as a supplement to chemical control experiments (e.g. Alford, 1985). Pitfall traps consisted of plastic beakers (74 mm internal lip diameter) sunk into the soil and coated around their rims with a solution of Fluon GP1 (Plasticote Systems Limited) to form a *c.* 25 mm band which prevented trapped weevils from escaping. At one site, in 1984, adult carabids captured in the pitfall traps were also counted. Observations were made at six sites: A – Redmarley, Gloucestershire (1980); B – Wellington, Somerset (in 1980); C – Ilminster, Somerset (in 1982 & 1983); D – Milverton, Somerset (in 1984 & 1987); E – Bradford-on-Tone, Somerset (in 1984); F – Yeovil, Somerset (in 1989).

Results

At Site A, vine weevil adults were monitored from late May to mid-September 1980 and, at Site B, from late April to mid-September 1980. At both sites, peak numbers in pitfall traps occurred in mid-July. Dissections confirmed the presence of mature overwintered adults in April and May and the development of new-generation adults from about mid-June onwards (Table 1). Maturity of new adults tended to be reached by August although, contrasting with

data from later years and other sites, immature specimens were still evident at Site A in mid-August.

Overwintered adults captured in April or May laid eggs immediately when caged with black currant foliage. Newly emerged adults in mid-summer, however, laid eggs only after several weeks, during which time they fed frequently on older foliage; young, bright green leaves were not attacked. Newly emerged weevils reared under laboratory conditions did not feed for their first few days of life and began laying from 59 to 77 days after emergence (mean, 68 days). Over this period, these weevils consumed an average of 19.2 ± 4.27 cm^{-2} of leaf area.

Table 1. Maturity of adults of *Otiorhynchus sulcatus* at two sites in 1980.

Date	Immature		Maturing		Mature	
	Site A	Site B	Site A	Site B	Site A	Site B
25 April	-	0	-	0	-	1
15 May	-	0	-	0	-	1
19	-	0	-	0	-	0
30	0	-	1	-	1	-
18 June	0	-	1	-	-	-
19	-	10	-	0	-	1
25	1	-	0	-	0	-
27	-	5	-	1	-	0
7 July	6	-	0	-	2	-
11	-	29	-	2	-	0
16	50	-	0	-	0	-
21	-	8	-	2	-	0
23	6	-	4	-	1	-
6 August	6	-	5	-	9	-
11	-	0	-	0	-	4
20	11	-	1	-	28	-
3 September	0	-	0	-	10	-
10	0	-	0	-	1	-

At Site C in 1982, vine weevil adults caught after 17 June were at first reproductively immature. All adults found before this date had, presumably, overwintered. The proportion of 'new' adults with developed ovaries rose sharply at the beginning of July; by the end of the month, most individuals were fully mature. Peak activity was recorded from the end of June to the beginning of July, with numbers trapped typically declining noticeably in August. In 1983, young adults began to appear in mid-June. Large numbers appeared in pitfall traps at

the end of the month but activity then declined rapidly (Table 2). This decline was earlier than would normally be expected and, coupled with the presence many dead bodies on the plantation floor, appeared to be the result of a pesticide application. Subsequent analysis confirmed the presence of significant amounts of aldrin + dieldrin (0.620 mg/kg + 1.172 mg/kg respectively) in the carcasses, from a spray of aldrin applied in April by the grower in a futile attempt to kill overwintered larvae. In 1988, at another site in Somerset where many dead weevils were found, the grower denied that any pesticide had been applied but subsequent

Table 2. Decline in activity of 'new' adults at Site C (1983), following application of aldrin in April.

Date traps examined	Block 1 (10 traps)	Block 2 (5 traps)	Block 3 (10 traps)
29 June	85	80	133
6 July	33	6	42
12	7	1	3
21	3	1	4
28	0	0	4
4 August	0	0	0

Table 3. Numbers of adults of *Otiorhynchus sulcatus* and carabid beetles caught in pitfall traps at two sites in 1984.

Date	Site D (20 traps)		Site E (10 traps)	
	Weevils	Carabids	Weevils	Carabids
6 June	0	-	-	-
13	1	-	-	-
20	29	-	-	-
27	42	-	53	-
4 July	62	-	57	-
11	47	46	30	5
18	14	255	24	16
25 July	2	31	17	24
1 August	3	74	10	41

analysis of the dead weevils and of soil from the same vicinity confirmed the presence of aldrin residues in both (0.024 mg/kg in the weevil carcasses and 0.188 mg/kg in the soil).

At Sites D & E in 1984, most adult vine weevils were caught in pitfall traps in early July (Table 3); numbers then declined rapidly. At both sites, large numbers of adult carabid beetles were also found in pitfall traps (Table 3) from July onwards. Some were caught in the traps before this time, but these were not counted. Adult carabids recorded in the pitfall traps included: *Carabus violaceus* L., *Cychrus caraboides* (L.) and two species of *Nicrophorus* at Site A; *Abax parallelepidus* (Piller & Mitterpacher) and *Harpalus affinis* (Schrank) at Site B.

At Site F in 1989, large numbers of vine weevil adults were found during the late winter, hibernating beneath the partially buried remains of old, black Polythene mulch, their noticeably clumped distribution suggesting that an aggregation pheromone had some influence on their behaviour. Pitfall trap catches (Table 4) indicate the build-up of overwintered adults in the spring and their decline, followed by the commencement of a summer build-up of young adults.

Table 4. Pitfall trap catches of *Otiorhynchus sulcatus* adults at Site F in spring and early summer (1989).

Date trap emptied	Trap 1	Trap 2	Trap 3	Trap 4	Mean/trap/week*
12 April	1	2	2	1	1.3
19	3	4	2	4	3.3
26	13	4	2	3	5.5
5 May	22	20	5	10	11.1
16	36	31	13	15	15.1
26	29	19	9	11	11.9
9 June	5	3	0	3	1.4
20	11	10	1	4	4.1
30	6	8	4	4	3.9
13 July	22	41	14	8	11.4

* Number of days between counts, divided by 7.

Discussion

In the UK, the withdrawal of persistent organochlorine insecticides for control of pests such as vine weevil placed considerable pressure on growers of certain horticultural crops, especially currant, strawberry, containerized hardy ornamentals and glasshouse pot plants. Aldrin (once available for control of wingless weevils, including vine weevil) has not been recommended for use on fruit crops in the UK since the 1960s. However, this did not prevent its use in the 1980s by growers frustrated by escalating vine weevil problems. Neither aldrin nor DDT (all uses in the UK revoked in 1989 and 1984, respectively) is now available in the UK.

The cultural conditions under which black currants are grown offer an ideal environment for the survival of vine weevil. Bushes tend to be well sheltered and the pests are largely free from gross disturbance, except during mechanical harvesting and, perhaps, when pesticides are applied. Birds, notably rooks (*Corvus frugilegus* L.), readily devour the immature stages, pecking avidly around the bases of infest bushes, and adult weevils are sometimes devoured by small mammals. Carabid beetles, which are often abundant in black currant plantations (e.g. data in Table 3), will also feed on them. The extent of such predation in black currant plantations has not been quantified but it is tempting to suggest that, particularly when numerous, they are at least partly responsible for the rapid decline in weevil numbers that occurred during the post-emergence summer period within black currant plantations. However, migration away from emergence sites would also lead to a decrease in numbers trapped within plantations. No insect parasitoids were found attacking adults or larvae, but field-collected vine weevil adults were sometimes parasitized by nematodes (*Mermis* sp.).

Numbers of overwintered weevils active at Site F in spring 1989 appeared unusually high but the reasons for this were unclear, unless the black Polythene beneath which many sheltered improved their chances of survival. Whether the aggregation was linked to production of an aggregation pheromone is not known.

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Importance of overwintering adults to summer oviposition in Northern Ireland

Introduction

In the northern hemisphere, adult vine weevil (*Otiorhynchus sulcatus* (F.)) emerge from pupae in June and July (Stenseth, 1976) and undergo a period of pre-oviposition feeding and maturity lasting several weeks prior to oviposition (Cram, 1958). Larval growth is temperature dependent (Stenseth, 1979) so that the overwintering stage is variable.

In some cases, adults have been reported to survive through the summer, overwinter and lay eggs the following season during early summer. In Northern Ireland, overwintering adults have been reported from strawberry fields (Willis, 1964) and so may be expected to contribute to egg production. The duration and commencement of an egg-laying season may therefore depend upon the winter survival of adults.

Methods

The study site was a free-standing gravel bed at Loughgall, Co. Armagh, Northern Ireland which is used to grow-on a variety of ornamental pot plants. Oviposition was monitored by placing two parallel rows of ten polyanthus plants onto the gravel bed. Each plant was in a 12 cm pot and had a 1 cm layer of sieved (500 µm mesh) sand to act as a trap for deposited eggs. At the end of each sampling period plants were recovered, replaced and taken to the laboratory where the sand was washed off the plants and through a 500 µm mesh sieve, which retained the eggs.

Plants were first positioned on 3 March 1983 and examined every two weeks. From March 1984 to November 1986 they were examined weekly. All recovered eggs were placed on wet filter paper in a Petri dish so that proportional hatch (viability) could be observed.

The site was revisited in 1995 and similar observations on egg deposition made on a weekly basis commencing 12 April. In addition, an array of 40 blocks, each of 20

plants was also layed out on the gravel bed. One plant was collected at random from each block evey two weeks and larvae extracted from the compost by wet-sieving. The first larval samples were taken on 17 May 1995 and the last on 12 December 1995. Recovered larvae were weighed and allocated to one of 5 size classes (<2 mg, 2-15 mg, 15-60 mg, 60-100 mg > 100 mg).

Results

The mean duration of oviposition by vine weevil at this site was 21.4 wk and extended from 17 to 26 wk. The dates at which egg-laying commenced ranged from 1 May to 1 June and the pattern varied from year to year (Figs 1 and 2). The viability of eggs deposited in 1983-1986 spanned 0 to 100%, however the pattern of viability differed

Fig 1. Numbers (histogram) and viability (line) of eggs recovered from polyanthus plants in 1983 (a), 1984 (b), 1985 (c) and 1986 (d).

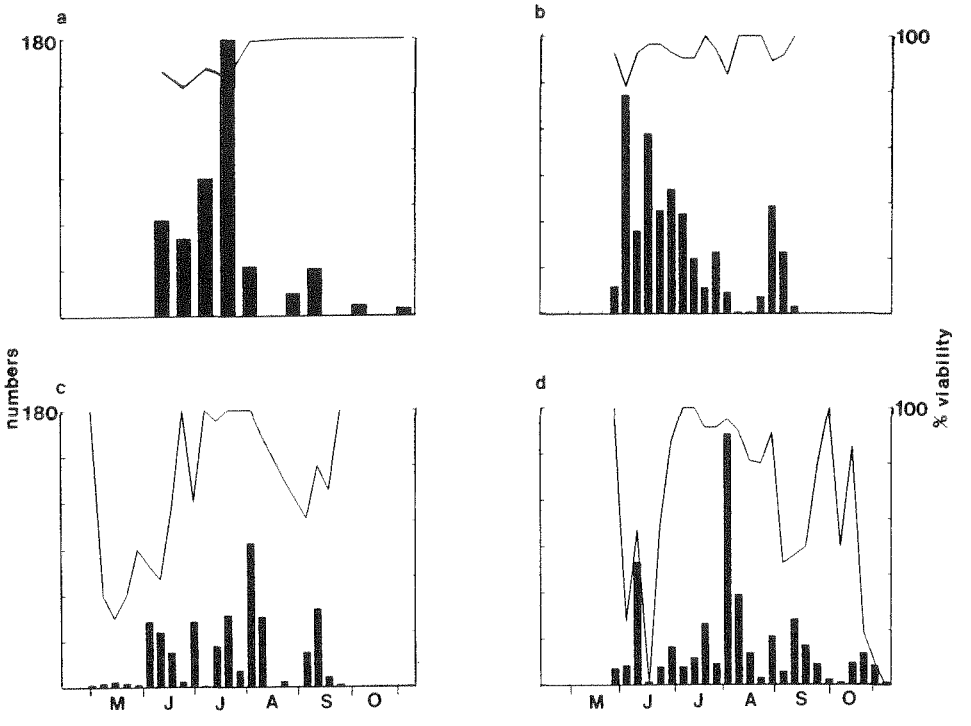
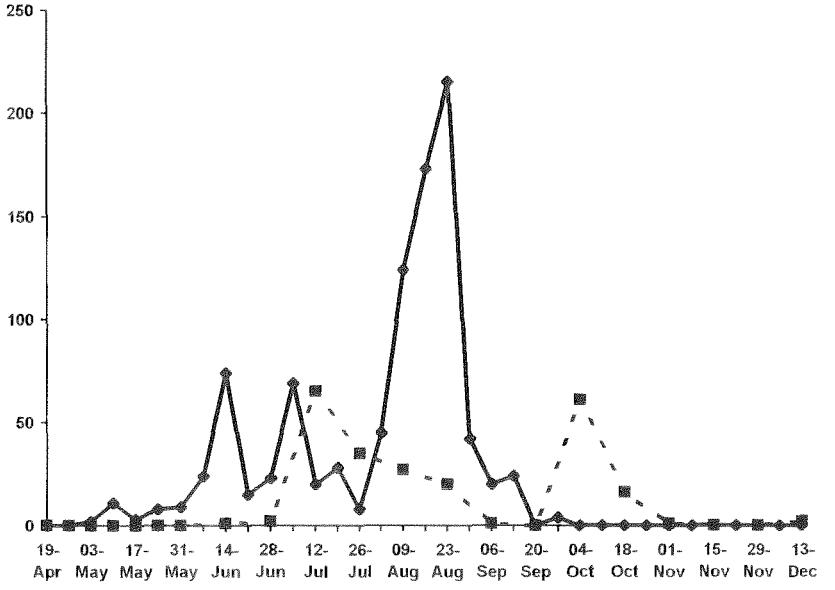


Fig 2. Recovery of eggs (◆) and vine weevil larvae <2 mg (■) from polyanthus plants in 1995.



between years with large numbers of eggs (1983 and 1984) and those with fewer (1985 and 1986). This difference manifested itself by a decline in peak viability towards the end of the season for the latter years. Viability in any one sampling period was related to the number of eggs produced:

$$V = 9.2 + 8.84(N) \qquad (r = 0.762;\ P<0.001)$$

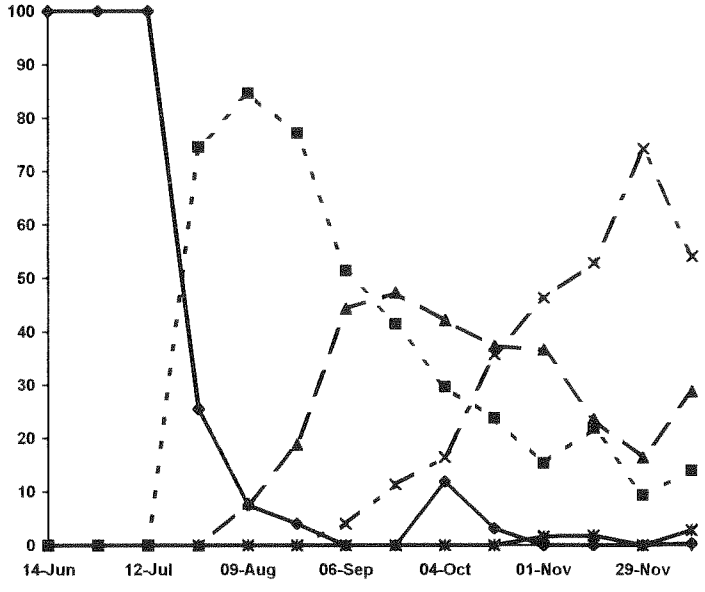
where V is arcsin transformed percentage viability and N is the square root of the numbers of eggs laid.

There was no evidence from the 1983-1986 data that the number of eggs laid in any one year was related to the number deposited the previous year. The total did, however, decrease in relation to the increase in the number of days with mean sub-zero temperatures since the previous egg-laying season ($r = -0.988$; $P<0.01$). Division of the total number of eggs laid into those deposited before the third week of July (i.e. eggs approximately attributable to overwintering adults - following Evenhuis, 1978; Garth and Shanks, 1978; Smith, 1932; Stenseth, 1976) and those laid after yielded correlations of -0.819 ($P<0.01$) and 0.024 ($P>0.05$) respectively, suggesting that winter severity was acting on overwintering adults. The start of oviposition (measured

as the number of days after 1 May) could also be related to the number of days with mean sub-zero temperatures (r = -0.988; $P<0.01$).

Inclusion of data from 1995 changed the correlation coefficients: for total egg numbers no correlation could be calculated; eggs deposited before third week of July became r = -0.598, $P>0.05$; eggs deposited after third week of July became r = 0.662, $P>0.05$; the start of oviposition became r = -0.885, $P<0.05$.

Fig 3. Frequency of weight classes of vine weevil larvae, expressed as percentage of population recovered from polyanthus plants in 1995. <2 mg (◆), 2-15 mg (■), 15-60 mg (▲), 60-100 mg (✖), >100 mg (✳).



The number of small larvae (<2 mg) recovered from around the roots of polyanthus plants in 1995 is indicative of recruitment into the population and are presented in relation to the oviposition pattern (Fig 2). Larvae were first recovered six weeks after the start of oviposition. The frequencies of the differing larval size classes recovered from these plants (Fig 3) suggests a pattern of growth such that the largest larvae (>100 mg) present in the autumn arise from these early larvae and, hence, from eggs deposited by overwintering adults.

Discussion

Vine weevil oviposition commenced in May or June of each year and this pattern is consistent with that expected from overwintering adults. Except for 1986, there was a trough in oviposition in August (1983-1986) or July (1995). This may represent the separation of egg recruitment into those deriving from overwintering and summer-emerging weevils, since newly emerged weevils could not be expected to produce eggs until August at the earliest (Stenseth, 1976). On this basis, some 54% (range 30-75%) of eggs recorded during this study arise from overwintering adults. These weevils therefore make a substantial impact on the pest problem and necessitate control over a prolonged period.

Adults overwinter in the crowns of plants and under debris (Willis, 1964) and so are exposed to ambient temperatures. Whilst a number of relationships seemed apparent from the 1983-1986 data only that of the date of first egg recovery with the number of days with mean sub-zero temperatures was sustained by adding the 1995 data. If this relationship can be further substantiated, it may afford a means of forecasting the onset of oviposition in Northern Ireland.

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Chemical ecology of adult vine weevil

Introduction

The larval and adult stages of vine weevil, *Otiorhynchus sulcatus*, attack many glasshouse and outdoor ornamental plants. There are a number of relatively successful methods of controlling larval damage, including the use of entomophilic nematodes. Control of the adults is more difficult because of their extended period of activity and, to decrease reliance on chemical pesticides, new integrated control strategies are required. Although biological control agents are available, these are most effective on container-grown plants or when crops are grown under glass. In outdoor nurseries, problems must be overcome in ensuring good contact between the insect and the control agent. A combined semiochemical strategy, in which the behaviour of the vine weevil is manipulated to maximise the effectiveness of the biological control agent, would be advantageous.

Studies on the chemical ecology of other curculionids have identified semiochemicals involved in host plant location, e.g. for the cabbage seed weevil, *Ceutorhynchus assimilis* (Blight *et al.*, 1995), and in aggregation behaviour such as is observed with the pea and bean weevil, *Sitona lineatus* (Blight *et al.*, 1991). Adult vine weevils, both in the field and in laboratory colonies, are known to aggregate in large groupings and it has been suggested that an aggregation pheromone may be responsible. The objective of this study is to investigate the chemical nature of vine weevil aggregation and to identify behaviourally active compounds, so that a complete stimulus can be used to lure the adults into traps containing a fungal pathogen such as *Metarhizium anisopliae*, which has strains known to be extremely effective against coleopterous insects.

A highly efficient approach to the identification of insect semiochemicals is to link electrophysiological recordings from the antenna to high resolution capillary gas chromatography (GC). The work on *C. assimilis* and *S. lineatus* initially employed the electroantennogram (EAG), but was further refined by recording from single cells within the antennal olfactory organs. In this study, the antennal morphology of the vine weevil is examined and EAG preparations are investigated. An assay to demonstrate

aggregation behaviour is devised and coupled GC-EAG of volatiles from weevils aggregating in artificial refuges is used to locate components with neurophysiological activity for identification and investigation in behavioural assays.

Methods

Insects. Adult vine weevils were maintained in the laboratory and fed on the foliage of yew, *Taxus baccata* (Taxaceae).

Collection and analysis of volatiles. Rolls of cellulose fibre (corrugated cardboard) on which adult vine weevils had been allowed to aggregate were extracted with redistilled ether. The product was vacuum distilled as described previously (Pickett & Griffiths, 1980) and sealed in glass ampoules under nitrogen. Volatiles were analysed and identified by GC and coupled GC-mass spectrometry (MS) (Pickett, 1990).

Chemicals. Compounds for EAG studies were obtained commercially or synthesised by standard methods and were diluted with purified hexane to give 10^{-5} g in 10 μl.

Antennal morphology. Excised heads of adult weevils were attached to an aluminium stub with double-sided tape. The specimens were gold-coated in a sputter coater (Emscope AE 1231) and examined using a scanning electron microscope (Hitachi S450).

Electrophysiology. Standard electrophysiological techniques were used to obtain EAG recordings from excised antennae. The coupled GC-electrophysiological system is described by Wadhams (1990).

Behavioural assay. Rolls of cellulose fibre (corrugated cardboard), 15 cm long × 10 cm wide, were used as artificial refuges. Two types of refuge were used in the assay: 'old' refuges, on which weevils from the stock culture had been allowed to aggregate for several weeks before removal, and 'new' refuges which had not been exposed to weevils. One of each was placed at either end of a Perspex box, 30 cm × 15 cm. A piece of yew foliage was laid between the two refuges and six weevils were placed in the box. There were ten replicates and the experiments were set up between 19.30 and 21.00 hrs, when weevils were active. In the following morning, lights were switched on and 15 minutes allowed for the weevils to hide. Weevils were assessed as hiding in an 'old' or a 'new' refuge or amongst foliage and numbers were analysed by Duncan's NMRT.

Results and discussion

The behavioural assay on *O. sulcatus* (Table 1) clearly demonstrated that cellulose fibre

Table 1. Behavioural bioassay with *Otiorhynchus sulcatus*.

Experiment number	Mean number of weevils per refuge*			
	'Old' refuge	'New' refuge	Foliage	S.E.D.
1	5.00 a	0.75 b	0.25 b	0.54
2	4.25 a	1.50 b	0.25 b	0.84
3	3.75 b	1.25 b	1.00 b	1.34
4	4.00 b	1.75 b	0.50 b	1.21
Overall means:	4.25 a	1.31 b	0.50 b	0.42

* Means followed by a different letter are significantly different (P = 0.05).

refuges previously occupied by adult weevils stimulated aggregation by other weevils, confirming the presence of an aggregation pheromone or other attractants. The vacuum-distilled ether extract of the refuges elicited significant EAG responses and coupled GC-EAG located 20 components having some electrophysiological activity. Three of the most active peaks were identified by GC-MS as being associated with hexanoic acid, heptanoic acid and vanillin (4-hydroxy-3-methoxybenzaldehyde). Identity was confirmed by peak enhancement on GC and each compound was shown, as an authentic commercial product, to have EAG activity (hexanoic acid: 0.4 mV $\pm$ 0.08; heptanoic acid: 0.3 mV $\pm$ 0.06; vanillin: 0.4 mV $\pm$ 0.05). Tentative identifications of a number of other electrophysiologically active peaks, including the unusual sesquiterpene hydrocarbon α-muurolene, have been obtained and the role of these compounds will be assessed in behavioural assays.

Preliminary EAG studies using known plant volatiles showed responses of 0.7-1.0 mV to racemic linalool, methyl salicylate, (*Z*)-3-hexen-1-ol, racemic 1-octen-3-ol, benzyl alcohol, 1-hexanol, (-)-myrtenal, 4-allylanisole and 2-phenylethanol. (*E*)-β-Farnesene, 4-pentenyl isothiocyanate and the solvent control (hexane) elicited less than 0.2 mV. These compounds represent a range of stimuli likely to be encountered by a phytophagous insect interacting with hosts and non-hosts. Specific semiochemicals mediating interactions of the vine weevil with its host plants will be identified by further GC-EAG studies.

The antenna of the vine weevil is geniculate and comprises a scape, pedicel and a flagellum, itself comprised of six antennomeres and a club. Constriction bands divide the club into four clavomeres. Scanning electron microscopy showed that the sensilla are much denser on the club and that more sensillar types occur there. Six types of cuticular structure were found. 1) *Sensilla chaetica*: these hairs have a wide diameter, are slightly tapered, bear deep longitudinal ridges and emerge from well defined sockets. They are found over the whole length of the antenna and subtend an angle of around 60° with the antennal surface, so that they protrude above all the other sensilla. On the club, they occur in a ring on the distal half of each clavomere. 2) *Sensilla trichodea*: curved hairs of narrow diameter, tapering to a fine point. They are longitudinally striated and occur on all antennomeres. 3) *Sensilla basiconica I*: smooth hairs, tapered to a fine point, bending in one direction halfway along their length and again, in the opposite direction, towards the distal end. These sensilla occur only on the club, where they are found in bands on the proximal region of clavomeres two and three. 4) *Sensilla basiconica II*: straight, smooth, peg-like sensilla with blunt tips, occurring only on the club. They are scattered over all the clavomeres, but occur mainly in bands on the distal portion of clavomeres one and two. 5) *Sensilla basiconica III*: cone-shaped pegs, emerging from raised areas of cuticle. These sensilla are few in number and scattered irregularly over the club. 6) *Domed sensilla*: these resemble campaniform sensilla in external morphology and occur in groups on the apical clavomere.

Sensilla chaetica and sensilla basiconica are found on the antennae of other curculionids; for *C. assimilis*, it is suggested that the former are involved in contact chemoreception (Isidoro & Solinas, 1992) and in *Hylobius abietis*, sensilla basiconica I and II have been shown to have an olfactory function (Mustaparta, 1975). The role of these sensilla in the chemically-mediated behaviour of vine weevil will be studied using single-cell recording techniques.

Conclusions

The study to date confirms that aggregation of vine weevils is stimulated by the presence of a pheromone or other attractants and shows that the vacuum-distilled ether extract of 'old' refuges contains neurophysiologically active components. GC-EAG of this sample has pinpointed active compounds, a number of which have subsequently been identified by GC-MS and comparison with authentic samples. Work is now in progress to

investigate the behaviour associated with these compounds and to identify other electrophysiologically active components from the refuges. Attempts are being made to define which of these arise directly from the refuges and which are produced as insect metabolites and may, thereby, comprise an aggregation pheromone.

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Casual experiences of vine weevil biology before, during and after carrying out control tests

Introduction

The vine weevil (*Otiorhynchus sulcatus*) has become one of the most significant pests in northern Germany's nurseries, especially in those which cultivate a wide range of host plants in pots or other containers. As a practical foundation of our nursery advice in the world's largest connected nursery area (>4,000 ha), in Schleswig-Holstein near Hamburg, we are carrying out chemical and biological control tests against the pest.

To obtain the required eggs, larvae and adults in viable and large enough numbers for these control tests, the rearing of a captive vine weevil population was necessary and useful. Rearing, breeding and the analysis of numerous control experiments gave the opportunity for studying the pest's biology in its different developmental stages. In general, observations were made casually but, nevertheless, they might give confirmation or show differences from observations made by author who have made comparable studies. Some of these experiences (from 1990 onwards) are presented here.

Methods

Rearing vine weevil under laboratory conditions

The method used for rearing a vine weevil population under controlled conditions must be favourable to the pest, and also economic for the people who have to do the work. The following method was used, under which conditions up to 500 adults, separated by origin and age, were kept:

- *source of population*: different origins, collected annually (mostly as larvae) from soil or substrata of pots and containers in infested nurseries;
- *cage*: several 20 × 20 × 10 cm plastic boxes, each covered with a gauze lid for ventilation and containing 75 weevils;
- *diet*: fresh foliage of *Prunus laurocerasus* 'Herbergii', placed at the bottom of each cage, renewed at intervals of 2–3 days; the foliage was also used by the adults for hiding

during the day.

- *temperature and humidity*: room conditions ± 18° C (in summer sometimes higher!) but lower at night, and between 55 % and 85 % r.h.
- *light*: normal daylight plus additional lighting in winter, from 19.30 hrs to 17.00 hrs.
- *water*: Petri dishes were placed in each cage and filled with absorbent paper to hold the drinking water;
- *egg collection*: removed periodically from the bottom of the cages
- *reproduction*: a) egg transfer to a cultural medium (peat moss) of containerized plants (mostly *Euonymus fortunei* cultivars because of their easy propagation), kept in large cages in the glasshouses; b) as further development from material in untreated control in tests after inoculation with eggs or larvae.

Results and discussion

Adult longevity

In June 1990, immediately after their emergence, 303 adults were placed under laboratory conditions. The oldest weevil died in November 1994, having reached an age of 4½ years (54 months). Thiem (1932) reported a maximum survival of 26 to 32 months. More than half of their origin or year of emergence (Figures 1 & 2). This observation corresponds to the statement by Thiem that adults normally survive for 18 months. Mortality in our cultures was particularly high after periods of oviposition.

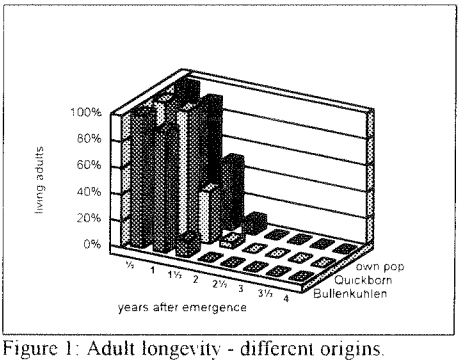

Figure 1: Adult longevity - different origins.

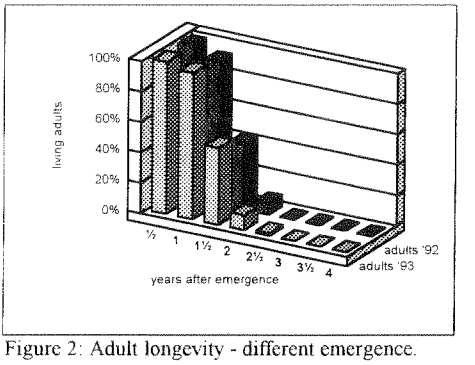

Figure 2: Adult longevity - different emergence.

Preoviposition, maturation feeding and egg laying (1993–1995)

– *Preoviposition*: under the prescribed rearing conditions, young adults needed 3–8 weeks after emergence before they produced their first eggs. These are within the range of 25 days (fed on *Taxus cuspidata*) and 62 days (fed on *Cornus florida*) cited by Maier (1981). In addition to its dependence on diet, the preoviposition time is related to temperature, Klingler (1959) giving the following details:

21–39 days for weevils collected from the glasshouse (15–21°C)

29–40 days for weevils collected outdoors (20°C)

9–21 days for overwintered weevils (18–20°C).

Unfortunately, the exact temperature range in our studies (although about 18°C) was not known.

– *Maturation feeding*: young (callow) adults needed to feed for about 2–7 weeks before they were able to lay eggs; older (overwintered) adults required 0–7 days, These results are similar to those of Brüning (1980), who found that callow weevils required 25 days at 24°C and 61 days at 12°C. Hering (1960) stated that an overwintered adult requires no feeding time before ovipositing. During the year, feeding by adults seems to be interrupted twice: in April/May/June and in October/November.

– *Egg-laying*: the period of egg laying appeared to be interrupted at the same time as feeding (namely, April/May/June and October/November), resulting in two peaks of oviposition in the year. In some cases, there was also a short interruption during the summer egg-laying period (possibly related to unsuitably high temperatures). An autumn interruption has already been reported by Klinger (1959) (in laboratory studies) and by Hering (1960) (in both laboratory and outdoor studies). In contrast, Stemseth (1979) reported three egg-laying periods in glasshouses of about 70, 90 and 60 days. Cram (1965) achieved continuous egg production for 30 weeks (range 27 to 32 weeks), when adults were fed on strawberry leaves. This may be a more suitable diet than the *Prunus laurocerasus* 'Herbergii' used in our studies.

Egg viability and hatching

Vine weevil eggs are of two kinds: white eggs and brownish eggs, the latter with melanized chorions. Montgomery & Nielsen (1979) proved that the former were infertile. This was also our experience and we used only melanized eggs for trials. Under optimum conditions, we achieved almost 90% egg hatch (at 25°C and 100% r.h. in the laboratory). When eggs were

added to culture media (peat moss), containing *Euonymus fortunei*, the hatching rate decreased to about 50%.

In one trial, egg hatch reached only 25%. This trial was done in summer 1995, which was very hot in Germany. For a long period, temperatures were relatively constant at 30°C, with only small outdoor and indoor fluctuations; the same was true in our plastic cages containing adults. These high temperatures probably affected viability of the eggs used to inoculate the culture media. Reduced egg survival above 27°C has been reported by Montgomery & Nielsen (1979).

Oviposition sites

Twenty-five adults were kept in a cage for 11 weeks, with an artificial tree as a carrier of fresh leaves of *Prunus laurocerasus* 'Herbergii'. Instead of using foliage for daytime refuges, the weevils were required to use a raised base plate on the floor of the cage. For egg laying, the weevils were offered three different, dampened media in Petri dishes. These, in increasing order of attractiveness, were: bark mulch (13 eggs laid), sand (74 eggs laid) and peat moss (151 eggs laid). Very few eggs were found beneath the bottom plate. This result suggests that adults prefer to oviposit in specific media. Hering (1960), however, found that eggs were laid in situations where adults spent the day.

Acknowledgement

I would like to thank Elke Mester for technical assistance.

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Modelling vine weevil population dynamics

Introduction

The vine weevil, *Otiorhynchus sulcatus* F., is an important pest of a wide range of crops in many countries. Over 140 known plant hosts have been identified (Warner & Negley, 1976). Damage is caused primarily by larval stages feeding on the roots of the host plant, although adult feeding on the foliage of the host can impair its appearance and, hence, value.

Traditionally, vine weevil has been controlled using persistent insecticides aimed at killing larvae and adults. However, difficulty in controlling vine weevil with some pesticides have been reported (Nielsen & Steinhauer, 1976) and it is often not possible to apply pesticides because of cultural considerations, e.g. on soft fruit emergence of adult vine weevils often coincides with the beginning of fruit harvest.

Consequently, much research has focused on the role/impact of natural enemies on vine weevil populations. Vine weevils have many natural enemies, including mammals, birds and amphibians, although most research has concentrated on predators, entomophagous fungi and parasitic nematodes. Varying levels of vine weevil control have been reported using natural enemies dependent upon several factors, in particular the synchronicity of susceptible vine weevils stages with suitable numbers of natural enemies.

Determining the phenology and availability of insect life stages susceptible to chemical and biological control is expensive and laborious. Computer models have been used to improve the process (Morgan & Solomon, 1993). Computer models can be used to not only predict the emergence of different life stages of a pest but also the impact of different control tactics upon populations of the pests (Campbell, et al., 1993).

The objective of this paper were to develop a computer model to study factors affecting the phenology and population dynamics of vine weevil.

The Model

A deterministic computer model was developed with temperature as its driving variable and a time step of one day.

The life cycle of the pest was represented for each life stage; eggs, larvae pupae and adults. Adults were further represented by two stages; pre- and reproductive mature adults. A method similar to a 'boxcar train' (de Wit & Goudriaan, 1976) was used to simulate development within and between stages and the fecundity of reproductive adults. All development and reproductive rates were calculated using data from Stenseth (1979).

Simulations

All simulation for this study started on day 1 (January 1st) and were initialised with 100 larvae (although the model contained algorithms to start simulations on different dates with varying numbers of all life stages).

In the first set of simulations the population dynamics of vine weevils were simulated using temperatures recorded in a Stevenson thermometer screen and at 5cm in soil. The temperature data were recorded from the meteorological station at Broom's Barn Experimental Station, UK in 1981 and 1982. The soil temperature data were used for subsequent simulations.

In the second set of simulations the effect of small change in temperature were investigated by increasing daily maximum and minimum by 1°C.

In the final set of simulations adult development rates, the duration of immature stages, survival of all life stages and fecundity were investigated. The impact of increasing adult development rates (20%), immature duration (20%) and mortality (5%), and decreasing fecundity (20%) on vine weevil abundance were tested. Additionally, the effect of increasing pupal mortality (30%) was also investigated.

The timing of first pupae, adult and reproductively mature adults and maximum number of larvae were used as performance criteria for the simulations.

Results

The emergence of pupae, pre-reproductively mature and reproductively mature adults occurred later in 1981 than in 1982; the emergence of pupae was about 6 days later, while the emergence of both pre-reproductively mature and reproductively mature adults occurred about 20 days later in 1981 than in 1982 (Table 1). Similarly, the cooler temperatures experienced in 1981 facilitated a smaller maximum number of larvae than in 1982 (36,686 in 1981 to 53,242 in 1982).

Increasing temperature had little effect on vine weevil population development; increasing daily maximum and minimum resulted in the emergence of pupae and adults being brought forward by a maximum of 5 days, while the peak number of larvae was increased by about 8% (Table 1).

Table 1. The development of population in vine weevils in 1981 and 1982, and the effect of changes in various population parameters on the phenology of life cycle events and larvae abundance.

Simulation	Emergence day of Pupae	Pre-reproductive adults	Reproductive adults	Maximum number of larvae
1981	82	166	205	36,686
1982	76	146	184	53,542
+1°C	72	143	179	57,898
+20% adult development rate	76	146	178	50668
+20% duration of immature stage	113	148	193	28598
+5% mortality rate of all stages	76	146	184	46013
+20% reproductive rate	76	146	184	50823
+30% pupal mortality rate	76	146	184	47369

Changing adult development rate, mortality, fecundity and pupal survival had no effect on the timing of pupal emergence and had little effect on the phenology of pre-reproductive adults, although increased pupal mortality delayed the emergence of pre-reproductive and reproductive adults by about 4 days (Table 1). Conversely, increased adult development rates brought forward the maturity of adult vine weevil by about 6 days (Table 1). The greatest effect on vine weevil population development was observed by altering the duration of

immature stages; the emergence of pupae and adults were delayed by about 25 and 11 days, respectively (Table 1).

Although altering each variable had little impact on the timing of events in the life cycle of vine weevil they did have varying effects on maximum number of larvae. The least effect occurred with altering adult development rates and reproduction; peak larvae abundance only decreased approximately 5% for both variables (Table 1). The greatest effect occurred by altering the duration of immature stages; maximum number of larvae was increased by about 50% (Table 1). Changing mortality rates of all stages and only pupae had intermediate effects; peak numbers were decreased by about 15 and 12%, respectively (Table 1).

Discussion

Computer models of the population dynamics of insect pests have been used in conjunction with crop breeding programmes to identify crop varieties with suitable characteristics for resistance to pests (Campbell, et al., 1993). The simulations of the vine weevil have indicated parts in the life cycle of vine weevil which could be manipulated in order to cause the largest reductions in the populations of the pest. Changes to the duration of immature stages, and to a lesser extent to the mortality of all stages and in particular to pupae, would cause relatively large reductions to abundance of the pest. Conversely changes in adult development rates and fecundity would have little impact on pest density.

Although the model is only in a prototype form it is already posing interesting questions in the development of vine weevil populations and has signalled those aspects of biology of the pest where rewards for further investigation are likely to be greatest, and those where additional study may be inappropriate.

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Reduction in vine weevil larval growth by mycorrhizal fungi

Introduction

The vine weevil, *Otiorhynchus sulcatus*, is one of the most important pests of soft fruit and horticultural crops in the U.K. For some years, the insect was successfully controlled with the persistent insecticides such as aldrin. However, these insecticides have serious effects on many non-target organisms, and the banning over recent years of aldrin and others such as DDT has left the horticultural industry in a very vulnerable position. Currently, the insect is moderately well controlled by a number of soil-applied insecticides, but it is generally accepted that complete reliance on chemical control is very dangerous, not only for environmental concerns but also because of the possibility of the pest becoming resistant to the compound.

Recently, research has focused on biological methods of vine weevil control. Weevil larvae may be killed by several species of nematode or by pathogenic fungi. The nematodes and fungi are very effective; however, they are expensive to produce and are very susceptible to variations in soil type, temperature, application time and host plant (Stimman *et al.* 1985). Nematodes are efficient in controlled conditions such as glasshouses but are less so in field-grown crops, such as strawberry. Chemical control in the field is moderately effective, particularly with the introduction of slow release insecticides. However, these chemicals are relatively immobile in soil, meaning that large parts of the root systems may be unprotected and thus attacked by weevil larvae. Specialised application methods have been developed for nematodes, but as with chemical control, these need to be continually re-applied. Once the nematodes have killed all the larvae, they too will die, as they cannot persist in soil without a host. As strawberry is a perennial crop and weevil attack is likely at almost any time, there is a serious need to develop a biological *protection* system, rather than a *control* system.

It has recently been found that naturally occurring arbuscular mycorrhizal (AM) fungi may protect plants against phytophagous insects (Gange & Bower in press), including vine weevil (Gange, Brown & Sinclair 1994). Their mode of action is unknown, but it is likely that plant resistance is enhanced by fungal-induced changes in plant biochemistry. Resistance to adult weevils has been reported in some strawberry clones (Moorhouse, Charnley & Gillespie 1992), but resistance to the more destructive larval stage is unknown. This paper aims, therefore, to discuss the potential of AM fungi as bioprotectants of strawberry against vine weevil.

Methods

Experiment 1

Sixty seedlings of *Taraxacum officinale* were planted singly into sterilized compost in 13cm pots. Mycorrhizal inoculum was added to half the pots by the incorporation of 2g of clay granules from a single-species culture of *Glomus mosseae*, with an infectivity of 430 ± 51 fungal propagules g^{-1}. Control plants received sterilized granules. Plants were grown for 16 weeks at 20°C and 18 L:D. After this time, plants were infested with newly hatched weevil larvae. These were added at the rates of zero, two and eight per pot, equivalent to 0, 150 and 600m^{-2}. There were 10 replicates of the six treatments (3 levels of weevil infestation × 2 levels of fungal infection). Plants were maintained at 20°C for 10 weeks, when root material was washed free of soil and the number of live larvae in each pot counted and weighed.

Experiment 2

Eighty strawberry plants (cv. Red Gauntlet) were produced by pegging a single runner into 13cm pots filled with sterilised compost. The plants producing the runners had been created in the same way, and all originated from one plant, thus giving clonal material. Before pegging, 2g of granules from a single species culture of *Glomus mosseae* or *G. intraradices* were added to the pots, in a layer 2cm below the surface. In the case of two-species inoculation, 1g of each culture was added. The infectivity of the granules was *G. mosseae*: 376 ± 49 and *G. intraradices* 402 ± 56 fungal propagules g^{-1}. Sterilised granules were added as a control, thus giving four fungal treatments in a factorial design.

Plants were allowed to establish for 10 weeks, when in each fungal treatment, 10 pots were or were not infested with 10 mature (brown) vine weevil eggs. There were thus 10 replicates of eight treatments (4 fungal treatments × 2 weevil treatments). Plants were maintained at 20°C for 10 weeks, after which time roots were washed free of soil and all live larvae counted and weighed.

Data were analysed using plants as replicates. Prior to Analysis of Variance, all data were tested for normality and homogeneity of variances. The arc sine transformation was used to normalise percentage data.

Results

In experiment 1, the infection of roots by *G. mosseae* reduced weevil larval survival in the low density larval pots by 52%, while in the high density treatments it was reduced by 40%, relative to controls (no fungus) (Fig. 1). These differences were highly significant ($F_{1,36}$ = 22.5, $P < 0.001$).

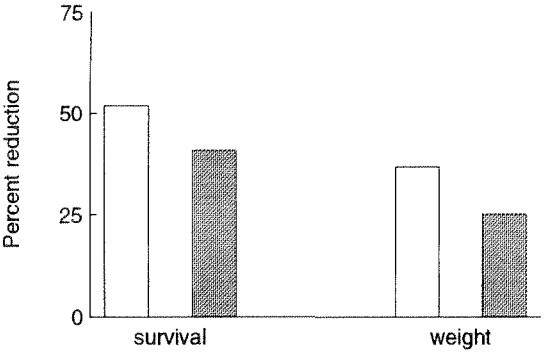

Fig. 1: Per cent reduction in vine weevil larval survival and weight by *Glomus mosseae* on *Taraxacum officinale*. Open bars: low weevil density ($150m^{-2}$); shaded bars: high density ($600m^{-2}$).

Weevil larval growth, as measured by wet weight at harvest was also reduced by the fungus. In low-density pots there was a 37% reduction compared with growth on fungus-free plants, while in high-density pots growth was reduced by 25% (Fig. 1). These again represented a significant effect of fungal presence on weevil performance ($F_{1,32} = 9.01$, $P < 0.01$).

In strawberry (experiment 2), *G. mosseae* again resulted produced reductions in larval growth and survival. In addition, *G. intraradices* when present as a single infection had comparable effects (Fig. 2). However, a notable feature of these results was that when both fungi were present together, the antagonistic effects on the insect disappeared, so that larval performance in this treatment were not different from controls. This meant that the percentage reduction in performance relative to controls was negligible (Fig. 2).

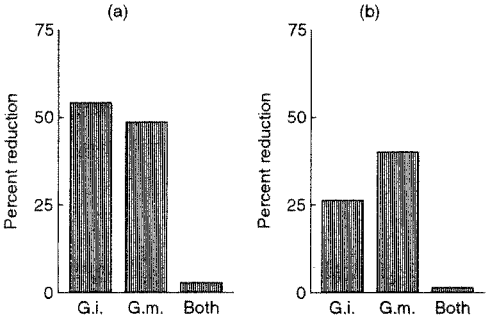

Fig. 2: Per cent reduction in vine weevil larval survival (a) and weight (b) by either *G. intraradices* (G.i.), *G. mosseae* (G.m.) or both fungi on strawberry.

When the survival and weight data were analysed, there was no significant main effect of either fungus, but there were significant interactions (survival: $F_{1,36} = 15.5$, $P < 0.001$; weight: $F_{1,36} = 16.9$, $P < 0.001$). Linear contrasts were performed to determine if each single infection mean differed from the controls. In all cases, these comparisons were significant ($P < 0.01$), and, thus, single infections were antagonistic to the larvae, whereas multiple infections were not (Fig. 2).

Discussion

It is clear that mycorrhizal fungi have the potential to halve vine weevil growth and survival. In field conditions, this result may be particularly important, because larvae need to attain at least the third instar (out of six) to overwinter successfully (Moorhouse *et al.* 1992). Therefore, larvae feeding on mycorrhizal plants would be much less likely to survive the winter, because of death from natural enemies, drought or frost.

One might expect strawberry, as a perennial crop, to be highly infected with mycorrhizal fungi in the field. However, a standard husbandry procedure is to sterilise a field to remove root-pathogenic fungi and weevils, followed by planting out of nursery-raised stock. Gange (unpublished) surveyed five such fields and found the average infection level of five cultivars to be less than 2% of the root system infected, after one year. This is extremely low compared with the level of 34% achieved in experiment 2. However, it is quite practical to inoculate strawberry plantlets with mycorrhizas at the nursery stage and in Finland this has led to increased growth and fruit yields in the laboratory and field (Niemi & Vestberg 1992). Indeed, the technology is now available to produce inoculum commercially for other crops such as tomatoes and cucumbers, producing benefits to growth and yield (Clover 1994). Mycorrhizas have great potential as biological growth enhancers; the fungal species have global distributions and the species identified as infecting strawberry in California and Finland also occur in UK soils.

However, the culturing of mycorrhizal fungi is problematic, as they can exist only in a symbiotic partnership with a host plant, in which carbon compounds are directed to the fungus. Therefore, consideration should be given as to the mechanism of resistance which these fungi induce in plant roots. *G. intraradices* and *G. mosseae* have been shown to induce the production of many compounds, including phenols, flavanoids, terpenoids and polypeptides in roots (Gange & Bower in press). Identification of the antagonistic chemicals and the mechanisms responsible for their production may enable us to produce varieties of strawberry which show resistance to this pest. Resistant varieties could then be grown in integrated control systems, making control by other biological means, such as nematodes or entomopathogens, more effective. In addition, the use of resistant varieties would lessen our

reliance on chemical control, meaning that inputs could be reduced, and reducing the probability of pesticide resistance arising.

Growers realise the urgent need for an alternative strategy for weevil control, which can be linked with reduced chemical inputs and, hence, costs (Anon. 1995). AM fungi could act as a novel biological protectant method for strawberries. The fungi are living systems which extend through the soil as the root grows, and will not die if weevils are absent. Unlike nematodes or pathogenic fungi, they can provide growth benefits even if the weevil is absent. In glasshouse crops, application of mycorrhizas is cost effective (Clover 1994) and in California, mycorrhizas have been shown to be of great benefit in the development of low-cost, high-yield organic strawberry production (Gliessman *et al.* 1994). Development of the techniques in a highly mycorrhizal crop such as strawberry will allow us to extend the knowledge to other situations, such as hardy nursery stock, where the weevil is a great pest and current control methods expensive.

Acknowledgement

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Studies on the survival of *Otiorhynchus sulcatus* larvae in peat substrates

Introduction

Occasionally, it is thought that pests, e.g. Sciaridae or vine weevils, may be spread into greenhouses or containerized prodcution areas by horticultural peat substrates. In contrast, substrate producers claim that even if there might be a case of contamination, larvae of *Otiorhynchus* spp. may not be able to survive the processing, storage and transport of substrates. Several experiments were designed to study the ability of *O. sulcatus* larvae to survive in dry and moist peat under two different temperature regimes (15 and 20°C) without additional food.

Methods

Several (3 litres capacity) containers were filled with 2 litres of peat substrate (Floradur B) each. One half of the containers additionally received 0.5 litres of tap water. In one part of the experiment, larvae (L3/L4) of *O. sulcatus* were taken from a culture and 10 individuals added per container. In another part of the experiment, five adult vine weevils were added per container and allowed to deposit eggs for one week; the weevils were then removed. Each treatment consisted of 10 replicates. After adding the larvae or removing the adult weevils, the containers were stored at constant temperatures of 15 and 20°C, respsectively. Larvae (L3/L4 and neonate) in control containers were fed on corms of *Cyclamen persicum*.

Results (Table 1)

No larvae were able to pupate in any treatment without the addition of food. Eggs of *O. sulcatus* hatched but, in the absence of food, the neonate larvae died within a few weeks. The length of survival of larvae was dependent on temperature. No larvae survived a period of 5 weeks in dry or moist peat substrates. At 20°C the length of survival was even shorter, compared with the 15°C treatment.

Table 1: Length of survival of larvae of *Otiorhynchus sulcatus* in peat substrates kept at different temperature and moisture regimes (% of control).

Larval stage	Time (weeks)	Survival at 15°C		Survival at 20°C	
		dry	moist	dry	moist
L3 and L4	2	-	-	33%	20%
	3	29%	22%	1%	0%
	4	18%	13%	0%	0%
	5	4%	1%	-	-
	6	0%	0%	-	-
Neonate larvae	2	-	-	7%	1%
	3	13%	13%	0%	0%
	4	7%	2%	-	-
	5	1%	0%	-	-
	6	0%	0%	-	-

Conclusions

These data indicate that spreading of larvae of *O. sulcatus* in fresh peat substrates is very unlikely. The probability that larvae will survive the procedures of processing and, additionally, storage and transport of a substrate may be considered extremely low. The more probable way of infesting horticultural crops with vine weevil, is through importation of contaminated plants or root balls or by spreading of adults.

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Seasonal activity and selection for host plants of several different *Otiorhynchus* species in Switzerland

Adults of *Otiorhynchus* were collected in about 100 private gardens in Switzerland from 24 August to 19 October 1994 and from 31 March to 02 June 1995. Five economically important species were found: *O. crataegi, O. ovatus, O. rugosostriatus, O. singularis, O. sulcatus,* Thirty-four plant species from 20 families were identified as potential hosts. Most hosts, including Ericaceae (*Rhododendron*), Primulaceae (*Primula*), Rosaceae (*Cotoneaster, Prunus, Rosa*), and Saxifragaceae (*Astilbe, Hydrangea, Ribes, Saxifraga,*), suffered noticeable leaf damage.

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Microbial control of vine weevil

Introduction

Over the past 10-15 years, vine weevil, *Otiorhynchus sulcatus*, has become a serious pest, mainly in ornamental plants and hardy ornamental nursery stock. The failure of chemical pesticides, the banning of persistent chlorinated hydrocarbons and the increasing distribution of the insect by infested potted plants have lead to an urgent need to develop new and safer pest control methods. Therefore, research on biological control strategies against vine weevil were started some years ago. In this paper, recent investigations on microbial control of vine weevil using entomopathogenic fungi are described.

Naturally occurring pathogens of vine weevil

Entomopathogenic fungi are the most common pathogens isolated from larvae and adults of *Otiorhynchus* spp. (Zimmermann 1981). These include *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *M. flavoviride*, *Paecilomyces farinosus*, *P. fumosoroseus* and *Verticillium lecanii*. The most important species causing heavy infestations of larvae in field populations are *M. anisopliae*, *B. bassiana* and *P. fumosoroseus* (Marchal 1977, Zimmermann 1981, Mietkiewski *et al.* 1992).

Laboratory experiments

Experiments in the laboratory to select virulent strains have been done by several authors (see Zimmermann & Simons 1986, Moorhouse *et al.* 1990). In bioassays, the species *B. bassiana*, *M. anisopliae*, *M. flavoviride*, *P. farinosus* and *P. fumosoroseus* were tested against eggs or larvae. In general, spore concentrations of 10^6, 10^7 or 10^8 ml^{-1} were used. All stages of vine weevil were susceptible to the fungi used; however, adults proved to be more resistant (Zimmermann unpubl.). In all trials, strains of *B. bassiana*, *M. flavoviride* and, especially, *M. anisopliae* showed the highest virulence. Poprawski *et al.* (1985) found that *M. flavoviride* was highly infectious to both weevil eggs and neonate larvae, with an LD_{50} of only 10 conidia ml^{-1}. In other experiments, strains of *M. anisopliae* also showed the highest efficacy

under soil conditions (see Moorhouse *et al.* 1990). Therefore, for further investigations in the greenhouse mainly isolates of *M. anisopliae* have been used.

Greenhouse experiments

Under glasshouse conditions, good to excellent control of *O. sulcatus* larvae after application of *M. anisopliae* conidia on a variety of plant species has been recorded (e.g. Zimmermann 1981, 1984; Stenzel 1992, Moorhouse *et al.* 1993b). Various application forms have been tested, e.g. prophylactic or curative soil treatments as a drench or an incorporation of spores into the soil. In general, a prophylactic use of spore suspensions (10^7 - 10^8 conidia ml^{-1}) or cell granules (1g/l substrate of BIO 1020) resulted in larval mortalities of about 80 - 100%, while a curative treatment several weeks after egg laying showed reduced levels of control (Zimmermann 1981, Moorhouse *et al.* 1990, 1993b). The dose rate is of major importance. Application of 50 ml per pot of an *M. anisopliae* suspension containing $2.8x10^6$, $2.8x10^7$ and $2.8x10^8$ spores ml^{-1} resulted in larval control of 28,5%, 34.5% and 82%, respectively (Zimmermann 1981). Comprehensive greenhouse studies have been conducted with BIO 1020, the first German fungal biopreparation based on *M. anisopliae*. This product was developed by BAYER company for microbial control of vine weevil. In 41 trials against various ornamental plants, the mean efficacy of BIO 1020 after application of 1g/l substrate was about 80% (Stenzel 1992). In all experiments, a long-term effect of *M. anisopliae* of several months was observed (Stenzel 1992, Moorhouse *et al.* 1993b).

Outdoor and Field experiments

There are only a few data on microbial control of *O. sulcatus* in outdoor or field experiments. Isolates of *B. bassiana*, *B. brongniartii* and *P. fumosoroseus* failed to significantly reduce larval populations on strawberry plants in a small-scale field trial (Soares *et al.* 1983). Lateron, Zimmermann & Simons (1986) reported a population reduction of about 30-50% in some outdoor experiments on strawberry and ornamentals after prophylactic treatment of *M. anisopliae*. The low levels of control may be due to suboptimal soil temperatures. Encouraging results from field experiments on strawberries have been obtained by Moorhouse *et al.* (1990). For example, after application of 100 ml of 10^7 or $5x10^7$ conidia ml^{-1}, larval numbers were reduced by 75.3% and 97.2%, respectively. Experiments on outdoor hardy ornamental nursery stock species were reported by Stenzel (1992) and

Moorhouse *et al.* (1993a), resulting in an efficacy of *M. anisopliae* of 68% and zero to 96%, respectively.

Important factors affecting the efficacy of microbial control

Besides the selection of virulent strains and the development of suitable formulations, there are other environmental and soil factors which may interfere with a fungal biopreparation and which may be responsible for the efficacy of an entomopathogenic fungus. These soil factors are: (1) Temperature, (2) moisture, (3) substrate, (4) pH and (5) the host plant.

The importance of **soil temperature** for the activity of entomopathogenic fungi against *O. sulcatus* has been pointed out by several authors (Soares *et al.* 1983, Moorhouse *et al.* 1990, Hartwig & Oehmig 1992,). Most isolates of *M. anisopliae* have a temperature range for growth of about 15° to 32°C, with an optimum of about 25°C. Laboratory and greenhouse experiments at different temperatures, e.g. 10°, 15°, 20° and 25°C, have demonstrated that most of the species/strains tested were highly infective at 20° and 25°C. However, at 15°C the efficacy against larvae of *O. sulcatus* often was reduced. No mortality was recorded at a permanent temperature of 10°C. However, Hartwig & Oehmig (1992) proved that at fluctuating temperatures from 5° to 20°C a short "warm phase" is sufficient to obtain infections.

The effect of **soil moisture** of BIO 1020 on the mortality of *Tenebrio molitor* larvae as test insect was studied by Hartwig & Oehmig (1992). They found that at normal (60% WC_{max}) and very moist (90% WC_{max}) soil conditions, no influence on the efficacy was observed. Under extreme dry conditions near the wilting point of plants (30% WC_{max}), only a slightly reduced activity of the cell granulate was noted.

The effect of different **soil substrates and potting media** on the efficacy of *M. anisopliae* was also tested. Moorhouse *et al.* (1992) found significant differences in the behaviour of four *M. anisopliae* strains in field soil and peat compost, e.g. curative applications of *M. anisopliae* conidia to strawberry plants in field soil reduced the number of *O. sulcatus* larvae by up to 94% while similar treatments in peat compost resulted in a mortality of 53%. On the other hand, Hartwig & Oehmig (1992) tested several commercial, horticultural substrates with no influence on the efficacy of BIO 1020 against *T. molitor* larvae.

Depending on their composition, soil substrates may also have different **pH** values which may influence the activity of a fungal pathogen. In general, *M.*

anisopliae is able to grow in a wide range of pH values. In greenhouse experiments, Zimmermann (1981) found that *M. anisopliae* was highly effective in an acid peat soil (pH 3.4-3.8) as well as in a neutral compost soil (pH 6.4-6.7). Also, Hartwig & Oehmig (1992) did not observe any influence of different soil substrates with pH values from 3.1-7.1 on the mortality of *T. molitor* larvae caused by BIO 1020. These findings indicate that pH is of minor importance.

The first observation on the influence of the **host plant** on the efficacy of *M. anisopliae* was given by Zimmermann (1984). He noticed that there was a reduced efficacy in potted cyclamen. In tests with *M. anisopliae* on a range of outdoor hardy nursery stock species, e.g. *Skimmia*, *Viburnum*, *Hydrangea* and *Thuja*, Moorhouse *et al.* (1993a) found that larval control was species dependent. However, in 41 greenhouse experiments on various ornamental plants, BIO 1020 did not show - except on ivy - a significant difference in its efficacy (Stenzel 1992).

Conclusions

During the last 10-15 years, experiments with entomopathogenic fungi against the vine weevil have demonstrated that an effective microbial control is possible. Especially, by prophylactic application of *M. anisopliae* into soil substrates, the larval numbers are effectively reduced, mainly under greenhouse conditions. Following the withdrawal of BIO 1020, new approaches for the development of microbial preparations are now necessary in order to have additional long-term biological control measures.

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Prospects for biological control of black vine weevil (*Otiorhynchus sulcatus*) in nursery stock

Introduction

Control of black vine weevil still is a worldwide problem in many ornamentals and in some fruit crops such as blueberries, cranberries and strawberries. This pest cannot be controlled completely, despite the use of several chemicals. A diminished weevil population can build-up very quickly since the weevils reproduce in great numbers. The adult weevils are active only during the night and damage to the foliage, being of little importance, is often overlooked. Not until the plants start wilting because the larvae have damaged the roots is the extent of the problem realised. At this stage control of the pest is too late to prevent economic damage to the crop. Therefore, many growers spray the foliage on the whole nursery weekly and apply soil chemicals regularly.

New active chemicals providing effective control of adult weevils are rare. Biological control of adult weevils is not yet successful. *Metarhizium anisopliae* and a new strain of *Bacillus thuringiensis* have potential to control weevils but some application problems have to be solved to make them effective in commercial practice.

Most effort and progress for the control of this pest organism was achieved with the control of the larvae. Some new chemical substances are available and under development. Biological control with insect-parasitic nematodes is improving. One problem for effective control with nematodes outdoors is the low soil temperature in autumn and spring. The efficacy of many strains of nematodes is limited by soil temperature (Van Tol 1993a, 1993b, 1994; Westerman & Van Zeeland 1989, 1994). Biological control with the fungus *M.anisopliae* (a granular product of Bayer (BIO1020)) also proved to be effective (Reinecke 1990; Van Tol 1993a, 1993b) for larval control. The costs for marketing this formulation are still too high.

In this article the possibilities and problems for the use of some commercially available strains of *Heterorhabditis* sp. and *Steinernema* sp. in outdoor conditions are summarized.

Materials and methods

In the pot trials *Thuja occidentalis* 'Brabant' was used as a test plant in 1991 and 1992. In 1993 and 1994 we changed to *Waldsteinia ternata* as a test plant because the natural mortality of larvae was lower with this plant. The plants were potted in May in peat in one litre pots. In 1991 and 1992 we inoculated the plants three times with 15 eggs per pot at two-week intervals. In 1993 and 1994 we inoculated two times with 20 eggs per pot at a three-week interval. The inoculations started at the end of July. Each treatment was repeated 4 times, with eight plants per block. BIO1020 (a commercial formulation of *Metarhizium anisopliae*) was mixed in peat at a concentration of 0.5 or 1.0 g/l soil and incubated at 20°C for 14 days. Carbofuran (Curater lq.; a.i. 200 g/l) was drenched into the soil, one week before and one week after the last inoculation with eggs, at a concentration of 0.75 g a.i./m^2. The nematodes were applied in the last week of September at a concentration of 500,000 nematodes per m^2. In the trial with application of nematodes at different times, the nematodes were applied on 22 September, 5, 14, 21 and 28 October respectively. All the treatments with carbofuran and the nematodes were applied in 25 ml water per pot. In 1991 and 1992 nematodes were applied a second time at the end of October. The soil temperature in the pots was registered with a datalogger (Rologg NT1). At the end of November the number of larvae per pot and the instar composition were recorded. The results were statistically analysed with ANOVA.

In the field trials we *Thuja occidentalis* 'Brabant' was used as a test plant in 1991 and 1992. In 1993 and 1994 we changed to *Taxus baccata* as a test plant. The trials were performed on a peaty soil. In 1991 and 1992 we inoculated three times at two-week intervals, with 50 eggs per plant. In 1993 and 1994 we inoculated twice at a four-week interval. In 1993 we inoculated two times 40 eggs and in 1994 40 and 20 eggs respectively. Carbofuran (Curater lq.) was applied twice in three litre water per m^2 (0.75 g a.i./m^2) (see pot trial). BIO1020 was mixed in peat soil at a concentration of 10 g/l and incubated at 20°C for 14 days. 10 liter of this mixture was mixed in the top 10 cm soil per m^2 before planting the test plants (see pot trial). The nematodes were applied with a watering-can. Three litres of water per m^2, containing 1,000,000 nematodes, were applied in the last week of September. In the trial with different inoculation times, the nematodes were applied on 22 and 27 September and 5, 14 and 21 October respectively. Temperature registration was performed as in the pot trials. At the end of February the number of larvae per plant and the instar composition were recorded.

The results were statistically analysed with ANOVA.

Results

Only the results of the commercially available nematode strains in the Netherlands at this moment are shown, including some results with *Metarhizium anisopliae* (BIO1020).

The results in Table 1 show that there was some variation in efficacy between the years for the nematode strains. This variation was not found for BIO1020. The reason probably was the variation in the composition of the population of weevil larvae at the moment of application of the nematodes (Table 2). In 1993 and 1994 there were relatively more small larvae (L2 and L3) found in the pots than in 1991 and 1992. Smaller larvae are less well parasitized by these nematodes. This is due to rejection of the larvae by the nematodes and because of the reduced chance of finding larvae in the soil. The variation in the composition between the years was caused by a slower development of the larvae in *Waldsteinia* (test plant in pots in 1993 and 1994) than in *Thuja* (test plant in pots in 1991 and 1992). In the field we did not find the same effect as in the pots. Most probably this was because the development of the larvae did not vary so much between the years (results not shown). BIO1020 was mixed in the soil in spring and infects eggs and all instar larvae equally well. Therefore, the results do not vary between the years.

Carbofuran applied as a drench to the soil gave variable results in pots (0 to 98% control) and a consistent result in the field (~40% control). The insect-parasitic nematodes Larvanem and Nemasys H (*Heterorhabditis* sp. (NWE)) gave good control of the larvae in pots and field provided that the majority of the population of the larvae were not too small. The strain Nl-H-F85 (Larvanem) seems to be the better strain compared with UK-H-211 (Nemasys H). The nematodes of Exhibit (*Steinernema carpocapsae*, strain US-S-25) gave only moderate control in pots and no control in the field. BIO1020 (*M. anisopliae*) performed well in pots in every year. In the field the control was limited (50 to 60%). Remarkable was the positive effect of the combination of BIO1020 and Nemasys H in the field (86% control).

Figure 1 shows the results of an outdoor pot and field trial where nematodes were applied at different times. The results confirm the laboratory and climate chamber experiments. As soon as the soil temperature dropped below 12°C there was no control of larvae. Application of nematodes within one day before temperature dropped below 12°C was enough to control the larvae in pots with Larvanem. Nemasys H needed a few days at this temperature to control the

larvae. Both strains were able to reduce the older larval population significantly when soil temperature was less than one day over 12°C after application. In the field the result with Nemasys H were the same as in the pots. In the field, however, the control level was higher than in the pots. Again, the high number of small larvae in the pots was probably the reason for this effect.

Table 1: Percentage reduction of larvae of *Otiorhynchus sulcatus* compared to untreated in pot and field trials from 1991 to 1994.

year	1991		1992	1993		1994	
treatment	pot *	field	pot *	pot *	field	pot *	field
untreated	0 a	0 a	0 a	0 ab	0 a	0 a	0 a
carbofuran	95 cd	40 ab	0 a	98 f	33 b	51 bc	39 b
Nemasys H	96 cd	73 c	88 c	55 c	72 c	40 b	40 b
Larvanem	99 d	-	99 c	78 d	60 bc	65 c	94 c
Exhibit	69 b	-	56 b	38 b	0 a	-	-
BIO1020[1]	85 c	52 b	89 c	92 ef	62 bc	-	-
BIO1020[2]	-	-	93 c	72 cd	-	-	-
BIO1020[2] + Nemasys H	-	-	-	91 ef	86 d	-	-

[1]-Application at a concentration of 1 g/l soil.
[2]-BIO1020 was applied at a concentration of 0.5 g/l soil.
*-In 1991 and 1992 we used *Thuja* as a test plant and in 1993 and 1994 *Waldsteinia*.

Table 2: Composition of the population of the larvae of *Otiorhynchus sulcatus* at the end of the pot trials.

Year	n[1]	%L2[2]	%L3[2]	%L4[2]	%L5[2]
1991	2.2	8	24	31	37
1992	2.4	7	21	21	52
1993	7.7	63	34	3	3
1994	3.3	32	38	30	0

[1]- n is the number of larvae found per pot in the control.
[2]- instar scale used is egg, L1 to L5, pupae, adult.

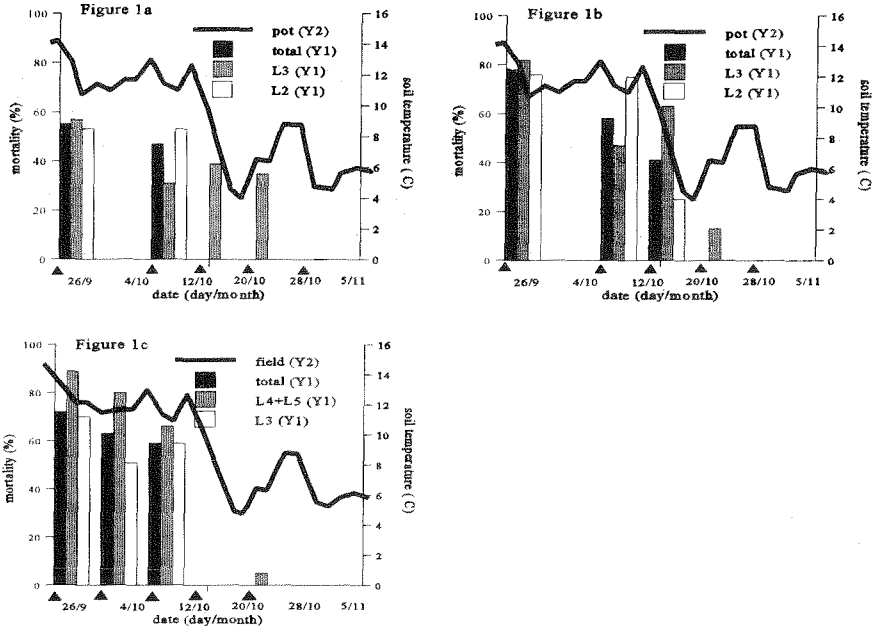

Figure 1: Reduction of *O. sulcatus* with 1A) *Heterorhabditis* sp.(NWE)(UK-H-211) in outdoor pots, 1B) *Heterorhabditis* sp.(NWE)(Nl-H-F85) in outdoor pots and 1C) *Heterorhabditis* sp.(NWE)(UK-H-211) in the field. ▲= the day of application of the nematodes. L2 to L5 are the different larval instars (scale = egg, L1 to L5. pupae). The lines show the soil temperature and the bars the mortality of the larvae.

Discussion

Biological control of the larvae of black vine weevil can be effective. It is, however, important to choose the right nematode strains for application in outdoor conditions. In North-west Europe the soil temperature in autumn and spring is usually low. Temperatures between 10 and 15°C are normal at this time of the year. Application of nematodes is necessary in these periods when soil temperatures are low. The weevils stop laying eggs when the temperature drops and late applications will give better control because bigger larvae are better parasitized by the nematodes. The results of several laboratory, pot and field trials show the importance of the size of the larvae on the level of control. Spring application is useful only for control of larvae in hedges and stock plants since growth damage will be limited in these plants. Young nursery plants, however, will suffer from severe growth reduction or worse; plant death will occur with this control strategy. Low soil temperatures imply that only cold-tolerant strains of insect-parasitic nematodes can be used to control

larvae of black vine weevil. The two *Heterorhabditis* strains (Nemasys H and Larvanem) are effective at these outdoor conditions, and a few days of 12 to 13°C is adequate to get maximum control. The strain of *Steinernema carpocapsae* (Exhibit) is not adapted to the conditions described above, and there is a poor control in pots and no control in the field. The effect of soil temperature on the efficacy of several nematode strains show that *Heterorhabditis* sp. (NWE) is effective down to 12°C and *Steinernema carpocapsae* (US-S-25) is not effective below 15°C (Schirocki & Hague 1994; Van Tol 1993a, 1993b, 1994, 1996). The behaviour of this type of nematode in soil is also limiting its efficacy. *Heterorhabditis* belongs to the group of cruisers and *Steinernema carpocapsae* to the group of ambushers. Cruisers are nematodes that actively forage for a prey while ambushers have a sit-and-wait strategy. *Steinernema carpocapsae* is therefore specialized on insects moving around in the top soil layers and *Heterorhabditis* for insects deeper in the soil (Campbell & Gaugler 1993; Grewal et al. 1994; Kaya et al. 1993; Lewis et al. 1992, 1993). Temperature limit and ambushing behaviour of this *Steinernema* strain make it unreliable for control of black vine weevil in outdoor conditions in the Netherlands.

Metarhizium anisopliae as a granular product (BIO1020) would be an excellent product for control of larvae in pots. It is effective against all instars and eggs of black vine weevil and can be applied at the start of a growing season. Intensive monitoring for weevils and larvae is no more needed when applying this product. In the field the efficacy is lower. However, combination of the fungus with *Heterorhabditis* in the field is giving the maximum control possible. Although a procedure for registration of BIO1020 has been started in the Netherlands registration now will not be sought, due to high production costs.

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A strategy for control of black vine weevil (*Otiorhynchus sulcatus*) in a Integrated Pest Management programme in nursery stock

Introduction

For introduction of Integrated Pest Management (IPM) on nurseries it is necessary to avoid regular chemical sprayings on the whole nursery to control pests. For control of black vine weevil on a nursery in IPM it is difficult to avoid these sprayings when the pest is not monitored intensively. Experience with local sprayings against this pest based on careful monitoring are sparse and no clear strategy is available for control of black vine weevil in IPM on nurseries. Only a few include control of black vine weevil in IPM in nursery stock (Dolmans 1994; Mulgrew 1990; Van der Horst & Van Tol 1996). Avoiding adult weevil control in IPM and controlling only the larvae as an alternative is expensive, if performed on the whole nursery and can cause serious environmental problems when huge amounts of chemicals are applied to the soil.

In this article the possibilities and problems for the use of insect-parasitic nematodes in outdoor conditions on a nursery are described, and the possibilities and problems for control of black vine weevil in an IPM programme discussed.

Materials and methods

In 1993 we started the introduction of integrated pest management (IPM) on a field nursery in Boskoop. The nursery, size 1.2 ha, consisted of approximately 200 different plant species and cultivars. Every week (starting in March) the nursery was monitored for pests and diseases. With the grower, we decided each week about the control strategy. The grower registered weekly what, when and where he sprayed. Our strategy was to spray with chemicals, not hazardous to natural enemies. If no such chemical was available chemicals having no long-term effect on other organisms and those polluting the environment as little as possible were used. For control of black vine weevil we used wooden planks and *Euonymus fortunei* 'Dart's Blanket' as a monitoring aid. Where weevils and/or feeding damage on the *Euonymus* occurred we sprayed locally with acephate (Orthene; a.i. 80%), sometimes combined with diflubenzuron (Dimilin lq.; a.i. 480 g/l). Dimilin makes the weevils sterile for a long period

(Frers 1993; Zepp et al. 1979). In September Larvanem (*Heterorhabditis* sp. (NWE) (Nl-H-F85)) was applied to the soil in hedges, stock plants, and on places where weevils were found throughout the season. This nematode strain proved to be most effective for control of larvae in the field (Van Tol 1996). Larvae were collected on the nursery, one week after nematode application, and checked for parasitization.

Results

On the nursery no increase in the total number of adult black vine weevils was found after two years of IPM. In 1994 the weevils emerged in a short period in June because of the warm spring and summer. In 1993 the emergence from the soil was more extended over the summer. This resulted in a reduction in use of insecticides of 40% compared with 1993 (Figure 1). The total reduction of pesticides used against black vine weevil was 85% in 1993 and 92% in 1994 (Figure 1). When no weevils were found in September and October and no feeding damage occurrde on the trap plant *Euonymus fortunei* we decided not to spray. In 1992 (before IPM), the grower sprayed the whole nursery eight times with several insecticides to control black vine weevil.

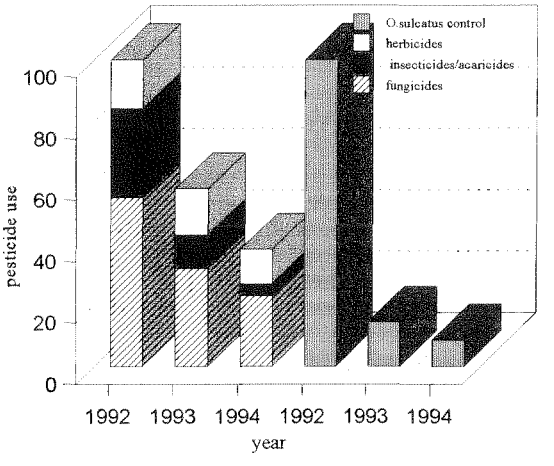

Figure 1: Relative pesticide use before (1992) and after (1993 and 1994) introduction of IPM on a field nursery growing shrubs and trees on peat. Pesticide use showing relative values: 1992 is set to 100. First 3 bars = total pesticide use; last 3 bars = insecticide use for *O. sulcatus* control.

In 1993 and 1994 the grower also sprayed eight times but only locally where we found weevils and/or feeding damage on the *Euonymus*. This strategy resulted in a built-up of populations of predators and parasitoids of several other pests on this nursery. In 1993 we still had to spray against several aphids. In 1994 the natural enemies controlled aphids almost completely. This resulted in a reduction of 81% compared with the introduction year of IPM (1993) on this nursery. Caterpillars were controlled with *Bacillus thuringiensis*. For the control of the two-spotted spider mite (*Tetranychus urticae*) fenbutatinoxide and hexythiazox were used. These chemicals are not harmful to predators of mites. In 1994 an increase in predators such as *Amblyseius potentillae*, *Phytoseiulus* sp., *Anthocoris* sp. and *Feltiella acarisuga* (syn. *Therodiplosis persicae*) was noted. In several plant species this resulted in good control and no chemicals were used.

The relative use of insecticides against black vine weevil stabilizes around 40 to 50%; however, the use of pesticides to control aphids and spider mites is still decreasing because the control by natural enemies is increasing strongly in the second year after introduction of IPM on this nursery. In 1993 the savings on insecticides were 66% and, in 1994, 87% compared with 1992 (Figure 1). The total reduction in pesticide use was 42% in 1993 and 61% in 1994 on this nursery (Figure 1).

The grower never uses soil insecticides to control black vine weevil. In 1993 and 1994 we used *Heterorhabditis* sp.(NWE) (Larvanem) to control the larvae locally. One week after application the grower collected larvae to check the infection rate. Approximately 75% was parasitized.

The costs of crop protection in plant production were 1.5% of the total costs in 1992 and 2.6% in 1994, labour being the most important cost factor in IPM. The cost of monitoring raises these costs by more than 100%. The direct costs of control (chemical and biological) are not rising in IPM. The main cost of control was the use of the insect-parasitic nematodes (more than 50%).

Discussion

Nematode products are still relative expensive for growers and, at present, this makes it impossible to apply the nematodes over the whole nursery. Applying nematodes on places with relative high infestations of black vine weevil would be more efficient and cost saving for the nurseryman. For this strategy it is important to monitor intensively on a nursery during both spring and summer. This strategy was tested in a IPM programme on several nurseries. After two years of IPM we were able to control black vine weevil on the nursery described in this article. No soil chemicals were applied. Locally, we sprayed the foliar to control the adult weevils in summer; in autumn we applied *Heterorhabditis* to spots where weevils were more often found in the summer period. This strategy resulted in 90% reduction of chemicals applied to control black vine weevil. This strategy made it possible also to control other pests in an integrated way. After two years of IPM natural enemies on this nursery increased to numbers large enough to control several aphid species almost completely. The reduction in insecticide use ranged from 66% in 1993 to 87% in 1994. The total reduction in pesticide use was 61% in 1994.

A problem for IPM and especially for black vine weevil control on nurseries is the time needed for monitoring. The high cost of monitoring is an important obstacle for widespread introduction of IPM at this time. Monitoring weevils with planks and trap plants is time consuming and there is no relation known between the number of weevils found, the feeding damage and the actual number of weevils on the nursery. Therefore, more work has to be done on developing useful traps and kairomones for quantitative assessment of black vine weevil infestations. Kairomones would also make selective control of black vine weevil possible, using less chemical on small areas.

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Selection and use of entomopathogenic nematodes against vine weevil

Introduction

In many cultures - especially in tree or grape-vine nurserys, ornamentals under glass or in strawberries in the field - the larvae of vine weevil (*Otiorhynchus sulcatus*) cause severe damage to the roots. The adults feed on the leaves of many plant species and reduce marketability, especially of ornamentals. Owing to a increase in the availability of effective chemical insecticides, there is now an increased incidence of damage in such cultures. Biological control can offer two methods to combat black vine weevil larvae: (1) application of entomopathogenic fungi, mainly *Metarhizium anisopliae*, into the soil or growing substrate (see Zimmermann, 1996) and (2) entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* (Nem., Rhabditoidea) which are commercially available and easy to handle. Control of vine weevil (larvae and pupae), using different strains of these nematodes, many is reported in papers (e.g. Van Tol, 1993). Backhaus (1994) reviewed recent activities in *Otiorhynchus* control by heterorhabditids. Despite a relatively good control of the larvae (about 60-95%), there are still some obstacles, e.g. insufficient activity at low temperatures, high numbers of nematodes necessary to achieve high efficacies, a restricted shelf life of formulated nematode products. Some recent approaches to enhance the efficacy of entomopathogenic nematodes and nematode products are presented in this paper.

Action of entomopathogenic nematodes

For a better understanding of possible improvements of certain traits in nematodes a short view on the life cycle is given. Infective juveniles (third instars), so called 'dauer larvae', of heterorhabditid and steinernematid nematodes actively find and penetrate their potential hosts in the soil. The juveniles invade the haemocoel of the host and release their symbiotic bacteria into the haemolymph. As far as known, every species of heterorhabditid and steinernematid nematodes possesses its own bacterial symbiont, belonging to the genera *Photorhabdus* and *Xenorhabdus*, respectively. These bacteria multiply rapidly in the haemolymph and kill their hosts within a few days. After one or more reproductive cycles in the cadaver, the nematodes again form infective juveniles, which emerge from the cadavers and spread in the surrounding soil.

The efficacy of the nematode/bacterium complex may be enhanced by selection of certain traits of the nematodes themselves or of their symbionts. Especially in the genus

Heterorhabditis, much recent work has led to a better understanding of the biology, giving starting points for manipulation of different traits.

Cold active nematodes

Weevils of the genus *Otiorhynchus* are widespread in the holarctic region and well adapted to low temperatures. Most larvae overwinter in the soil and feed on the roots of many (cultivated) plants at soil temperatures higher than 6°C. However, the commercialized steinernematid and heterorhabditid strains, e.g. *Steinernema carpocapsae* 'ALL' and UK-isolates of *Heterorhabditis* produce adequate reduction of the larvae only at about 15°C (Schirocki & Hague, 1994). Therefore, strains should be available which have a good control potential, especially at low soil temperatures.

Grewal et al. (1994) demonstrated that some steinernematids have a wide temperature range for infection, establishment and reproduction; e.g. *S. feltiae* infects host larvae from 8° to 30°C and *S. carpocapsae* infects from 10° to 32°C. *Heterorhabditis* sp. (HF 85), a cold active natural Dutch strain, showed good control at low temperatures in containers but most of the other tested isolates of *H. bacteriophora* and *H. megidis* were inadequate in the field, though they still gave equal or better control than the chemical standard carbofuran (Van Tol, 1993). In recent years, several heterorhabditids were isolated from soils of North-West-European countries (e.g. Griffin & Downes, 1991). The minimum temperature of these *Heterorhabditis* isolates to infect or kill insect larvae was between 5° and 7° (Griffin & Downes, 1991). Because only a few infective juveniles invade the host larvae at those temperatures, the total number of killed hosts was low. Therefore, Griffin & Downes (1994) selected heterorhabditids to improve infectivity using 13 North-West-European strains of *Heterorhabditis*. After nine selection cycles, the LT 50 was lowered from 10° to 5°C and the heredability for this trait was estimated as 0.33. With such strains, larval control would be possible even in early spring or late autum.

Improvement of host finding, penetration, and further traits

To achieve good control under glass as well as in the field, about 0.5 x 10^6 juveniles/m^2 have to be applied to the soil. The nematode population declines sharply soon after application and only a small percentage of the juveniles may penetrate and kill the host larvae (SMITS, 1996). Because of high product costs, possible reductions of this initially large number of nematodes have been studied. Miduturi et al. (1994) compared the effect of 1000, 3000, 10000 and 30000 infective juveniles in 250 ml pots on larval mortality. The lowest dose of *Heterorhabditis* sp. (HF 85) resulted in a high mortality 10 to 20 days after application, whereas *S. carpocapsae* achieved a similar mortality at 3000 to 10000 juveniles. These findings confirm the results of Van Tol (1993), that *Heterorhabditis* sp. (IH) from Italy and *S.*

carpocapsae (S25) from USA are inferior to the North-West-European strains of *Heterorhabditis*. Selection for higher efficacy of the nematodes and/or their bacterial symbionts have been tried in recent years. This includes higher activities of the juveniles, better host finding and an increased pathogenicity of the symbionts.

Burnell & Dowds (1996) summarize the research activities for genetic improvements of the nematodes and their symbiotic bacteria. In order to increase the pathogenicity of the nematode/bacterium complex, Gerritsen & Smits (1994) combined several axenic strains of *Heterorhabditis* with the symbionts of other nematode strains. Nematodes of *Heterorhabditis* sp. (North-West-European group) are unable to grow and multiply with *H. bacteriophora* bacteria. No combination achieved better results than the natural nematode/bacterium complex. Clarke & Dowds (1994) tried to improve the growth rate of different *Photorhabdus* strains from North-West-European *Heterorhabditis* sp. without success. The growth rate of strains from Ireland, the Netherlands and Russia were found to be very similar at the tested temperatures. This implies that the isolation of a wild type strain of *Photorhabdus* with an improved growth rate at low temperatures is unlikely. Until today, trials for genetic improvement of the symbiotic bacteria did not succeed.

Enhanced host-finding was realized in a hybridized strain of *S. carpocapsae* (Gaugler et al., 1991). This strain shows a significant response to larvae of *Galleria mellonella* in soil, up to a distance of 20 cm, compared with 3.5 cm in wild type strains. Host-finding in other targets was also enhanced but vine weevil not included. Westerman (1995) studied the vertical migration of 21 *Heterorhabditis* isolates. The ability to migrate vertically into the soil is an important trait for vine weevil control, because the larvae feed in soil depths between 2 and 15 cm (max. 30 cm). Most North-West-European isolates of *Heterorhabditis* sp. and *H. megidis* (HO1) migrated better than *H. zealandica* or *H. bacteriophora*. The presence of a *G. mellonella* larva on the bottom of the test tubes increased the downward movement, but the reactions of the nematodes varied markedly between different batches. These tests show that selection of natural isolates for rapid downward movement is possible, an important trait for an effective and rapid control at all soil depths. On the other hand, Hanula (1993) demonstrated a marked difference between laboratory studies in soil columns and those in undisturbed field soils. *S. carpocapsae* (ALL) was the only strain that killed the larvae at 20 cm depth, whereas *H. bacteriophora* (C1) did not infect larvae below 12.5 cm.

Formulation and shelf life

Commercialized nematodes are formulated in different ways. Aqueous suspensions in small containers are the best for practical use. They allow the preparation of the spray suspension for application on small plots, e.g. on potted plants under glass. Water-dispensable granular formulations (Grewal et al., 1995) are simple to use and have a prolonged shelf-life up to

5-6 months for *S. carpocapsae* at room temperature. But there is still a need for formulations of heterorhabditids and steinernematids with shelf lives longer than 6 months at room temperature.

Production

The production of entomopathogenic nematodes has to be simplified and improved in order to reduce the product costs. *Steinernema* species are produced in large quantities by fermentation techniques. Also, for *Heterorhabditis*, such techniques are close to practical use (Ehlers, 1994). These techniques should also be adapted to the production of improved strains or promising new isolates.

Conclusions

In recent years, some traits of entomopathogenic nematodes infecting vine weevil have been improved by selection and breeding, and new strains or species have been isolated from natural soils. Especially, cold-active strains are important to control the larvae of *O. sulcatus* early in the year and late in the autumn, when the soil temperature is low. Enhanced host-finding or penetration rates may reduce the number of nematodes necessary for effective control. New formulations prolong the shelf-life, and fermenter techniques may reduce the product costs.

Entomopathogenic nematodes reduce populations of *O. sulcatus* as good as conventional insecticides or even better. Side effects on naturally occurring fauna in the field are negligible (Buck & Bathon, 1993; Koch & Bathon, 1993). Therefore, entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* should be chosen for vine weevil control.

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Detection of predation on vine weevil by natural enemies using immunological techniques

Introduction

Many studies have investigated the role of carabid beetles and other polyphagous predators in influencing the community structure of phytophagous arthropods. However, direct field observation is difficult, owing to the small size and cryptic nature of both the predators and prey species. Dissection and physical examination of the gut contents of these predators is difficult and laborious, and impossible in the case of predators that consume only the liquid parts of their prey. Immunolological techniques, based on polyclonal antibodies, have been employed in a number of studies to look at predator-prey relationships in a range of species. However, the antisera often cross-reacted with non-target species. This problem can be reduced by cross-absorbing the antiserum with predator proteins but production of a polyclonal antiserum which is specific to one genus or species is difficult. The relatively new use of monoclonal antibodies in this field minimises the problem of lack of specificity. Monoclonal antibodies have now been used to identify species-, stage- and even instar-specific prey.

This paper reports the development of a polyclonal antiserum that recognises all vine weevil developmental stages. Secondly, it describes the production of a panel of stage-specific monoclonal antibodies which are able to distinguish between vine weevil eggs, larvae and adults. Their future use in extensive screening of polyphagous predators present in vine weevil-infested strawberry and blackcurrant crops is discussed.

Methods

The polyclonal antiserum was raised against whole-body macerates prepared from mature vine weevil larvae. Cross-reactions between the antiserum and non-target species were reduced by cross-absorption with extracts made from starved individuals of four common carabid species. Immunoglobulin (IgG) was prepared from the antiserum by ammonium sulphate precipitation, followed by ion-exchange chromatography.

The monoclonal antibodies were produced by three separate fusions, one against each developmental stage, following a protocol modified from Galfre and Milstein (1981). Supernatant screening of positive hybridomas was performed against homologous and heterologous vine weevil antigens, and against predator antigens, using an indirect ELISA (Voller *et al.*, 1976). Only cell lines that demonstrated no cross-reactivity with predator species were cloned.

Results

Preliminary cross-reactions with non-target species were successfully eliminated by cross-absorption, without significantly reducing the sensitivity of the polyclonal antiserum to vine weevil, as indicated in Figure 1. ELISA responses of the cross-absorbed IgG (at concentrations of 10 µg/ml and 2 µg/ml) with non-target species were low compared with vine weevil antigens, and would not produce false positives. The polyclonal antiserum was raised against vine weevil larvae, but recognised all developmental stages.

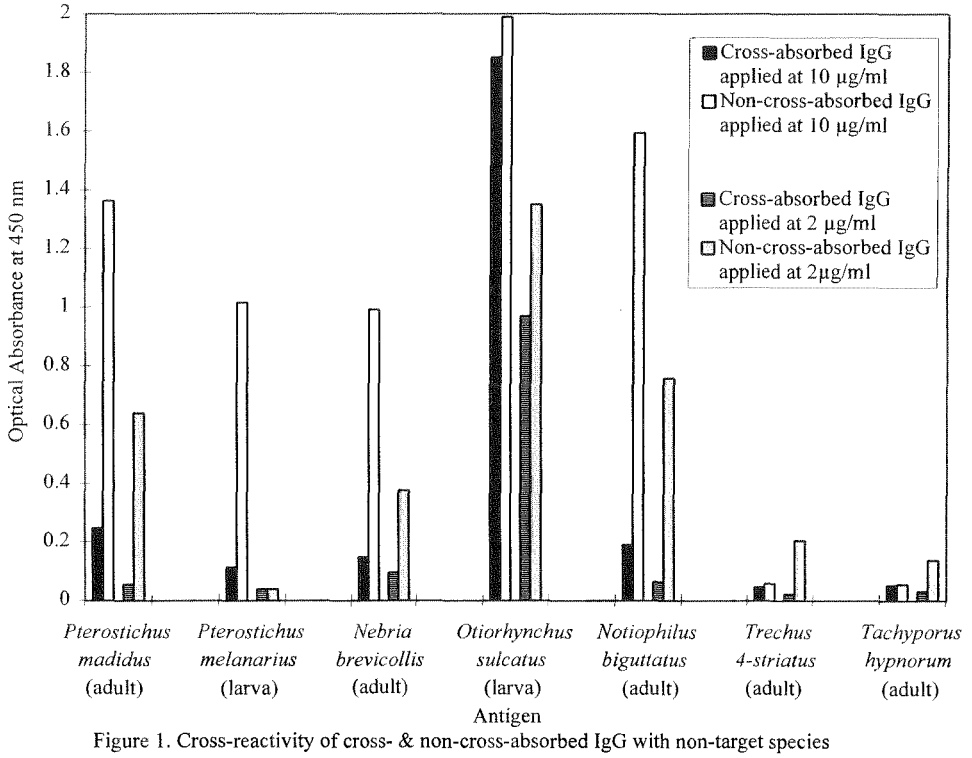

Figure 1. Cross-reactivity of cross- & non-cross-absorbed IgG with non-target species

To produce the monoclonal antibodies, three separate fusions were carried out, one against each of the three vine weevil stages. Of the 239 positive hybridomas screened against predator antigens, 89 were vine weevil-specific. Table 1 lists the final panel of MAbs produced and their specificities.

Table 1. Specificity of MAbs produced

Monoclonal Antibodies	Specificity
EMA 161, EMA 162	E
EMA 130, EMA 131, EMA 135, EMA 151, EMA 152	A
EMA 134, EMA 149, EMA 154	L,A
EMA 159, EMA 160	E,A
EMA 122, EMA 150	E,L,A
EMA 133	E,L,A + all predators

E, L, A = eggs, larvae, adults of vine weevil, respectively

Discussion

Initially, the polyclonal antiserum cross-reacted with some adult (e.g. *Pterostichus madidus*) and larval (e.g. *Pterostichus melanarius*) Coleoptera (Figure 1); to a lesser degree, it also cross-reacted with some non-coleopteran species (e.g. *Forficula auricularia*) (results not shown). Cross-absorption of the IgG removed cross-reactions with non-target species to a background level, while losing little of the IgG vine weevil activity.

The polyclonal antiserum recognised all developmental stages of vine weevil but was incapable of differentiating between them. This limited the use of the antiserum. A number of polyphagous predators found in soft fruit plantations, including *Tachyporus hypnorum, Notiophilus biguttatus* and *Trechus quadristriatus* are of interest as potential predators of eggs and newly hatched larvae. Medium-sized carabids, such as *Nebria brevicollis* and *Pterostichus* spp., have been found to feed on larger vine weevil larvae and adults in the laboratory, but the development of MAbs has provided a method to investigate what is occurring in the field.

Fifteen MAbs were developed to recognise different developmental stages of vine weevil. Stage-specific MAbs were raised against vine weevil egg and adult antigens. A number of MAbs in the final panel recognised two stages, while others recognised all three. In addition, EMA 133 recognised all predator species screened and, consequently, was developed for use as a positive control.

Few other workers have used monoclonal antibodies in field predation studies. Symondson and Liddell (1993) have developed a MAb capable of distinguishing arionid slugs from those of other genera. Hagler and co-workers have successfully produced MAbs to egg antigens of three different pest species. In each case, the MAbs recognised adult females of the same species (Hagler *et al.*, 1991, 1993, 1994). The highest degree of MAb specificity has been achieved by Greenstone and Morgan (1989), who produced an ELISA system capable of identifying predation on the fifth instar of *Heliothis zea*.

This is the first study to produce a panel of MAbs as a diagnostic probe to determine which developmental stages are subject to predation. We propose to divide each predator extract into three portions and screen each against a MAb with a different specificity in an indirect sandwich ELISA. An ELISA plate will be coated with the polyclonal antiserum to capture any vine weevil antigens, and an egg-specific, an adult specific, and a larva-plus-adult specific MAb, will identify predators that have fed on eggs, larvae or adults, as well as those that have eaten eggs-plus-larvae. A predator that has fed on an adult vine weevil would elicit the same response in the multiple-ELISA as one that has fed on an adult and larva; it may be possible to differentiate between these two possibilities on the basis of which vine weevil stages were present in the field at the time of sampling. Similarly, a predator that had fed on both eggs and adults could not be distinguished from one that had fed on all three stages within the antigen detection period, although the latter is improbable.

Prior to the use of these MAbs on field-collected samples, further screening is required against alternative prey species, including other *Otiorhynchus* species, and against other stages of predator species, particularly their eggs. Following further laboratory tests, it is intended to exploit these MAbs as a tool in evaluating the natural enemies of *Otiorhynchus sulcatus* in soft-fruit plantations.

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Biological control of *Otiorhynchus sulcatus* with *Steinernema* nematodes, under field conditions in northern Germany.

Introduction

In north-west Germany, the vine weevil (*Otiorhynchus sulcatus*) is of economic importance, especially in tree nurseries. Serious damage on a wide range of woody ornamentals is most frequently caused by the root-feeding larval stage. *Rhododendron spp.* and *Taxus baccata*, which are of major importance for nursery productions in this region, are among the preferred host plants (von Reibnitz and Backhaus 1993).

Chemical control methods failed to obtain sufficient results, particularly in soil and substrates with high amounts of organic matter. The use of insecticides is more and more restricted by the German plant protection act. Therefore, the development of entomopathogenic nematodes of the genus *Heterorhabditis* or *Steinernema* as a biological control agent against larvae of *Otiorhynchus sulcatus* has advanced rapidly over the last few years (Simons 1981, Kakouli et al. 1993)

The use of the nematodes in outdoor production is limited by the soil temperature. The nematodes infect larvae readily at temperatures arround 8° C, but the associated bacteria as active agent will not be able to multiply below 12° C (Ehlers 1991, Backhaus 1994). Due to the life cycle of *O. sulcatus* control of larvae has to take place in early spring (March/April) and in autumn (September/October). During these months the soil temperatures in northern Germany are often below 12° C and exceed this limit only for a few hours a day. Therefore, the following experiments were carried out to test the efficiency of *Steinernema* nematodes in outdoor production under the influence of natural weather conditions of this region.

Material and Methods

Two-year-old *Taxus baccata* were planted in 2-litre pots (13 cm × 13 cm × 12,5 cm) using a loamy soil. Each pot was inoculated with 5 larvae (L3) of *O. sulcatus*. The larvae were kindly provided by Urania Agrochem GmbH, Christinenthal. Two days after the larvae had invaded the soil, containers were dug-in on a open field stand.

One or three weeks, respectively, after infestation with larvae, the pots were treated with suspensions of entomopathogenic nematodes. In all experiments commercially available nematodes of the species *Steinernema carpocapsae* (Exhibit G25; Ciba Agro, Frankfurt) were used. The pots were treated with 500,000 nematodes per m^2 of soil area. Nematodes were dispersed on the soil surface in 30 ml tap water per pot. Control plants received 30 ml of tap water per plant. Each treatment was replicated 18 times. Fourteen and 28 days after treatment the pots were checked to assess the ratio of dead/alive larvae. Soil temperature in the pots was measured by a sensor from a nearby weather station.

Results

Several experiments following the method described above were carried out. Table 1 and 2 show the results of two examples in which *Steinernema* nematodes were applied on 18 April and on 2 May 1995.

In the first experiment, 14 days after the treatment in control pots 84 % of the larvae were recovered. In treated pots, 74 % of the larvae were found again. Thirty-nine larvae were still alive but 36 larvae were already infected by nematodes, because they died one week after incubation under room temperature (Table 1). Exhibit G25 showed an efficiency of 46 % related to the recovery rate in the untreated control.

Twenty-eight days after treatment only 48 % of all larvae were recovered, while in untreated pots 79 % of the larvae were found. This indicates that many parasitized larvae had already disintegrated. In the treated pots only 3 of the 43 found larvae were still alive. The *Steinernema* nematodes showed an efficiency of 95 %.

Similar results were recorded in the second experiment, when the nematodes were applied two weeks later, on 2 May, although the effect of the nematodes occured faster compared with the

results of the first experiment. Fourteen days after treatment, 63 larvae could be found again and only 9 larvae were still alive (Table 2). Five of the surviving larvae were parasitized and died one week after incubation under room temperature. At this date, and 28 days after treatment, efficiencies of 86 % and 93 %, respectively, were recorded.

Table 1. Rates of recovery and number of dead/alive larvae 14 and 28 days after application of *Steinernema* nematodes on 18 April 1995

Treatment	Days after treatment	Rate of recovery (%)	Number of larvae (n = 90)		
			alive	dead	not found
Untreated	14	84	72	3	15
Exhibit G25	14	74	39*	28	23
Untreated	28	79	65	6	19
Exhibit G25	28	48	3	40	47

* 36 larvae died one week after incubation under room temperature.

Table 2. Rates of recovery and number of dead/alive larvae 14 and 28 days after application of *Steinernema* nematodes on 2 May 1995

Treatment	Days after treatment	Rate of recovery (%)	Number of larvae (n = 90)		
			alive	dead	not found
Untreated	14	79	65	6	19
Exhibit G25	14	70	9*	54	27
Untreated	28	68	60	1	29
Exhibit G25	28	33	4	26	59

* 5 larvae died one week after incubation under room temperature.

Discussion

The results of the experiments show that the use of *Steinernema* nematodes is an promising method to control larvae of *O. sulcatus*. The rates of recovery and the numbers of surviving and dead larvae indicate how parasitized larvae die and disintegrate over a period of four weeks. The different efficiencies, which were recorded two weeks after treatment in both experiments, can be attributed to the influence of the natural changes in soil temperature. When nematodes were applied on 18 April the effect was delayed, because after treatment the soil temperature exceed 12° C for only for a short period between 22 and 26 April. During the first half of May, the soil temperatures rose to 20° C. Therefore, the full efficiency was not achieved until 28 days after treatment. In the second experiment, which was carried out two weeks later on 2 May, the nematodes already showed a marked effect 14 days after application.

The results indicate that the use of *Steinernema* nematodes in outdoor production in April is posssible. The effect of an early application may be delayed, but it helps to reduce densities of larvae.

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The cinnamic acid derivative cinnamamide as a repellent against the vine weevil *Otiorhynchus sulcatus* (Coleoptera: Curculionidae)

Introduction

In the UK, the vine weevil, *Otiorhynchus sulcatus*, has become the most serious insect pest of hardy ornamental nursery stock and soft fruit in the 1990s (Moorhouse *et al.*, 1992). Damage to plants is caused by the larval stages which feed on roots and crowns, and by adult weevils feeding on foliage. The adult feeding activity causes characteristic leaf notching, thereby reducing plant quality and marketability, especially in ornamentals (Moorhouse *et al.*, 1993). On other plants (e.g. vines) adults may also cause yield losses by feeding on the developing fruit (Cone, 1963). Adult *O. sulcatus* are all females, and reproduction occurs parthenogenically. After adult emergence, the nocturnal, wingless adults remain near the plant from which they emerged, although some individuals may move much longer distances and infest neighbouring fields. The adults are particularly difficult to control using insecticidal sprays, since the nocturnal movement of individual adult weevils to feed on the leaf canopy of plants is somewhat irregular, necessitating a repetitive programme of spraying if significant control is to be achieved. In addition, the larvae and adults can survive temperatures as low as -3°C for 90 days without significant mortality (Stenseth, 1987) and can survive commercial cold storage in the compost of potted plants. Problems caused by vine weevils have been increasing over recent years after the withdrawal of the insecticide aldrin, and as a result of certain changes in horticultural practices. The chemical insecticides currently available for vine weevil control in the UK are carbofuran, chlorpyrifos, fonofos and gamma-HCH, and these products are aimed mainly at killing the soil-dwelling larval stages. Moreover, these chemicals have reduced efficacy compared with aldrin, are relatively ineffective against adult stages and, because they are not target specific, may cause unwanted effects in non-target soil dwelling organisms, including beneficials. For example, chlorpyrifos is extremely harmful to the natural enemies of vine weevil such as ground beetles and earwigs (Labuschagne, 1994a). More recently, entomopathogenic nematode products have been developed and are now widely used for vine weevil control, especially in protected horticultural systems. However, nematodes also have their limitations, namely reduced efficacy at lower temperatures, leading to poor performance outdoors. Moreover, nematodes kill only larvae and pupae; they have no effect on eggs or adults.

Non-lethal chemicals that deter vine weevils from feeding would have considerable potential as benign methods of reducing adult feeding damage. Secondary plant substances (e.g. phenylpropanoids, phenols and terpenoids) have a profound effect on food-plant selection by many polyphagous herbivores. The presence of such chemicals in specific plant species may serve as a warning that the plant is toxic, and encourage herbivores to feed on other plant species. Certain rhododendrons are resistant to vine weevil attack, and Doss (1984) found that these species have scales on the leaves that contain essential oils (predominantly sesquiterpenoids) which can deter feeding by vine weevils.

Studies undertaken at this laboratory have demonstrated that one class of phenylpropanoid precursors, the cinnamic acids, deter feeding in several vertebrate and invertebrate species. Cinnamamide is active against birds (Crocker *et al.*, 1993; Crocker and Reid, 1993; Watkins *et al.*, 1995), mammals (Crocker *et al.*, 1993) and slugs (Watkins *et al.*, 1996). The aim of the present study was to determine whether this compound would also deter feeding by adult vine weevils on strawberry leaves in a two-choice feeding experiment.

Methods

Experimental animals

The vine weevils used in all experiments were collected from the field as late-instar larvae and reared on strawberry roots (cv. Aromel). Once the adults had emerged, they were kept individually in clear, sealed, plastic pots (diameter 55 mm) at 20°C with a 14 hr light and 10 hr dark (14L:10D) photoperiod, and fed on fresh strawberry leaves.

Treatment of strawberry leaves with cinnamamide

Similarly sized strawberry leaves (cv. Aromel, area approx. 600 – 900 mm^2) were used in these studies. There were two treatments: untreated (control) and cinnamamide-treated leaves. The cinnamamide formulation consisted of finely milled particles of cinnamamide (Aldrich Chemical Co., Gillingham, Dorset, UK.; 25% w/v) dispersed in water using a non-ionic surfactant (Atlox 4911, ICI Surfactants, Cleveland, UK; 1.15% w/v) and stabilised using xanthan gum (Kelzan S, Kelco International Ltd., London, UK; 0.2% w/v). Treatment of leaves was achieved by applying 5.5 µl cinnamamide formulation onto each side of the strawberry leaf, using a small paintbrush, to give application rates of 1.0%, 0.5% and 0.1% w/w cinnamamide per leaf.

Test procedure

The experiment was set up as a two-choice test. The areas of one untreated and one cinnamamide-treated strawberry leaf were calculated by determining their area on graph

paper. These leaves were both placed inside a clear, sealed, plastic pot (diameter 55 mm) on filter paper (Whatman No.1, 55mm diameter). One adult vine weevil that had previously been starved for 24 h, was introduced into each pot. The pots were maintained at 20°C and 14L:10D for five days. After this time, the areas of the leaves were again calculated. There were 20 replicates at 1.0% w/w, 15 at 0.5% w/w and 11 at 0.1% w/w.

In addition, a further set of 20 leaves were kept in the same conditions as above for five days, but unexposed to vine weevils. The loss of area of these control leaves due to desiccation was calculated and used to correct the final figures for the leaf area consumed by the weevils.

Results

When strawberry leaves were kept in plastic pots, but remained unexposed to vine weevils their area was reduced by a mean of 8.6% after five days.

Analysis of the data from the two-choice test showed that when cinnamamide was applied to strawberry leaves at 1.0% w/w there was a highly significant reduction in adult vine weevil leaf consumption (65.9%) compared with the control leaves ($t = 4.64$, 38 df, $P<0.001$). At 0.5% w/w cinnamamide there was also a reduction in damage (63.5%) compared with the control but this was less significant ($t=2.19$, 28 df, $P<0.05$). At the lowest application rate (0.1% w/w) damage was not reduced significantly ($P>0.05$) (Table 1).

Table 1. Mean leaf area consumed ($mm^2 \pm SE$) of untreated and cinnamamide-treated strawberry leaves after five days exposure to adult vine weevils in a two-choice test.

Application rate for cinnamamide on strawberry leaves (% w/w)	Control	Cinnamamide treated	Percentage damage reduction
1.0	272 ± 34	92 ± 19	65.9
0.5	107 ± 28	39 ± 14	63.5
0.1	59 ± 26	31 ± 13	47.1

Discussion

The present studies have demonstrated that cinnamamide effectively deters feeding by vine weevil adults when applied to strawberry leaves at 1.0% w/w, although the effect was less pronounced at lower dosage rates. Notwithstanding the substantial reduction in damage to strawberry leaves conferred by cinnamamide in comparison with damage sustained by untreated leaves, the overall consumption of strawberry leaves appeared to decrease with the application rate of cinnamamide (Table 1). Thus, greater quantities of both treated and control leaves were eaten by the weevils in the experiments where cinnamamide was applied at 1.0% w/w, and much less leaf was eaten by the same insects in the experiments where cinnamamide was used at 0.1% w/w. This difference was probably artefactual and may have reflected the difference in age of individual weevils, since the experiments were done in the order shown in Table 1. The apparent differences in leaf consumption may also have been a result of using the same weevils, sequentially, in each of the three tests; those used in the 1.0% test had not previously been exposed to cinnamamide, whereas those in the 0.5% w/w and 0.1% w/w tests had. Thus, it is possible that the insects could have developed a gradual learned aversion to cinnamamide, which may have reduced their inclination to feed even on untreated strawberry leaves.

When given a choice, the vine weevils avoided the cinnamamide-treated leaves. The choice test used in these experiments reflects the situation in the natural environment where alternative foods are likely to be available. If the vine weevils can be discouraged away from the crop plant then there is a possibility that they will be driven to feed instead on surrounding weed species. Adult vine weevils are more predisposed to be polyphagous than larvae (Neilsen and Dunlop, 1981) and will readily consume a range of weed species if the desired host plant is absent (Moorhouse *et al.*, 1992). In addition, these displaced adults might be more likely to select an oviposition site away from the crop plant. Since the larvae are relatively immobile, it is likely that they would not then migrate back into the crop.

The use of a repellent such as cinnamamide has many advantages over the chemicals currently available for vine weevil control. In particular, cinnamamide has low toxicity to both vertebrates and non-target arthropods. For this reason, cinnamamide would be unlikely to affect natural enemies of vine weevil, and would not present any significant hazard to humans when the plants are handled. Moreover, cinnamamide has been shown to be an effective, non-lethal repellent in other insects, e.g. *Lacanobia oleracea* (Lepidoptera: Noctuidae), *Pieris brassicae* (Lepidoptera: Pieridae), *Sitobion avenae* (Hemiptera: Aphididae) (H.J. Mosson, unpublished results) and slugs (Watkins *et al.*, 1996). This chemical may have potential, therefore, to protect a much wider range of horticultural plants from damage by invertebrates.

We have yet to isolate and identify the mode of action of cinnamamide in invertebrates. In vertebrates, cinnamamide appears to act instantaneously, causing irritation in the oral cavity, and through a post-ingestion malaise also induces a learned aversion (Watkins *et al.*, 1994). Identifying the site(s) and mode of action of cinnamamide in invertebrates would enable the more effective targeting of these deterrents.

The effective dose of cinnamamide (1.0% w/w) found in this study equates to a high and expensive application rate. Subsequent experiments need to look at factors such as formulation, effectiveness on different types of plant, and the effects of weathering on the persistence of cinnamamide in the field.

Notwithstanding the effectiveness of cinnamamide as a protectant against plant damage caused by adult vine weevils, it is clear that even highly effective feeding repellents will do little to reduce the overall number of vine weevils. A strategy for vine weevil control will have to combine biological, physical and chemical methods as well as varietal resistance or tolerance to the pest (Labuschagne, 1994b). Cinnamamide has low toxicity to the natural enemies of vine weevils and would be highly compatible, compared with conventional chemicals, for use with entomopathogenic nematodes and other parasites and pathogens. Cinnamamide could, therefore, provide a valuable tool in an integrated pest management strategy for vine weevil.

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The effect of low temperature on survival of *Steinernema carpocapsae* in *Otiorhynchus sulcatus* larvae

Introduction

Steinernema carpocapsae (UK isolate) has been shown to infect vine weevil, *Otiorhynchus sulcatus*, larvae at temperatures ranging from 14 to 33°C (Schirocki and Hague, 1994). Soil temperatures in the UK are between 10 and 15°C in late summer and spring, times when larvae are present in the soil. Nematode application is recommended at soil temperatures above 13°C. Therefore, treatments are restricted to late August or early September, when the majority of the larvae have hatched, or in May when soil temperatures are high enough but the larvae have not started to pupate (Sampson, 1994). Nematode application in late summer or early autumn may result in nematode invasion at temperatures around 15°C but larval mortality may be reduced if soil temperatures drop before the bacteria/nematode can develop. Grewal *et al.* (1994) suggested that nematodes might be able to survive within the hemocoele of insects during the winter and resume development, causing mortality, when temperatures rise in spring. Live vine weevil larvae containing nematodes have been found in winter and they died soon after their transfer to the laboratory (Oakley, 1994).

The following experiments were done to investigate the ability of *S. carpocapsae* to survive in *O. sulcatus* larvae at low temperatures and subsequently cause mortality when temperatures rise. Data on *Galleria mellonella*, a known susceptible host, are also presented

Material and Methods

Survival of *S. carpocapsae* in *O. sulcatus* larvae:

A dosage of 400 infective juveniles (IJs) of *S. carpocapsae* (UK isolate) was applied in 0.5 ml of water to 4 g sterile moist sand in multi-well tissue culture plates 2.5 cm in diameter. Dishes used for the control received water only. One *O. sulcatus* larva was placed in each dish and

kept at 22°C for 3 hours. Larvae were then rinsed with water and transferred to nematode-free wells filled with moist sterile sand. Replication was 12-fold; treatments were as followed:

Treatment A: exposure at 5 or 10°C for 10, 20, 30 or 40 days

Treatment B: exposure at 5 or 10°C for 10, 20, 30 or 40 days then at 22°C for 120 hours

Larval mortality was recorded after the completion of each treatment. The number of nematodes infecting the vine weevil larvae was determined for treatment B. Control larval mortality refers to treatment A only. Statistical analysis was done using the CoStat (1986) programme. All nematode data were transformed to square root before analysis of variance.

Survival of *S. carpocapsae* in *G. mellonella* larvae:

A dosage of 400 IJs of *S. carpocapsae* (UK isolate) was applied in 1 ml of water to filter paper in 9 cm Petri dishes. Dishes used for the control received water only. One *G. mellonella* larva was placed in each dish and kept at 22°C for 2 hours. The replication was 12-fold. Larvae were then rinsed with water and transferred to dry filter paper in multi-well tissue culture plates 2.5 cm in diameter, and treated as follows:

Treatment A: exposure at 22°C for 48 hours

Treatment B: exposure at 10°C for 5, 10 or 20 days

Treatment C: exposure at 10°C for 5 or 10 days then 22°C for 48 hours

Larval mortality was recorded at the end of each treatment and the number of nematodes establishing counted. The number of dead nematodes per larva was determined in those treatments where there was 100 % mortality.

Results

S. carpocapsae did not kill *O. sulcatus* larvae during exposure at 5°C except for one larva after 20 days (Table 1). However, mortality increased when larvae were transferred to 22°C. Vine weevil larvae died during exposure to 10°C; after increasing the temperature to 22°C for three days there was only a minimal increase in larval mortality. Larval mortality did not exceed 50 % in any treatment because of low initial nematode infection.

Table 1. The percentage mortality of *O. sulcatus* larvae after initial exposed to *S. carpocapsae* at 22°C for 3 hours followed by treatments at: A, 5°C or 10°C for 10 to 40 days; B, 5 or 10°C for 10 to 40 days followed by 120 hours at 22°C.

	5°C		10°C	
	Treatment		Treatment	
Days	A	B	A	B
10	0.0	25.0	0.0	16.7
20	8.3	50.0	16.7	25.0
30	0.0	33.3	50.0	50.0
40	0.0	33.3	16.7	8.3

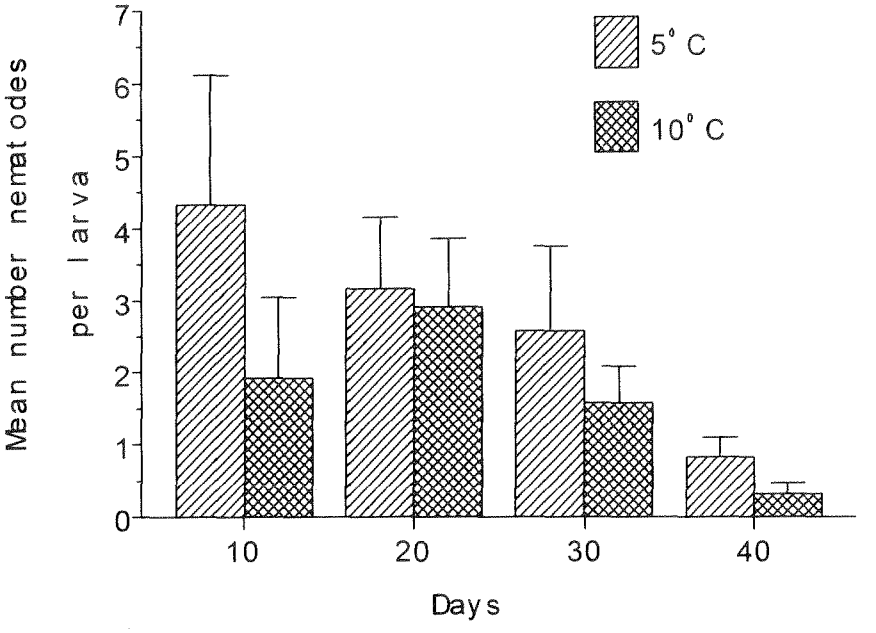

Figure 1. Mean number of nematodes per *O. sulcatus* larva after initial exposure to *S. carpocapsae* at 22°C for 3 hours followed by treatments at 5 or 10°C for up to 40 days followed by 22°C for 120 hours

The number of *S. carpocapsae* surviving in *O. sulcatus* larvae treated at 5 and 10°C declined with time of exposure to these temperatures (Figure 1; $P<0.05$), indicating that nematodes had disappeared after initial invasion. Exposure temperature did not significantly influence the mean number of nematodes per larva.

S. carpocapsae inside *G. mellonella* larvae was able to cause host mortality at 10°C (Table 2): after 20 days, all larvae were dead (treatment B). When larvae were transferred from 10°C to 22°C after 5 and 10 days, larval mortality was 100 % (treatment C). All larvae in the control survived.

After an initial nematode exposure for 2 hours at 22°C and subsequent treatment at 22°C for 48 hours the number of nematodes found in *G. mellonella* larvae was greater than in treatment B or C (Table 2) which suggests that invading nematodes had disappeared when larvae were exposed to 10°C. The percentage of nematodes dead in treatment B and C were significantly greater than in treatment A (Table 2).

Table 2. The percentage mortality of *G. mellonella* larvae, the number of nematodes established and the percentage of dead nematodes in cadavers after initial exposure to *S.* carpocapsae at 22°C for 2 hours followed by treatments at: A, 22°C for 48 hours; B, 10°C for 5, 10 or 20 days; C, 10°C for 5 or 10 days followed by 48 hours at 22°C.

Treatment	Days at 10°C	% larval mortality	No. nematodes established (±SEM)	% dead nematodes
A	0	100	21.5 ±8.3	4.2
B	5	0	--	
	10	17	--	
	20	100	6.3 ±1.4	40.3
C	5	100	10.6 ±3.0	20.6
	10	100	5.7 ± 1.6	40.3

SEM = standard error of the mean

Discussion

Grewal *et al.* (1994) have suggested that IJs may survive inside an insect throughout the winter and then develop to kill the host in the spring when the temperature rises. The results presented here for a very susceptible host, *G. mellonella*, confirm this suggestion. However, IJs which enter the host die with time while the temperature remains below the level at which the nematode and bacteria will develop. In *O. sulcatus*, infection was not as high as for *G. mellonella* and, therefore, mortality never reached 100 % when the temperature was raised at the end of the treatment. However, mortality did increase when larvae were transferred from 5°C to 22°C, and IJs disappeared in the vine weevil larvae kept at both 5 and 10°C.

Since a large proportion invading IJs die and disappear with time, when exposed to 5°C and 10°C, it seems unlikely that the survival of *S. carpocapsae* in insect hosts is a major factor contributing to mortality of vine weevil larvae in the spring. It would be useful to investigate other nematode species/isolates which may be more lethal to *O. sulcatus* larvae with respect to overwintering in larvae.

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Effects of *Steinernema carpocapsae* on the carabid beetle *Poecilus cupreus* in a laboratory test

During the last few years, *Steinernema carpocapsae* was applied to various crops. Subsequently, a wider range of uses for entomopathogenic nematodes will be expected in the field. However, the ecological effects of field applications of entomopathogenic nematodes on non-target arthropods are as yet unknown. Therefore, investigations on the effects of entomopathogenic nematodes on non-target arthropods in the laboratory were begun at the Office for Plant Protection in Berlin in 1995. The effect of *S. carpocapsae* was examined on different developmental stages of *P. cupreus*.

Adults

Six plastic boxes containing clay soil with nine adult *P. cupreus* per box were wetted with a solution of nematodes (10^3infective juveniles/ml). The control boxes were wetted with water. The boxes were placed in a climate room with a temperature of 20°C, a photoperiod of 16:8 (L:D) and a light intensity of 1000 lx. The beetles were fed with fly pupae (*Calliphora sp.*) twice a week. The experiment was repeated once more. The condition of beetles in treated and untreated boxes was observed for 15 days, but there were no observable differences between the treatments.

Eggs

One hundred eggs of *P.cupreus* per experiment were treated with a solution of 1 ml containing 10^3 infective juveniles per ml. Water was used for the control. All the eggs were kept seperately in plastic tubes. The numbers of eggs hatching was recorded and was the same in both treatments.

Larvae

One hundred first- and one hundred final-instar larvae of *P.cupreus* were treated with *S. carpocapsae*. Glass tubes, each containing one larva and 50 ml of peat were treated at the rate of 4000 nematodes per tube. The control tubes for this experiment were each treated with 1 ml of water. The insects were kept at 20 °C and in darkness.

The effect of nematodes on larvae was determined as number of emerged adults, compared with a water-treated control.
After treatment of the first larval stage with entomopathogenic nematodes only 2.9 % successfully produced adults, compared with 33 % for the untreated. The treated final-instar larval stage was reduced significantly by about 75 % compared with the control. The experiment was repeated twice.

In further investigations, the effect on the mortality of various dose rates of nematodes will be checked.

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Prospects for chemical control of black vine weevil (*Otiorhynchus sulcatus*) in nursery stock

Introduction

Only a few chemicals give acceptable control of black vine weevil. These are, however, mostly highly persistent and cause unacceptable environmental pollution. In the Netherlands carbofuran was the only chemical registered for use in the soil to control the larvae; to control the adult weevils acephate and actellic were registered. Carbofuran and actellic will probably loose their registration. Although biological control is showing possibilities for control of larvae in soil the costs of these products are still too high for their widespread use. Control of adult weevils is possible only with chemicals. For this reason there is still a need for new effective and selective chemicals. In this article some results of recent trials with old and new active chemicals are described.

Materials and methods

Chemical control of larvae - pot and field trials 1987 - 1994

In the pot trials *Thuja occidentalis* 'Brabant' was used as a test plant in 1991 and 1992. In 1993 and 1994 we changed to the perennial *Waldsteinia ternata* because natural mortality of larvae was lower with this plant. The plants were potted in May in peat. In 1991 and 1992 the plants were inoculated three times with 15 eggs per pot at two-week intervals. In 1993 and 1994 we inoculated two times with 20 eggs per pot at a three-week interval. Inoculations started at the end of July. Each treatment was repeated 4 times with eight plants per block. All chemicals, except for ethoprofos (Mocap 20GS) and chlorpyrifos (suSCon10), were drenched into the soil, one week before the first and one week after the last inoculation with eggs. The liquid chemicals were applied in 25 ml water per pot. Ethoprofos was applied as granules on top of the soil of each pot. Chlorpyrifos (suSCon10) was mixed in the peat before potting the plants. The adhering soil between the roots of the plants was removed before the plants were potted in the soil with SuSCon10. In November each plant was checked for the number of living larvae and the different instars were recorded.

In the field trials *Thuja occidentalis* 'Brabant' was used as a test plant in 1991 and 1992. In 1993 and 1994 we changed to *Taxus baccata*. Trials were performed on a peaty soil. Each treatment was repeated 3 times with 5 plants per block (17 plants on 1 m^2 planted per block). In 1991 and 1992 we inoculated three times at two-week intervals with 50 eggs per plant. In 1993 and 1994 we inoculated twice at a four-week interval. In 1993 we inoculated two times 40 eggs and in 1994 40 and 20 eggs respectively. Inoculations started at the end of July. All liquid chemicals were applied twice in three litres of water per m^2. Ethoprofos and chlorpyrifos (suSCon10) were applied as described in the pot trials. SuSCon10 was mixed in the top 10 cm soil layer prior to planting. In a two-year trial we tested the effectiveness of SuSCon10 in the rootball of the plants. Plants were either planted directly in the field with SuSCon10 in the top 10 cm soil or potted with or without SuSCon10 for one year and planted in the field in the second year. Weevil eggs were applied in the second year in the field as previously described for 1993. *Thuja occidentalis* 'Brabant' was used as a test plant. The other measurements were performed as in the pot trials. The trials were harvested at the end of February. The three products tested and shown in Figure 1 are Curater lq. (carbofuran; 37.5 l(a.i.20%)/ha), suSCon10 (chlorpyrifos; 375 or 750 g(a.i.10%)/m^3) and an experimental product (fipronil; 12.5, 25 or 50 g(a.i.80%)/ha). Other tested chemicals are summarized in the results.

Chemical control of adult weevils

Chemicals were sprayed on foliage of *Rhododendron* 'Catawbiense Grandiflorum'. On day 0 (directly after drying of the leaves), 3 and 7 after the spraying top leaves were cut and fed to weevils in small boxes. Leaves were placed in small tubes with water to prevent drying of the tissue. Each box contained 10 weevils. Each treatment was repeated 4 times. Temperature was kept at 20°C. The boxes were checked for dead weevils on 1, 3, 7 and 21 days after feeding the weevils. Only the chemicals tested that were effective are shown in Table 1. Condor SC (methyl-parathion) was tested in a different way because of its slow release properties. In small closed boxes the weevils would die mainly from the evaporated active ingredient and not because of eating leaves treated with the chemical. Therefore, the weevils were tested outside in open boxes with a shelter on top. The boxes were 'greased' with Fluon to prevent weevils from leaving the boxes. The weevils were fed leaves on 0, 3, 7 and 14 days after spraying.

Table 1: Chemicals tested for control of black vine weevil adults

Chemical	Product name	Dose	a.i.
acephate	Orthene	0.75 g/l	80%
benfuracarb	Oncol 200EC	1 ml/l	200 g/l
carbofuran	Curater lq.	1 ml/l	200 g/l
furathiocarb	Delthanet 400EC	1 ml/l	400 g/l
methamidifos	Tamaron	3 ml/l	228 g/l
methyl-parathion	Condor SC	1 m/l	240 g/l
pirimifos-methyl	Actellic-50	1 ml/l	500 g/l

Results

Chemical control of larvae

From 1986 until now several chemicals have been tested for control of larvae in the soil. Chemicals such as benfuracarb, carbofuran, etrimfos, fonofos, furathiocarb, tefluthrin and terbufos have given variable results. In pots there was complete control or no control at all. In the field there was moderate or no control. Research with these chemicals was stopped because of the unreliable control and because of the environmental polluting properties of these chemicals. Granular application of ethoprofos was successful in pots but not in the field. The same was true of imidachloprid. For both chemicals the marketing companies in the Netherlands decided not to start a registration procedure because of the low effectiveness in the field. Flucycloxuron, tested in 1991, showed no control in all the trials performed. Figure 1 shows the results of the slow-release formulation of chlorpyrifos (suSCon10) and of fipronil. In pots chlorpyrifos was effective at a concentration of 375 g/m^3. In the field trials control was insufficient at all tested concentrations. Fipronil, a new chemical produced by Rhône-Poulenc, showed high efficacy at the lowest tested concentration in pot trials. In the field trials results were variable (40 to 80%) but better than with carbofuran or chlorpyrifos. The effect of presence or absence of suSCon10 granules in the rootball close to the stem of plants was tested in a 2-years trial. Figure 2 shows the results. When granules of suSCon10 were mixed in the field soil at a concentration of 750 g/m^3 (but not in the pots in the previous year) or when the granules were mixed in the pots in the first year (but not in the field soil in the second year) there was less than 30% control of the larvae. SuSCon10 mixed in the soil and in the rootball before planting at a concentration of 750 g/m^3 gave 60% control in the

second year after application. However, the best control (80%) was reached when the liner was treated in the first year in pots followed by application in the field in the second year in spring when the plants were planted in the field. 375 g/m³ of suSCon 10 was not effective.

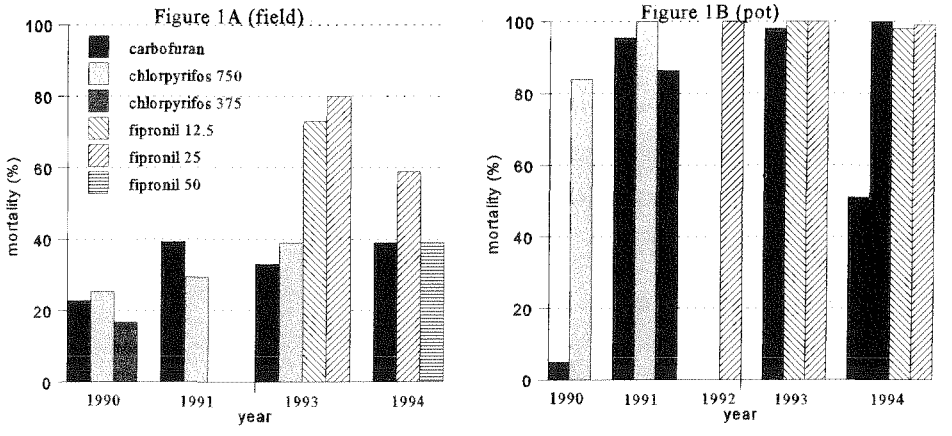

Figure 1: Mortality of larvae of black vine weevil (*O. sulcatus*) after a soil application of several chemicals in A) the field and B) in pots. Fipronil 12.5=12.5 g/ha; fipronil 25=25 g/ha; fipronil 50=50 g/ha. Chlorpyrifos 375=375 g/m³ and chlorpyrifos 750=750 g/m³ of suSCon 10.

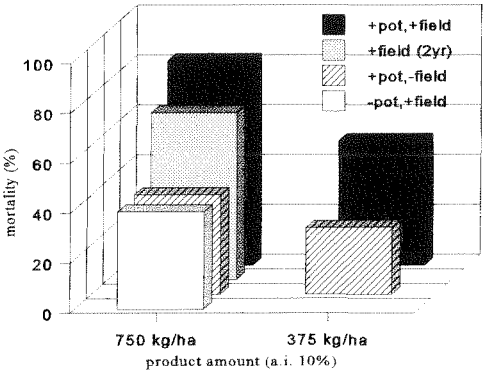

Figure 2: Influence of presence (+) or absence (-) of chlorpyrifos (suSCon 10) in the rootball of *Thuja occidentalis* on mortality of black vine weevil larvae after two year (1 year pot + 1 year field or two years field).

Chemical control of adult weevils

The chemicals bromofos, chlorpyrifos, chlorfenvinvos, fonofos, fosalon, isofenvos, mercaptodimethur, propoxur and tefluthrin gave no control. Ethofenprox, carbaryl and etrimfos gave 30 to 50% control in the first day after spraying but no control on 3 or more days after spraying. The results with the other chemicals are shown in Figure 3.
Acephate, pirimifos-methyl and methamidifos give 80 to 100% control in the first day after spraying. Efficacy, however, decreased very quickly and seven days after spraying control has ceased almost completely. Methyl-parathion gave 90 to 100% control up to 3 days after spraying. After 7 days there was no more control. Carbofuran, furathiocarb and benfuracarb were the only chemicals that showed persistence and 7 days after spraying 40 to 80% of the weevils were killed.

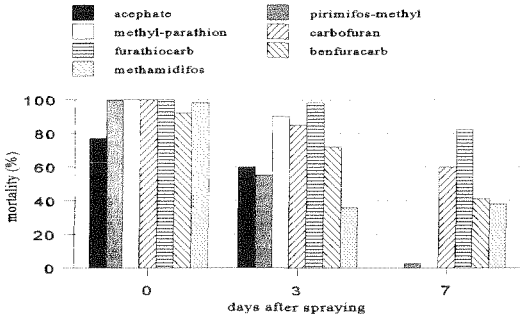

Figure 3: Mortality of adult weevils of *Otiorhynchus sulcatus* in time after spraying with several chemicals.

Discussion

The results show that two chemicals are effective for controlling vine weevil larvae. Chlorpyrifos (suSCon 10) is now registered in the Netherlands for application in pots at a concentration of 750 g/m³ in the first year for liners followed by mixing 375 g/m³ in the potting soil in the following year. In the field efficacy was too low to apply for a registration. Fipronil is a new chemical that has been tested now for several years. The application for registration will start this year. This chemical is effective in pots and in the field.
For control of adult weevils only methyl-parathion (Condor SC) was registered a few years ago. The other effective products are too harmful to other organisms and persistence in soil is too great. Fipronil, imidachloprid and, possibly, a new *Bacillus thuringiensis* strain will be tested further for control of adult weevils.

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Vine weevil adulticides

Introduction

In the United States, production of container-grown nursery plants is an intensive endeavour with a production cycle of 2–5 yr. These high-value crops and their short production cycle make relatively expensive chemical amendments to media or entomopathogenic nematode applications economically viable options for control of vine weevil (*Otiorhynchus sulcatus*). However, control of vine weevils in field-grown hardy ornamental nurseries is particularly difficult. Crops of *Taxus*, *Thuja* or *Tsuga* take up to 10 years to produce premium saleable material. Currently registered soil-applied insecticides have insufficient residual activity to be practical for such a long production cycle, and soil temperatures are generally too low for growers to consider applying nematodes for biological control. For these reasons, foliar sprays of organophosphate or pyrethroid insecticides principally are relied on to kill adults during their preovipositional stage in field-grown nurseries. The following study in 1995 contrasted the efficacy of conventional adulticide standards (acephate and bifenthrin) with experimental materials, including bait-formulated cryolite.

Methods

A 5-yr-old Christmas tree planting of *Picea pungens* was chosen as a model system because the field (E. Windsor, CT) was known to have a large population of vine weevils and the growing conditions were identical to those for non-irrigated field-grown nursery crops. A latin square design with six treatments was used because two orthogonal gradients, the original source of infestation and the grower's previous spray coverage, were expected to influence weevil density. Each plot consisted of the area taken up by trees planted in a 3 × 4 rectangle, with dimensions of 5.5 × 6.1 m. Some of the trees had been harvested in 1994; however, it was expected that larvae would complete development at sites where trees had been cut, and 2 –11 (avg. 5.9) trees remained per plot. During early June, 490 m of aluminum flashing strip (15 cm wide) was installed as exclusion barriers separating plots, with the bottom 5 cm buried into the ground and the top 5 cm coated on both sides with motor grease. Pitfall traps were constructed of 475 ml plastic cups (8.5 cm diam.) with the top 2 cm coated with an oily

lubricant. Two pitfall traps were installed near corners of each plot. Legs were cut into the rim of pots (4 litres), which were then inverted over pitfall traps to prevent them from filling with rain.

Treatments consisted of four foliar and one bait application of insecticides (Table 1), and an untreated control. Positive controls were acephate and bifenthrin, the current nursery standards. Foliar-applied cryolite was included in the trial to provide a direct comparison with the bait formulated cryolite and with conventional foliar sprays. Another new material, chlorfenapyr (AC303,630), had excellent activity against vine weevil in the laboratory (RSC, unpublished data), and it represents a new class of insecticides (pyrroles) with good properties for resistance management. The dosage of acephate was probably too high in the 22 June treatment, and cryolite and chlorfenapyr rates too low, so the 13 July and 10 August applications used new dosages. Foliar applications were made with a high-pressure hydraulic 100-litre research sprayer at a spray volume of approximately 4700 litres ha^{-1}. Bait was mostly scattered under foliage on the ground, with the remainder distributed throughout the plot.

Table 1. Vine weevil adulticides tested in field-grown Colorado blue spruce.

		Treatment date	
Active ingredient	Formulation	22 Jun	13 Jul; 10 Aug
acephate	Orthene 75S	3.7	1.5
bifenthrin	Talstar 10W	0.18*	0.22
chlorfenapyr	Pirate 360F	0.18	0.34†
cryolite	Cryocide 96W	22	45
cryolite	Cryolite 20% Bait	4.5	9

* Rates are expressed in kg [a.i.] per ha

† The last treatment used the 240F formulation

Pesticide effects were evaluated in three ways: weekly pitfall trap catches, timed counts of dead weevils, and soil samples to recover larvae. Pitfall trap catches were made weekly from 22 June to 7 September to measure adult survival. Dead weevils found in the traps were removed from the experimental plots, while live weevils were released. Mortality was

estimated with 4-min timed counts of dead weevils made 1 wk following each treatment. All dead weevils were removed from plots following mortality evaluations to reduce the overlapping influence of sprays. To evaluate larval populations, 10 kg (11 litres) samples of soil were taken on 14 November and 4 December from two trees within each plot. Samples were dug after the trees were cut, providing a 20 cm wide, 40 cm long, and 12 cm deep volume of soil from within the former dripline region. Soil samples were sieved through a No. 10 soil screen to recover all larvae.

Results

There were statistically significant treatment effects on weevil mortality (Table 2) following the first and second applications ($F=13.6$; $df=5, 20$; $P<0.0001$; $F=7.99$; $df=5, 20$; $P<0.001$, respectively). Following the first application, treatment efficacy was ranked (in decreasing order): acephate, bifenthrin, cryolite bait, chlorfenapyr, and cryolite spray, and all treatments significantly differed from the control. All materials in the second application were significantly different from the control, even with the new dosages. The general pattern for efficacy was essentially the same as for the first application; however, there were no significant

Table 2. Treatment means following application of vine weevil adulticides in field-grown Colorado blue spruce.

Active ingredient	Dead adults* 6/29	7/20	8/17	Live adults	Larvae*
acephate	55.0 a	46.0 a	28.7	146 a	5.04 a
bifenthrin	36.9 ab	29.7 a	21.6	204 a	1.84 a
chlorfenapyr	15.2 ab	11.6 a	18.6	118 a	3.89 a
cryolite spray	14.0 c	28.8 a	27.8	256 a	2.33 a
cryolite bait	20.7 c	30.8 a	30.1	112 a	2.85 a
control	2.7 d	5.4 b	13.2	391 b	14.81 b

* Reported means are back-transformed from square root (x+0.5) data; means within columns followed by the same letter do not significantly differ ($\alpha=0.05$; Student-Newman-Keuls' test).

differences between treatments. Following the third treatment, there were no significant differences between treatments and the untreated control ($F=1.93$; $df=5, 20$; $P=0.13$).

Insecticide treatment significantly reduced total weevils caught in pitfall traps (Table 2; ($F=6.64$; $df=5, 20$; $P<0.001$). The basic pattern was inversely proportional to the mortality estimates. Thus, there were high counts of live weevils found among less effective treatments, and lower counts for treatments causing higher mortality. Overall, there was significant difference only between the control and all the treatments. Treatment effect was also significant for the number of larvae recovered in soil samples (Table 2; $F=4.54$; $df=5, 20$; $P<0.01$); like trap catches, differences in larval counts were detected only between the control and the insecticidal treatments.

Discussion

None of the adulticide treatments was completely effective, in spite of the effort to obtain thorough coverage and a relatively close spray interval. The degree of population suppression observed with all the treatments would be adequate for crops in which low populations may be tolerated. However, the lack of complete control would be a concern for any crop that requires a higher degree of pest cleanliness, especially for maintaining a quarantine.

There are several problems inherent to attempting control of adults in large, field-grown nursery crops. The extended adult emergence requires multiple applications of any foliar insecticide to interrupt development of several cohorts. Not all adults are active each night, so short residual materials (e.g. acephate) may not be entirely successful (Nielsen and Montgomery, 1977). Clonal populations can immediately express resistance traits, most recently evident from recovery following knock-down by pyrethroids (Nielsen, 1983).

The need for adequate coverage of foliage with spray treatments often leads to serious over-application or over-dilution of pesticides, and often has the unintended consequence of causing secondary outbreaks of non-target pests, such as mites, scales and aphids. Bait formulations of insecticides for controlling vine weevil, first investigated by Floyd Smith (1932), appear to be a promising alternative to conventional foliar treatments. Baits, especially

when attractive, can bring a pest in contact with a toxicant, thus minimizing the active ingredient required for control. In this trial, the bait formulation provided the same degree of adult mortality with only 20% as much active ingredient as the foliar spray. The apple fiber-based bait used in this trial requires some degree of hydration to be palatable to vine weevils. This may be a limitation for non-irrigated crops, because the peak of adult emergence happens to occur when summer drought is common. In spite of severe drought conditions during this trial, we obtained positive results. Further enhancements in baits, either through incorporation of better attractants, phagostimulants, or toxicants, or through application to irrigated crops, could cause higher adult mortality and correspondingly improved reductions in larval populations.

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The development of controlled-release technology to give long-term control of vine weevil larvae in ornamentals.

Introduction

Until 1989, it was common nursery practise in the UK to incorporate aldrin into the growing media for most container-grown hardy ornamental nursery stock (HONS) and some other horticultural plants. This gave generally good control of vine weevil larvae (*Otiorhynchus sulcatus*) for the two or three years plants spent on the nursery. With the banning of aldrin in the UK in 1989, later than in most other European countries, the pest rapidly became re-established and in some areas reached near-epidemic proportions.

A replacement for aldrin was needed which could also be incorporated into growing media to give an equally long period of control. Although there were approved uses for fonofos, for incorporation or drenching, and carbofuran granules for surface application, neither gave reliable control of larvae beyond the season of application. Nematodes were a biological alternative but they too are short-lived and have temperature limitations.

Like aldrin, the replacement needed to be incorporated into growing media at the time of potting, normally from early spring to summer, to retain its effectivenss until the hatch of vine weevil eggs in late summer / autumn, and to maintain control in the original rooting area for a further one, or possibly two, growing seasons.

The development of a controlled release formulation of chlorpyrifos

Chlorpyrifos was known to control vine weevil larvae, and in the UK there were approved uses as a single drench treatment on strawberries and forest transplant lines. However, formulations existing at that time gave only short-term control, and for container-grown ornamentals were not suitable for incorporation into growing media to give control for more than one growing season. There were additional risks from phytotoxicity.

The product suSCon ® Green was developed as a controlled-release (CR) granular insecticide formulation containing 10% chlorpyrifos. A similar CR formulation containing the same active ingredient was first developed for use in sugar cane by Incitec Ltd, Australia, now known as Crop Care Australasia Pty Ltd. Other suSCon formulations using different active ingredients have been registered for a range of uses throughout the world, including forestry.

The patented process involves incorporating the active ingredient into a polymer matrix that is extruded and pelletised into granules. Commercial formulations of these products have granule sizes of 1 to 2mm depending on the target pest, intended use and rate and method of application.

After application of the granules, the active ingredient is released via leaching and diffusion processes. This establishes a zone of active ingredient concentration around each granule. As the active ingredient degrades, the concentration is maintained by continued and controlled release of active ingredient from the granules. Release periods of 6 months to 3 years are possible, depending on the components of the formulation and the granule size.

Trials in the UK with suSCon Green against vine weevil larvae started in the late 1980's. This was a specially developed formulation, with spherical granules of 1mm diameter, giving approximately 1,500 granules per gram of product. This size of granule was chosen to ensure the optimum number of 'killing points' for the larvae. Chlorpyrifos is not root systemic and has a low water solubility. Therefore, it does not dissolve and move laterally into the surrounding growing media. The mode of action is primarily by contact, with a small amount of vapour effect. Even and thorough mixing of the correct dose of suSCon Green is, therefore, essential to ensure control of vine weevil larvae. The granules are coloured dark green, which helps in monitoring the eveness of mixing, and also ensures they can be found relatively easily should there be need to check the dose rate.

® suSCon is a registered trademark

Evidence has also shown that suSCon Green will control vine weevil larvae with certainty only at neonate and early-instar stages. If older larvae are introduced to growing media containing the product, a high proportion are likely to survive. This means that the product must be incorporated into growing media at all stages of plant potting or re-potting to ensure control at or soon after egg hatch.

Results from trials

In 1989, sponsored efficacy trials were begun by ADAS at several centres in the UK, on plants grown on for three seasons. The results from some of these trials were presented in a poster display at the first International Workshop on vine weevil. A second series of ADAS trials, jointly sponsored by the Horticultural Development Council, was started in 1990.

Rooted cuttings were potted into treated growing media in 1990, re-potted into larger pots in 1992, with the new growing media also treated, but not potted on further in 1993.

Table 1 Control of vine weevil larvae on *Cotoneaster bullata* at ADAS Leeds, 1990-93.

(Egg incoculation - 30 eggs per pot, single annual application.)

	Peat / grit			Peat / bark/ grit		
Treatment	**1990/91**	**1991/92**	**1992/93**	**1990/91**	**1991/92**	**1992/93**
g a.i./m3	**Larvae (mean/pot)**			**Larvae (mean/pot)**		
suSCon Green 75	0	0	0.1	0.5	0	0
suSCon Green 100	0	0	0	0	0	0
suSCon Green 150	0	0	0	0	0	0
fonofos 43.3	0	0	0	0.1	0	0.1
untreated	8.9	2.8	19.1	11.1	9.5	12.5

Table 2 Control of vine weevil larvae on *Thuja plicata* at ADAS Reading, 1990-93.

(Egg inoculation - 1990 and 1991, 30 eggs per pot, single application. 1992, 80 eggs per pot divided into three applications.)

	Peat / grit			Peat / bark/ grit		
Treatment	**1990/91**	**1991/92**	**1992/93**	**1990/91**	**1991/92**	**1992/93**
g a.i./m3	**Larvae (mean/pot)**			**Larvae (mean/pot)**		
suSCon Green 75	0.5	0.4	0.4	0.3	1.0	3.5
suSCon Green 100	0	2.1	1.8	0	0.6	1.7
suSCon Green 150	0	0	0.4	0.1	0.4	0.7
fonofos 43.3	3.0	16.8	16.7	1.2	15.3	19.5
untreated	4.4	21.6	32.3	5.4	16.3	46.0

The results (Table 1 and 2) show that all three rates of suSCon Green were almost completely effective for three growing seasons at the Leeds site, with a moderate level each year of young larvae. At Reading, with a generally higher level of larvae infestation, all rates were effective for three seasons in the peat/grit growing medium, but slightly less effective in the third season with peat/bark/grit. By contrast, although fonofos was effective at Leeds, with the higher pest pressure at Reading it was much less effective.

Critical trials for phytotoxicity was carried out on a range of hardy plants in 1989 and 1990 at HRI, Efford. These showed that only one species was damaged at the commercially recommended rates of 750 g - 1kg suSCon Green /m3. Tests were also carried out on a wide range of plants on commercial nurseries to establish the level of safety under different growing conditions.

Trials have also been carried out with suSCon Green in other countries in northern Europe and in the USA, partly to meet efficacy requirements in obtaining official registration. Although different growing media were used in these trials, the response at similar dosage rates have been similar to the results in the UK.

Commercialisation

The product suSCon Green was approved for use in the UK in early 1993, and has thus been available for use by the HONS industry for three growing seasons. It was quickly adopted by growers as an alternative to aldrin, especially when raising young plants. Use has increased since, and it is now generally regarded as the standard control to use for growing media incorporation for hardy ornamental plants, especially those species and genera most susceptible to vine weevil.

The product is now registered for use in the Netherlands, Belgium, Irish Republic and France, all areas where attacks by vine weevil on hardy ornamental plants can be severe. In most of these countries, where aldrin had been banned a number of years earlier, there was less of a tradition of growing media incorporation as in the UK. The most common form of control in some countries is still overhead spraying against adults. Adoption by growers of the compost incorporation technique using controlled-release granules has been slower, but use is expected to increase as the benefits are realised.

Future Developments

This paper describes the first application of a controlled-release granular technology to combat an insect pest, by incorporation into growing media in plant containers. Previous applications of the same technology have been on outdoor crops, sometimes using systemic insecticides such as carbosulfan.

In principle, the system offers a number of advantages, including the elimination or reduction of more frequent overhead sprays, drenches or short-term granular applications. This can help improve both environmental and operator safety, in addition to providing economic benefits for growers.

Controlled-release technology of this type, offering such variables as granule size, changes of release pattern, and pesticide type offers a range of opportunities for the container-growing of ornamentals for the future. In addition to other target pests, there are several soil-borne diseases which might be satisfactorily controlled in a similar way, and methods by which uses of the technology might be extended are being studied.