Project title:	A review of vine weevil knowledge in order to design best-practice IPM protocols suitable for implementation in UK horticulture
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Project leader:	Jude Bennison, ADAS
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Date project commenced:	13 October 2013
Date project completed (or expected completion date):	May 2014

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

This project collated relevant current knowledge on vine weevil biology and control, identified key gaps in knowledge and provides impartial, best practice IPM programmes suitable for commercial adoption. Recommendations for knowledge transfer are given.

Background

Vine weevil is one of the most serious pest problems in UK soft fruit and hardy nursery stock crops. Adult damage to leaves and presence of larvae around roots can make ornamental plants unmarketable. Root damage caused by larvae in both ornamental and soft fruit crops leads to reduced plant vigour and yields and if damage is severe, to plant death. Chemical control of the pest is now difficult on ornamentals due to withdrawal of the most effective persistent products for use in growing media. Chemical control on soft fruit crops is mainly limited to using foliar sprays against adults, which gives unreliable control.

Growers are under pressure to reduce pesticide use and are increasingly adopting biological pest control methods within Integrated Pest Management (IPM) programmes. Biological control methods for vine weevil now available for use on both soft fruit and ornamental crops include insect-pathogenic nematodes and fungi. Growers need more knowledge about the pest biology and the biological methods in order to gain optimum control in their various production systems.

Summary

Objective 1. Collate current knowledge of vine weevil biology and control and identify key gaps in understanding

Task 1.1. Interviews with key industry representatives to identify currently used vine weevil management strategies and their success, and perceived gaps in knowledge

A total of 29 UK industry representatives were interviewed, including seven growers of hardy nursery stock (HNS), one grower of protected ornamentals, seven growers of soft fruit, eight consultants in the ornamentals and/or soft fruit industries and six suppliers of biological and chemical controls for vine weevil. Only growers who experienced vine weevil problems on their farms or nurseries during 2013 were interviewed. Only one of the growers of HNS relied on a pesticide control programme, all other growers of ornamentals and soft fruit used IPM programmes for management of vine weevil and other pests. Two case studies are summarised below, giving an example grower of HNS and a combined case study of the soft fruit growers who used current 'best practice' IPM programmes.

HNS

Components of the grower's IPM programme for vine weevil management included:

- On both protected and outdoor containerised plants, use of the entomopathogenic nematodes *Steinernema kraussei* (Nemasys L) in cool conditions (5-12°C) and *Heterorhabditis bacteriophora* (e.g. Nemasys H) in milder conditions i.e. 12°C or above was considered to be successful. Nematodes are applied as a drench to all vine weevil-susceptible plants and all plants in propagation. Monitoring of vine weevil larvae around roots is done by knocking out pots to guide autumn application timings but typically applied in weeks 36 and 42/43. Further monitoring of infected or healthy larvae is done following application. Nematode viability is checked using a microscope before application. All supplier application recommendations are followed. Run-off onto the floor from large, densely-spaced plants is a practical application problem and the grower is interested in the development of a specialised applicator to overcome this problem.
- The entomopathogenic fungus, *Metarhizium anisopliae* (Met52) is mixed into the substrate used in plug trays in propagation and is considered as successful in these conditions as no larvae have been detected. Substrate temperature is monitored. Gaps in knowledge identified were more guidance on suitable temperatures and moisture levels for efficacy and potential side effects of fungicides.
- Pymetrozine (Chess WG) is used under protection (daytime application) for adult control when monitoring indicates that adults are feeding. This seems to be effective, judged by monitoring damage on Euonymus 'bait' plants and by night-time crop walks to monitor for adults. Adult sprays are normally applied in April (for overwintered adults) and June or July (for new adults), depending on monitoring.
- Thiacloprid (Exemptor) is used in the growing media used to pot plugs up into all long-term liners potted after 1 July and for potting up highly susceptible saleable plants, bought-in plants with adult feeding damage and re-potted crops with a history of infestation.

Soft fruit

A combined case study of the growers interviewed is presented, as although most growers used similar programmes, some individual growers used one or more adapted or additional IPM components which justify presenting:

- All the growers used entomopathogenic nematodes and considered them to give satisfactory control when used in substrate-grown crops (strawberry, raspberry and blackberry) but to give poor control when used in field-grown crops, thus nematodes are seldom applied to soil-grown crops. Application is mainly through drip irrigation systems as it is much less labour-intensive than drenching, however drenching is sometimes used e.g. to infested strawberry tray plants or to large pots used for growing blueberries. Dripper efficiency is monitored using dye and some growers also check numbers of nematodes at the start and end of the irrigation system. Nematodes species used, as in HNS, are *S. kraussei* (Nemasys L or Exhibitline sk) or *H. bacteriophora* (Nemasys H, Larvanem, Nematop or Exhibitline h) depending on the time of year and temperatures. Efficacy is monitored by checking for live and infected larvae 2-4 weeks after application.
- Most growers use recommended nematode rates, in one or two applications in August or August and September) and again in April if live larvae are seen and temperatures are suitable. Several growers in Scotland have successfully used a 'little and often' method with lower rates (one fifth or half-rates) applied monthly, often between April and October. This strategy has been advised by Syngenta Bioline, following unreliable control given by recommended rates applied in autumn and spring, possibly due to overlapping vine weevil generations. Research to validate this approach compared with conventional nematode timings is justified.
- The current formulation and recommended incorporation method for Met52 is not suitable for use in soft fruit. Most strawberry crops are grown in coir, delivered in solid blocks in bags for wetting up, so incorporation is not possible. Most beds used for soil-grown strawberry crops are made up in autumn for spring planting, thus the product would run out of persistence by the following autumn when vine weevil larvae would be present, and in the second year's cropping when most vine weevil problems occur. Raspberry plants are cropped for 3-4 years and thus Met52 incorporation into the mixed coir substrate and chopped roots of previous crops would not give sufficient persistence. Growers would be interested in a liquid formulation that could be applied through drip irrigation. One grower had successfully used Met52 in a sawdust mulch (using EAMU 1997/2011) in spring on potted blueberry and considered this to have given successful control of any young larvae hatching from eggs laid into the mulch.
- Most growers used insecticide sprays at or just after dusk on warm, still nights for adult weevil control, including chlorpyrifos (Dursban WG or Equity), thiacloprid (Calypso) or pyrethroids such as lamda-cyhalothrin (Hallmark). Most growers

reported poor control, with only one grower reporting dead adults after spraying chlorpyrifos to the base of raspberry canes.

- Five of the seven growers interviewed applied a chlorpyrifos drench to strawberry crops after harvest in October to November, particularly on older, soil-grown crops where vine weevil numbers have built up due to the impracticality of using nematodes. Drenches were reported to give variable control of larvae.
- Cultural control methods used included using barrier glue on table top legs to prevent weevil adults crawling up to strawberry crops, removing polythene mulches on raised beds which was reported to significantly reduce weevil populations and choosing isolated sites away from infested areas to plant new crops.

Task 1.1, 1.2 and 1.3. Systematically retrieve relevant peer reviewed scientific literature, retrieve 'grey' literature and collate and summarise key relevant information.

A search of international scientific publications and 'grey' literature (such as HDC, Defra and HortLINK funded project reports, USDA funded research reports and conference proceedings) identified over 560 papers or reports with relevant information on vine weevil biology and management. These publications were grouped together in a database and each one was read by the project team and summaries of key knowledge were written up as a comprehensive report (given in the Science Section of this report) which collated current understanding of vine weevil biology and management. Key knowledge or technology gaps were highlighted. The report is split into the following five sections:

- Vine weevil biology and behaviour, monitoring and forecasting
- Biological control with entomopathogenic nematodes
- Biological control with entomopathogenic fungi
- Other non-chemical methods including predators and other natural enemies, plant extracts and botanical biopesticides, cultural control methods
- Chemical control, relevant to currently approved products in the UK or those with potential for future approval

Objective 2. Identify opportunities for the delivery of existing knowledge to support implementation

HDC intends to fund activities to communicate key information reported in the review to growers and other industry members. The report summarises knowledge transfer methods previously and currently used for communicating knowledge on vine weevil biology and control. These include HDC reports, website, factsheets, HDC News articles, Crop Walkers Guides, grower IPM workshops funded by HDC, Defra and others, consultancy provided by ADAS, other consultants and biological control suppliers, product labels and leaflets and supplier websites. When growers, consultants and suppliers were interviewed about their current management strategies in Objective 1, they were also asked to comment on the effectiveness of these knowledge transfer methods and which they would find most helpful in supporting the implementation of vine weevil control strategies. Full details of previously and currently used methods and industry feedback are given in the Science Section. Taking into account feedback from the industry, the following methods are suggested for communicating relevant knowledge and IPM protocols to growers in each relevant sector:

- HDC News article(s)
- Presentations at relevant grower meetings
- Vine weevil seminars or workshops in England, Scotland and Wales.
- Factsheets to be updated for both soft fruit and HNS/protected ornamentals
- Vine weevil section on the HDC website, designed to allow easy navigation and access to key information, seasonal action points and practical tips.
- Emails / texts to growers with vine weevil alerts and action points
- Practical demonstration of current best-practice application methods for vine weevil control on a soft fruit farm and HNS nursery.

Communication plans and research priorities to fill gaps in knowledge will be confirmed after discussions with key HDC staff and the industry representatives.

Objective 3. Design 'best-practice' IPM protocols suitable for implementation on susceptible crops in each relevant horticultural sector

Using the information on vine weevil biology and control collated in Objective 1, two flow charts were produced, one for containerised ornamentals and one for soft fruit, summarising key decisions and options for vine weevil management within an IPM programme. Each chart is presented in two parts, one for early season and the other for mid-late season (see Figures 1a and 1b (ornamentals) and 2a and 2b (soft fruit). Options for the various components of the IPM programmes are summarised in Table 1.

Table 1. Summary of components of IPM programmes for containerised ornamentals and soft fruit

IPM component	Containerised ornamentals	Soft fruit
Monitoring	Check around roots for larvae March-November, check again 2-4 weeks after nematode application to guide repeat applications Check for adult activity and damage April-October	
Cultural control	Dispose of badly infested plants and growing media, keep weeds controlled and maintain nursery hygiene	As for ornamentals, also consider removing polythene mulch, and using barrier glue on table-top legs
Entomopathogenic nematodes - timing	Apply as drench in April if live overwintered larvae found, repeat in August-November to control larvae hatching from summer and autumn-laid eggs if temperatures suitable (2 applications may be needed)	In substrate crops, apply by drip-irrigation in April if live larvae found and temperatures suitable, repeat in August-September (2 applications may be needed). Or consider the 'little and often' approach (low rates applied monthly April-October). Research is justified to validate this approach.
Entomopathogenic nematodes - temperatures	*Steinernema kraussei* (Nemasys L, Exhibitline sk) 5-30°C *Heterorhabditis bacteriophora* (Nemasys H, Exhibitline h) 12-30°C *H. bacteriophora* (Larvanem) 14-33°C *H. bacteriophora* (Nematop) minimum 12°C Mix of *Steinernema carpocapsae, S. feltiae* and either *H. bacteriophora* or *H. megidis* (SuperNemos) minimum 10°C	
Met52	Consider incorporation in growing media for spring/summer pottings. Minimum temperature for activity against larvae 15°C. Unlikely to be effective against larvae hatching September-November from late-laid eggs	Consider EAMU 1997/2011 for use in a mulch, e.g. to plants in large pots
Chemical control - adults	Consider foliar spray(s) against adults in April-May (overwintered adults) or June/July (new adults). Chess WG (EAMU 2834/2008 for protected ornamentals) or Steward (EAMU 2905/2008 for outdoor ornamentals) are more IPM-compatible than other pesticides and showed promise in HDC semi-field trial. (Lower, on-label or other EAMU application rates than those in the above EAUMUs have not been tested). Efficacy in commercial conditions needs validation.	Timing as for ornamentals. Chess WG (EAMU 2834/2008 for protected crops) or Steward (EAMU 2905/2008) on outdoor, uncropped soft fruit where a 1-year harvest interval is possible i.e. plants in propagation) are more IPM-compatible than other pesticides. Comments on efficacy at rates in other EAMUs as for ornamentals.
Chemical control - larvae	Consider thiacloprid (Exemptor) incorporation into peat-based growing media. Imidacloprid (Imidasect 5GR or Intercept 5GR only in peat-based growing media in glasshouses, do not move outside until after flowering).	Consider chlorpyrifos drench to strawberry after cropping if sufficient soil moisture and temperatures above 5°C

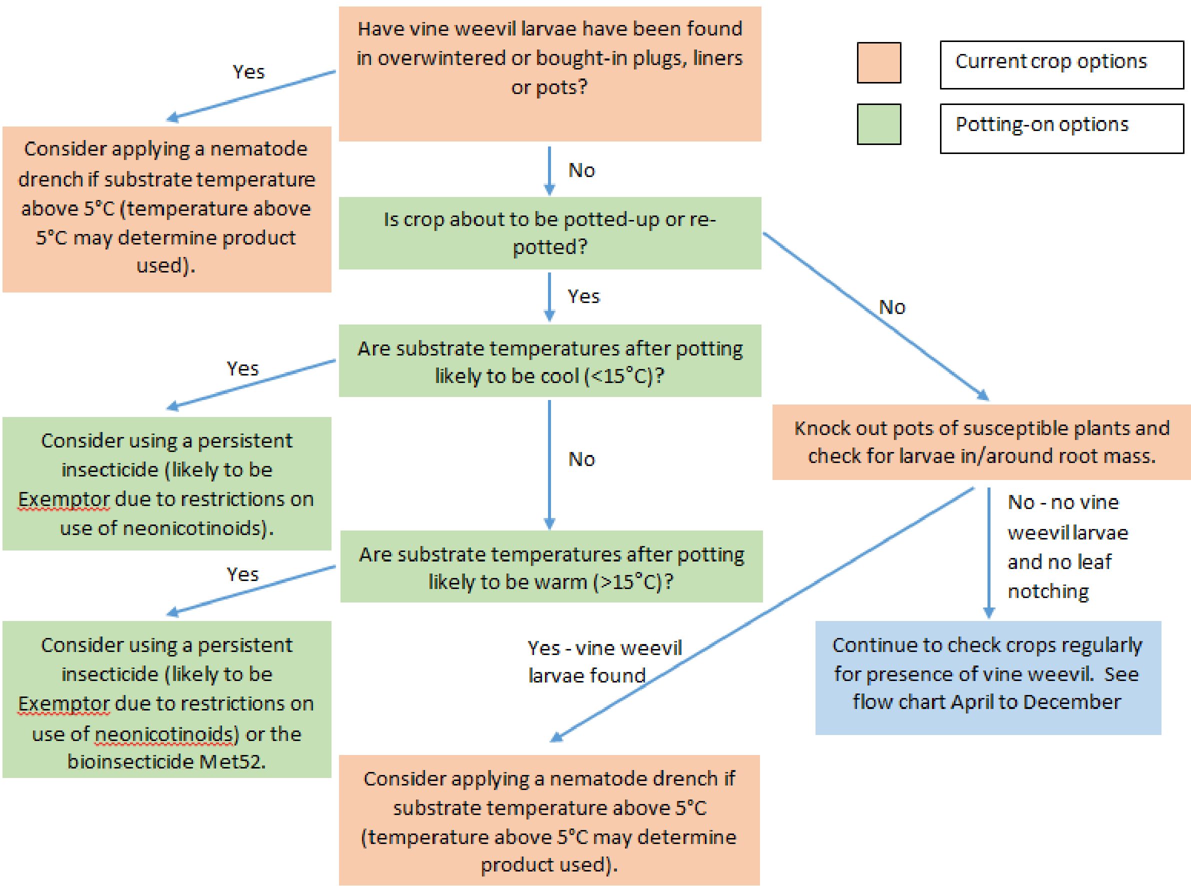


Figure 1a. Early season (January to April) decisions in vine weevil management on susceptible containerised ornamentals.

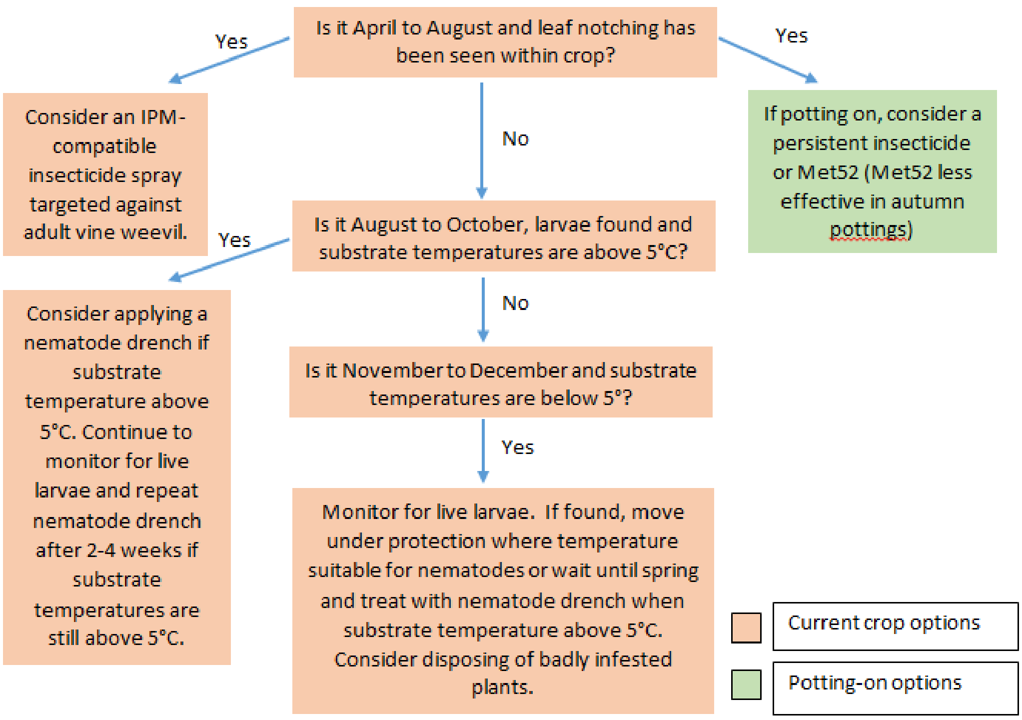
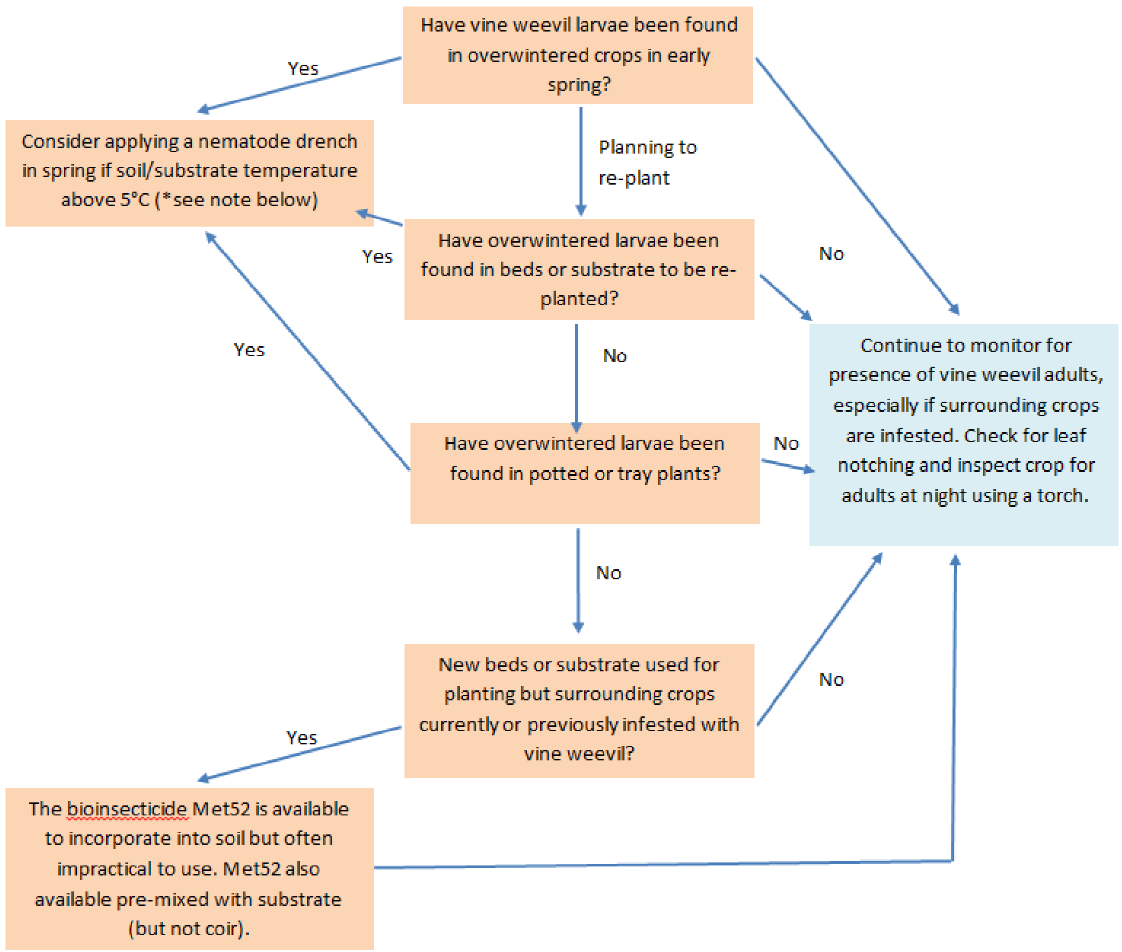
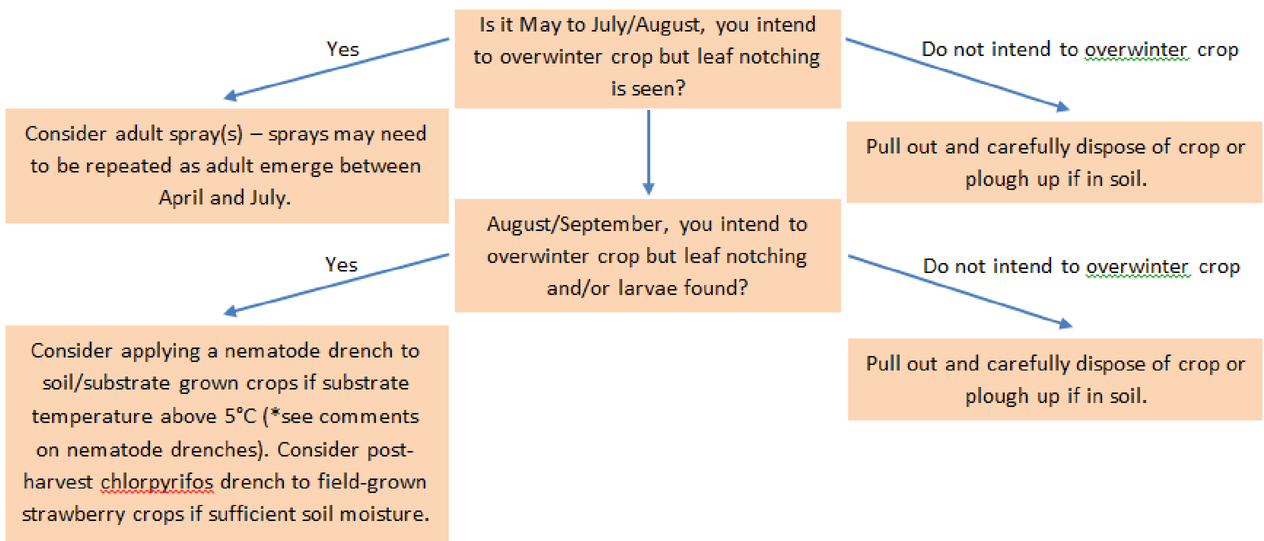


Figure 1b. Mid to late season (April to December) decisions in vine weevil management on susceptible containerised ornamentals.



*Nematode drenches are most likely to be applied through drip irrigation. The temperature above 5°C may determine the species/product used. Nematode drenches are likely to be more effective in substrate than in soil-grown crops.

Figure 2a. Early season (February to April) decisions in vine weevil management on soft fruit crops.



*Nematode drenches are most likely to be applied through drip irrigation. The temperature above 5°C may determine the species/product used. Nematode drenches are likely to be more effective in substrate than in soil grown crops.

Figure 2b. Mid to late season decisions (May to September/October) in vine weevil management on soft fruit crops.

SCIENCE SECTION

Introduction

Vine weevil is one of the most serious pest problems in UK soft fruit and hardy nursery stock crops. The adult damage to leaves and presence of larvae around roots can make ornamental plants unmarketable. The root damage caused by larvae in both ornamental and soft fruit crops leads to reduced plant vigour and yields and if damage is severe, to plant death. Chemical control of the pest is now difficult on ornamentals due to withdrawal of the most effective persistent products for use in the growing substrate. Chemical control on soft fruit crops is mainly limited to using foliar sprays against adults, which gives unreliable control.

Growers are under pressure to reduce pesticide use and are increasingly adopting biological pest control methods within Integrated Pest Management (IPM) programmes. Biological control methods for vine weevil now available for use on both soft fruit and ornamental crops include insect-pathogenic nematodes and fungi. Growers need more knowledge about the pest biology and the biological methods in order to gain optimum control in their various production systems.

This project collated relevant current knowledge on vine weevil biology and control, identified key gaps in knowledge and provides impartial, best practice IPM programmes suitable for commercial adoption. Recommendations for effective knowledge transfer are given.

Objectives:

1. Collate current knowledge of vine weevil biology and control and identify key gaps in understanding
2. Identify opportunities for the delivery of existing knowledge to support implementation
3. Design 'best-practice' IPM protocols suitable for implementation on susceptible crops in each relevant horticultural sector
4. Provide plans for communicating relevant knowledge and IPM protocols to growers in the relevant sectors

Materials and methods

Objective 1. Collate current knowledge of vine weevil biology and control and identify key gaps in understanding

Gaps in current understanding of vine weevil biology and control were identified by interviewing key industry representatives (growers, consultants and suppliers) and through completion of a systematic literature review and collation of current knowledge.

Task 1.1. Interviews with key industry representatives to identify currently used vine weevil management systems and their success, and perceived gaps in knowledge

A total of 29 industry UK representatives were interviewed:

- Seven growers of hardy nursery stock
- One grower of protected ornamentals
- Seven growers of soft fruit
- Eight consultants in the ornamentals and/or soft fruit industries
- Six suppliers of biological and chemical controls for vine weevil

Representatives covered a geographical spread in England, Scotland and Wales. Only growers who experienced vine weevil problems on their farms or nurseries during 2013 were interviewed. The interviews were done in person on farms or nurseries, or on the telephone, or by email. A questionnaire was prepared in consultation with ADAS soft fruit and ornamentals consultants and with the two grower representatives to ensure that the interviews were structured to discuss all relevant issues. These issues included:

- Details of currently used vine weevil management programmes including cultural, biological and chemical control methods and application timings and systems.
- Adaptation of programmes according to different crops and production systems such as field-grown or protected, substrate type, age of crop, irrigation methods, whether or not IPM is used for other pests as well as vine weevil.
- Success rate of the different vine weevil control methods used.
- Any gaps in knowledge or understanding about successful vine weevil management

Task 1.2 Systematically retrieve relevant peer reviewed scientific literature

Peer reviewed literature was obtained firstly by database searching for papers containing the terms '*Otiorhynchus sulcatus'* and 'vine weevil' using Web of Knowledge (http://wok.mimas.ac.uk/), Scopus (http://www.info.sciverse.com/scopus) and Cab Abstracts (http://www.cabi.org/). Both Web of Knowledge and Scopus only go back to 1970 and CAB Abstracts to 1973. In order to overcome this limitation the references included in key research papers and reviews were used to access relevant older literature. The most recent review of vine weevil biology and control was completed by Moorhouse *et al.* (1992) and this provided access to relevant pre 1970 literature. The project team also had access to the earlier vine weevil review by Smith (1932).

Task 1.3 Retrieve 'grey literature'

'Grey' literature was obtained from a range of sources, including HDC, CRD and HortLINK funded project reports; USDA ARS funded research; International Organisation for Biological Control (IOBC) proceedings; other conference proceedings (in Web of Knowledge). Dr Denny Bruck from the USDA Horticulture Crops Research Unit, Oregon, who has led most of the USDA vine weevil research in the last decade, supplied information on the USDA 'grey' literature.

Task 1.4 Collate and summarise key relevant information

References identified through the literature review were grouped together in a database (EndNote). This allowed easy management and searching of references. All the publications were then screened, removing duplicates and records that were not relevant to vine weevil biology and/or current vine weevil management. The key information from each reference was exported into an Excel format in order to produce referenced summary tables of available information. Information from other reviews recently completed by the project team were included in the tables; this avoided any duplication of effort and added value to the project. Tables were produced in the Excel spreadsheet for the different components that could form an IPM programme, plus underpinning information on vine weevil biology. These components included:

- Vine weevil biology – life cycle, development rates, thermal biology, oviposition behaviour, feeding behaviour of larvae and adults (including interaction with plant variety i.e. host plant resistance), semiochemicals, aggregation behaviour and dispersal.
- Monitoring techniques and population forecasting.
- Control with entomopathogenic nematodes
- Control with entomopathogenic fungi
- Potential control with bacteria and other microbiological control agents
- Predators and other natural enemies
- Chemical control of adults and larvae (relevant to currently approved products in the UK or those with potential for future approval)

- Potential for control with ‘natural’ insecticidal products (phytochemical biopesticides based on plant extracts)
- Cultural control methods

In the summary table in the Excel spreadsheet, for each reference, the type of environment in which the research was done was recorded (laboratory experiment, semi-field experiment, protected crop, open field scale). Papers concerning vine weevil control methods were scored to indicate the success of each approach. The summary table was designed (using filters) to allow a quick searching and visualization of the information collected. Over 560 publications were read, evaluated and included in the Excel database. This made the review systematic and reduced bias and has not been used before to review the biology and control of vine weevil.

In addition, after each of the papers and reports were read by the project team, summaries of the key knowledge from all relevant papers were written up as a comprehensive report which synthesized current understanding of vine weevil biology and management. Key knowledge and gaps in our understanding of vine weevil biology and/or management techniques were highlighted in this report.

Objective 2. Identify opportunities for the delivery of existing knowledge to support implementation

Task 2.1 Review and collate previously and currently used methods for communication of current knowledge on vine weevil management and consult growers on the effectiveness of these knowledge transfer methods

Knowledge transfer methods previously and currently used for communicating knowledge on vine weevil biology and control were summarised. These included:

- HDC reports, website, factsheets, HDC News articles and other communications
- Grower seminars and workshops funded by HDC, Defra and others
- ADAS and other consultancy channels
- Product labels and leaflets, websites and industry events provided by suppliers of chemical pesticides and biological control agents for vine weevil control

When growers and consultants were interviewed about their current vine weevil management strategies in Task 1.1, they were also asked to comment on the effectiveness of the various knowledge transfer methods, which ones they prefer and which they would find most helpful in supporting the implementation of vine weevil control strategies. They were also asked to highlight any gaps in supply of information and to suggest potential improvements.

Task 2.2 Identify more effective knowledge transfer methods

The results of Task 2.1 were used to identify which knowledge transfer methods growers preferred and which they would find most helpful in supporting the implementation of vine weevil control strategies.

Objective 3. Design 'best-practice' IPM protocols suitable for implementation on susceptible crops in each relevant horticultural sector

Task 3.1 Draft protocols

Using the combined information on vine weevil biology and control options collated in Objective 1, two flow charts were produced, one for containerised ornamentals and one for soft fruit, summarising key decisions and options for vine weevil management within and IPM programme.

Task 3.2 Confirm protocols after consultation with key industry representatives

The IPM flow charts were confirmed after discussing them with the project industry representatives.

Objective 4. Provide plans for communicating relevant knowledge and IPM protocols to growers in each relevant sector

Task 4.1 Draft communication plans

Using the results of work completed in Objectives 2 and 3, draft plans were developed for the effective communication of relevant knowledge on vine weevil biology and control and the best-practice IPM protocols to growers and other members of the horticultural industry.

Task 4.2 Confirm communication plans

The draft communication plans will be confirmed after discussions with the key HDC staff, the project industry representatives and other industry members in relevant sectors. The proposed plans have been included in this project report.

Results

Objective 1. Collate current knowledge of vine weevil biology and control and identify key gaps in understanding

Task 1.1. Interviews with key industry representatives to identify currently used vine weevil management systems and their success, and perceived gaps in knowledge

A summary of the combined interviews is given below.

Crops with vine weevil problems

Ornamental and soft fruit crops in which vine weevil was a problem are shown in Table 1.

Table 1. Ornamental and soft fruit crops in which vine weevil problems were reported in the grower interviews.

Protected ornamentals	Hardy nursery stock	Soft fruit
cyclamen	Acer	blackberry
	Alchemilla	blueberry
	Astilbe	raspberry
	Bergenia	strawberry
	Chaenomeles	
	Clematis	
	Cordyline	
	Cornus	
	Erodium	
	Escallonia	
	Euonymus	
	Ferns	
	Fragaria	
	Fuchsia	
	Geranium	
	Geum	
	Heuchera	
	Hydrangea	
	Liriope	
	Photinia	
	Phyllitis	
	Primula	
	Saxifrage	
	Sedum	
	Skimmia	
	Spirea	
	Tiarella	
	Vinca	
	Waldsteinia	
	Various conifers	

Incidence and severity of problems and any effects of production systems

When asked was vine weevil a resident pest on the farm or nursery and whether vine weevil caused significant financial losses, growers and consultants reported the following:

Pot plant grower: first problem with vine weevil in 2013 for 10-15 years, due to buying in infested cyclamen plugs, but no financial losses due to nematode application after potting when the problem was identified.

HNS growers and consultants: Two growers reported no financial losses as control with either pesticides or an IPM programme was sufficient. Four growers using IPM reported some financial losses although it was difficult to quantify; one of the growers estimated 3-5% losses. Production systems considered by growers and consultants to have affected vine weevil problems included:

- Conifer hedges and broadleaved shrubs and trees around nursery were regarded as a source of weevils on some nurseries but not others. One grower regarded natural predators to be suppressing weevils in hedges.
- Plants considered less susceptible to vine weevil damage can still be a source of vine weevil if control measures not used.

Soft fruit growers and consultants: All growers used an IPM programme, one grower reported no losses at present and the remaining six growers reported some losses, with 100% losses being possible on older crops. Factors considered to have affected vine weevil problems included:

- Use of polythene mulch on strawberry soil beds encouraging vine weevil populations and leading to faster development rate; removal of polythene reported to considerably reduce severe infestations
- Migration of adult weevils between crops or from non-cropped areas
- Table top strawberries less susceptible than those grown in raised beds. Use of barrier glue on table top supports for strawberry may have reduced infestations on one farm
- Crops kept for more than one year, or fields used for many years for soft fruit production have more problems
- Fewer problems on large farms with isolated rented sites
- Nematodes being less effective on soil-grown crops
- Blueberry being particularly susceptible to vine weevil
- Hedges and local gardens regarded as source of adult weevils by some but not others

- Weeds e.g. rosebay willow herb acting as host plants around field margins

Use of entomopathogenic nematodes in an IPM programme

Growers, consultants and suppliers were asked how successful use of nematodes has been in IPM programmes, what species and application methods and timings they used or recommended, what factors were regarded as affecting success, how they monitored for efficacy and what they considered to be the major challenges or gaps in knowledge. The main points are summarised below.

Success rate

- Most suppliers and consultants considered that depending on the growing system and the application method, a single application of nematodes should usually give 80-90% control. Effective application and timing are key to success. Nematodes are more effective when applied to substrate-grown crops then to field-grown crops, due to adequate irrigation in substrate crops and to problems with some field soils inhibiting nematode movement and survival.
- Nematodes are used more widely in the soft fruit industry than in hardy nursery stock. In soft fruit, applications to substrate-grown crops (strawberry, raspberry, blackberry and blueberry) have proved more effective than those to field-grown crops and therefore there is much more use of nematodes on substrate-grown crops. Application is mainly through drip irrigation (using drippers onto the crop or using T-tape systems in raised beds), as this system is far less labour-intensive than using drenches. However, growers of soft fruit report that although vine weevil control in substrate crops is generally good, problems can still occur, especially in older crops where larger vine weevil populations have built up. Adequate drenching on large pots e.g. those used for blueberry can be difficult due to vine weevil larvae moving to the edges of the pot.
- Growers of HNS report more variable results. Those more experienced with using nematodes consider they give satisfactory or good control. A major problem is effective application, as very little drip irrigation is used in HNS production and nematode applications have to be done using drenches. Drenching plants with dense foliage that overhangs the substrate in the pots can lead to most of the nematodes ending up on the floor rather than in the substrate.
- Vine weevil is less of a problem on protected ornamentals than on HNS, although it can occur on susceptible species such as cyclamen and hydrangea. Growers of protected ornamentals can achieve good control with nematodes due to glasshouse

temperatures usually being suitable, also these growers tend to be more experienced with using IPM than growers of HNS and soft fruit. However, effective application can still be a problem on closely spaced pots with dense foliage.

Nematode species

- Most growers of both soft fruit and HNS use *Steinernema kraussei* in cooler conditions and *Heterorhabditis bacteriophora* in milder conditions. Growers are aware of the lower temperature tolerance of *S. kraussei.* The use of *H. bacteriophora* in milder conditions is largely due to its lower price than *S. kraussei* but also because some growers believe that *H. bacteriophora* is more effective than *S. kraussei* in warmer conditions. This is not backed up by the suppliers of *S. kraussei (*Nemasys L) who give its temperature range as 5-30°C compared with 12-30°C for Nemasys H (*H. bacteriophora*). Other *H. bacteriophora* products (which are different strains than that in Nemasys H) give the temperature ranges of 14-33°C (Larvanem), 12-30°C (Exhibitline H) or above 12°C (Nematop).
- There is some interest in nematode species mixes, e.g. when wising to target other pests in addition to vine weevil. One hardy nursery stock grower is considering using a mix so that leatherjackets can be controlled by a species mix including *S. feltiae*. SuperNemos is a new product with three species of nematodes (*S. carpocapsae, S. feltiae* and either *H. bacteriophora* or *H. megidis*) and has been used experimentally on some sosft fruit and HNS crops. A soft fruit consultant reported that this product seemed to give equivalent control to that given by Nemasys L and Nemasys H on substrate-grown strawberry.

Application rate, numbers and timings

- Most growers of both hardy nursery stock and soft fruit use recommended rates of nematodes. There is some use of higher rates on hardy nursery stock in severe infestations and several growers of substrate-grown strawberry in Scotland have successfully used lower (one fifth or half-rate- rates) applied monthly, often between April and October. The latter strategy has been advised by Syngenta Bioline, following unreliable control given by recommended rates applied in autumn and spring, possibly due to overlapping vine weevil generations under protection. This strategy is only used when nematodes are applied through drip irrigation as labour costs would be prohibitive if drenching.
- Most other growers of soft fruit apply full rate nematodes, using one or two applications in the autumn (in August or August and September) and again in the spring (April) if live larvae are seen.

- Some growers of HNS apply one or two applications in the autumn, others prefer to make only one application in the spring if live larvae are seen, due to the labour cost involved in drenching.
- Suppliers and consultants all agree that even when using full recommended rates of nematodes, more than one application is usually needed for optimum control, using either two applications in the autumn if temperatures are suitable for a second application, or one in the autumn and one in the spring. *S. kraussei* is widely used for late autumn and spring applications due to its lower temperature tolerance than other species.

Substrate temperature and moisture

- All growers interviewed who used nematodes were aware of the minimum temperature requirements of the different nematode species and many of them, particularly growers of HNS measure soil temperature to decide on which nematode product to use.
- Growers are also aware of the need to apply nematodes to moist substrate and to keep the substrate moist after application, although some growers were not sure how long after application this was necessary.

Application methods

- All interviewed growers are aware of the need to store nematodes at the correct temperature and to use before the expiry date.
- Growers were aware of the need to use a clean tank for application and to remove fine filters but there was some confusion over some details of correct mixing (see Gaps in knowledge section below).
- Nematode application to substrate-grown soft fruit crops is mainly through drip irrigation as this is much less labour-intensive than using drenches. T-tape systems are also used in raised beds, where two lines per bed are more effective in delivering the nematodes to the root zones than one, however, nematode applications to soil-grown crops are less effective than those to substrate-grown crops.
- Many soft fruit growers monitor delivery of water through the drippers using dye, and take extra steps if needed to improve water and nematode distribution, e.g. cleaning or replacing blocked drippers, ensuring drippers are in the best position to deliver nematodes to the root zones and providing additional drippers if needed, e.g. to raspberry pots.

- All nematode suppliers and consultants give advice on application methods, and one supplier (BASF) provide technical leaflets on storing and mixing nematodes, positioning of drippers and use of a Dosatron, which are available through their distributors (see Appendix-4).
- Drenches are still used in some soft fruit crops, e.g. to strawberry tray plants if infested with larvae, or occasionally to planted crops if infestations are severe. A grower of blueberry has used drenches rather than drip irrigation to ensure nematodes were applied to the large area of substrate in pots, having observed that vine weevil larvae can be found at the edge of the pots. Drenches have also been used in field-grown strawberry but with disappointing results.
- Nematode application in HNS and protected ornamental crops is nearly always done by drenching, as drip irrigation is little used in ornamental production. Most growers use a Dosatron and are aware of the need for constant agitation of the tank to avoid nematodes settling out. One HNS grower has adapted a Dosatron with an agitator on a wheeled trolley specifically for nematode application. Many HNS growers find drenching difficult, time-consuming and inefficient, particularly when trying to drench closely spaced plants with dense foliage. Irrigation is used as recommended after application if necessary, to wash the nematodes from the foliage onto the substrate, but when the foliage overhangs the substrate in the pots, many of the nematodes are likely to end up on the floor rather than in the substrate.

Monitoring methods

- Some growers check nematode viability (movement when a sub-sample is added to a dish of water) when the packs are received, using a low-power microscope.
- In addition to monitoring dripper efficiency using dye, a few growers also collect samples of nematodes at the start and end of the irrigation system to check numbers are similar.
- Most growers monitor for treatment efficacy 2-4 weeks after nematode application by knocking out pots or digging up plants and looking for live larvae around the roots. Some growers mark pots or plants with known larvae at the time of application and use these to monitor for efficacy.
- Although the red colour of *H. bacteriophora*-infected vine weevil larvae is easily recognised, larvae infected with *S. kraussei* are a less noticeable yellowish brown colour, and all nematode-infected larvae disintegrate after a few weeks, therefore growers are advised to check for live larvae in order to decide on whether to make a repeated treatment.

Problems with using nematodes and gaps in knowledge

- Shortage of some nematode products can occur, particularly for autumn application and this can lead to growers either having to delay application or to use a less preferred product.
- Some growers claim to have received nematodes with low viability in the past, which is why they now check nematode movement in a sub-sample on receipt, using a low-power microscope.
- Some growers are unsure of which nematode species to use for best effect. Many growers believe that *H. bacteriophora* is more effective than *S. kraussei* in warmer conditions, even though only one *H. bacteriophora* product (Larvanem) claims to have an upper temperature limit (33°C) higher than that of *S. kraussei* (30 °C).
- Some growers are unsure about certain mixing and application details e.g. filter sizes that are suitable for nematode application, acceptable pressures, whether or not nematodes should be mixed in the dark, whether water should be aerated during mixing, how long they will survive once mixed in water and what proportion survive application through drip irrigation. Not all product leaflets give all these details and some growers flagged up the need for clearer, best-practice guidelines.
- Application of nematodes to soil-grown soft fruit crops is unreliable.
- Growers of HNS find that drenching nematodes is difficult, time-consuming and inefficient. An improved system for application to pots is needed.
- Some growers are unsure how long after application the substrate needs to be kept damp, and how long after application monitoring for efficacy should be done.
- Some growers would be interested in using a method for monitoring vine weevil egg laying to help decide whether repeated nematode applications are needed.
- Growers are not sure about the compatibility of pesticides and fertilisers applied to substrate together with nematodes.
- Suppliers and consultants all agreed that growers always need more advice and information on optimum nematode application methods and that more technology transfer is needed e.g. seminars, workshops, demonstration days.
- Some suppliers and consultants suggested that more knowledge is needed on how the vine weevil life cycle can vary according to geographic location and whether grown outdoors or under polythene or in glasshouses, as this would inform more effective timings for nematode application.
- One supplier suggested that more knowledge is needed in the effect of new growing media (e.g. green waste) on nematode efficacy and the compatibility of incorporated biopesticides (e.g. *Trichoderma*) with nematodes.

Use of Met52 in an IPM programme

Growers, consultants and suppliers were asked how successful use of Met52 has been in IPM programmes, what application methods and timings they used or recommended, what factors were regarded as affecting success, how they monitored for efficacy and what they considered to be the major challenges or gaps in knowledge. The main points are summarised below.

Use in different crops and success rate

- Only two of the five growers of HNS interviewed who had used Met52 in 2013 reported satisfactory results, where the product was used together with other control methods including nematodes or pesticides in an IPM programme.
- Other HNS growers were either disappointed in the control given or have not tried it due to lack of confidence in the product.
- HNS consultants reported that Met52 performed quite well in 2011/12 but in 2013 gave very poor control on many nurseries.
- One cyclamen grower who had used Met52 at potting on untreated plugs was unsure of how successful it might have been, as vine weevil larvae were noticed around the roots shortly after potting on so were considered to have been bought in with the plugs, and were successfully controlled with a drench of nematodes.
- The current formulation and recommended incorporation method for Met52 is not suitable for use in soft fruit. Most strawberry crops are grown in coir, delivered in solid blocks in bags for wetting up, so incorporation is not possible. Most beds used for soil-grown strawberry crops are made up in autumn for spring planting, thus the product would run out of persistence by the following autumn when vine weevil larvae would be present, and in the second year's cropping when most vine weevil problems occur. Raspberry plants are cropped for 3-4 years and thus Met52 incorporation into the mixed coir substrate and chopped roots of previous crops would not give sufficient persistence. Growers would be interested in a liquid formulation that could be applied through drip irrigation. One grower had successfully used Met52 in a sawdust mulch (using EAMU 1997/2011) in spring on potted blueberry and considered this to have given successful control of any young larvae hatching from eggs laid into the mulch.
- One soft fruit consultant had advised its use in soil-grown strawberry, when it was incorporated when making up the beds, but most consultants and growers consider it impractical and expensive for soil incorporation.

- One grower of potted blueberry considered that use of Met52 in a sawdust mulch gave successful control and will use it again this year. The mulch was used in spring before vine weevil egg laying, and was thought to kill young larvae hatching from eggs laid into the mulch.
- Met52 suppliers recommend that the product should be used at each potting stage in ornamental production e.g. plugs, liners and pots, as part of an IPM programme, using additional vine weevil control measures such as nematodes and pesticides as appropriate. Substrate or soil temperature is highlighted as an important factor affecting success, with best efficacy above 15°C.

Storage before use

- The product (for grower incorporation) was stored in the fridge or pesticide store as before use.
- Ready-mixed growing media was stored in bales outdoors, usually for less than two weeks.

Application methods and timing

- HNS growers interviewed who had tried Met52 had either mixed the product themselves into substrate or bought it ready-mixed. The product was either just used in propagation (plug trays), followed by other control methods at potting up, or as used throughout production i.e. for plugs, liners and at potting on and was used in peat, bark and coir mixes.
- One soft fruit consultant reported limited use in soil-grown strawberry when making up the raised beds.
- One blueberry grower used Met52 in a sawdust mulch (using the EAMU) in spring.

Temperature and moisture

- Growers of HNS, protected ornamentals and soft fruit who used Met52 did not measure substrate temperature. Most were aware that the product had temperature limits but assumed that temperatures would be suitable when vine weevil larvae hatched. Growers and consultants are now more aware that Met52 is more temperature-sensitive than they previously thought and will take this into account in any future use.
- The cyclamen grower thought he would not use the product again now that he is aware that Met52 is slow-acting below 15°C as the crop tends to be grown cool.

- Growers did not take any precautions to keep substrate moisture high, although use in HNS propagation units would always be in high humidities. The blueberry grower reported that Met52 seemed to be effective even when used as a mulch in dry sawdust.

Monitoring methods

- One HNS grower checked the substrate in the propagation house for mycelium growth.
- All growers checked for live vine weevil larvae around the roots when they expected to see them.
- A mealworm test can be done to check for Met52 presence in treated substrate and guidelines for this are available from Fargro.
- Suppliers of treated substrate can keep reference samples to check for presence of Met52 if required.

Problems with using Met52 and gaps in knowledge

- Many HNS growers have been disappointed with Met52 control of vine weevil in 2013 and some have reported that they will not use it unless they are confident of the conditions in which it will provide effective control.
- HNS growers would like more guidance on temperature and moisture requirements of Met52 and on compatibility of fungicides.
- One grower asked whether a higher rate of the product was needed, although recognised that this would increase the cost.
- Met52 as a substrate or soil-incorporated treatment is impractical for use by most soft fruit growers and they would like a liquid formulation to apply through drip irrigation, as used for nematodes, together with good evidence of efficacy before they would consider its use.
- Soft fruit growers are interested in Met52 used as a mulch on pots or around raspberry stools.
- Consultants would like robust information on best-practice use of Met52, including temperature limits and durations needed. Many are reluctant to advise it as they are not confident of the success of an expensive product.
- The blueberry grower who was pleased with the control by Met52 as a mulch commented that it would be useful of a ready-mixed mulch was commercially available.

Use of chemical pesticides in an IPM programme

Growers, consultants and suppliers were asked how successful use of chemical pesticides has been in IPM programmes, what chemicals and application methods and timings they used or recommended, what factors were regarded as affecting success, how they monitored for efficacy and what they considered to be the major challenges or gaps in knowledge.The main points are summarised below.

Use of pesticides against adult vine weevil and success rate

- Not all HNS growers interviewed used pesticides for control of adults. Most of those that did were very aware of potential side effects on biological control agents used in IPM and used pymetrozine (Chess WG) or indoxacarb (Steward) by preference (as a result of HDC project SF/HNS 112). However, as a last resort, chlorpyrifos (Dursban WG) was used by one grower. Most growers found it difficult to judge success of treatment, although dead adults were seen after using Chess WG on one nursery and on another, only low numbers of larvae were seen after spraying plants with high levels of adult feeding.
- Two HNS consultants interviewed advised Steward, Dursban WG or lambda-cyhalothrin (Hallmark) and reported good control (Steward or Dursban WG) or variable control (Hallmark).
- Pesticides for adult control were used by most of the soft fruit growers interviewed. Thiacloprid (Calypso), chlorpyrifos (Dursban WG or Equity), or Hallmark were used. Most reported poor control, with only one grower reporting fairly successful control, however although this grower had seen dead adults after spraying, percentage kill was never 100% so repeated sprays were used on raspberry.
- Most soft fruit consultants advise the use of pesticides against adults, particularly if adult notching is severe. Pesticides advised are Dursban WG or Equity, Calypso, deltamethrin (Decis), Hallmark or pyrethrum. Reported success rate varied from poor to good.
- One soft fruit consultant reported the use of garlic through the trickle irrigation prior to spraying with Calypso or pyrethrum to ‘drive the adults out’ and reported very good control, with many dead adult weevils seen.

Time of spraying and awareness of adult activity at night

- Spraying was done when adult weevils or feeding damage was seen during crop walking.

- All consultants and most growers of HNS and soft fruit were aware that vine weevil adults are active at night. Time of spraying varied, with HNS growers spraying between 4 and 5pm or 8.30-9pm and most soft fruit growers spraying at or after dusk.
- One HNS adviser advised spraying in late evening rather than after dark due to the health and safety risk of spraying in the dark, and one soft fruit grower commented on the practical difficulty of spraying in tunnels in the dark.
- Warm, dry, still nights were chosen for spraying, both for suitable spraying conditions and also for maximum weevil activity.
- Timing was also dependent on harvest intervals and other treatments made within IPDM programmes

Spray coverage and use of adjuvants

- Growers of both HNS and soft fruit aimed for full crop coverage, using 1000 litres/ha when spraying for adults.
- Some soft fruit consultants advised only spraying the ground rather than the crop, to protect predators.
- Using Codacide as an adjuvant was reported to improve control by one soft fruit consultant. Other adjuvants used with unknown effects were silicon and vegetable oil wetters.

Monitoring methods for adult control

- Monitoring for fresh adult notching damage or for adults in hiding places in the crop was used by both HNS and soft fruit growers to decide when to spray against adults.
- One HNS grower uses Euonymus indicator plants to monitor for notching
- Crop walking at night to look for adults was done by one HNS grower
- Looking for dead weevils and for fresh notching after spraying were used to assess control success

Use of pesticides against vine weevil larvae and success rate

- Two of the soft fruit growers interviewed used no pesticides for control of larvae. The other five used a chlorpyrifos drench to strawberry crops after harvest in October to November, particularly on older, soil-grown crops where vine weevil populations have built up due to the impracticality of using nematodes. Drenches were applied when the soil was moist and when temperatures were above 5°C for maximum efficacy.

- Soft fruit consultants reported good success of chlorpyrifos drenches if carefully applied to soil in the right conditions, but this varied from farm to farm.
- Most HNS growers and the protected ornamentals grower interviewed have relied in the past on compost-incorporated insecticides such as chlorpyrifos (SusCon Green), fipronil (Vi-Nil) and imidacloprid (Intercept or Imidasect) for control of larvae. Now that the only insecticides available for compost incorporation are imidacloprid and thiacloprid (Exemptor), and the neonicotinoid restrictions are limiting the use of imidacloprid, most HNS growers will be limited to the use of Exemptor. The recommended rate for this product against vine weevil has recently been increased by the supplier and growers have not yet had experience of its efficacy at the higher rate. There is some grower concern over its potential systemic side effects on predatory mites and aphid parasitoids used in IPM programmes. Both imidacloprid products and Exemptor are currently approved for use only in peat-based growing media.
- HNS growers have also used a drench of imidacloprid as a curative control method, but due to the neonicotinoid restrictions this use will now be limited.
- Some HNS growers are still using a drench of chlorpyrifos as a curative control as a last resort. Although this is not recommended on ornamentals, it is still permitted under the Long Term Arrangements for Extensions of Use (LTAEU). Growers report this treatment to be effective against vine weevil larvae as long as enough drench is applied. However, ADAS consultants and a pesticide supplier reported concerns over potential chlorpyrifos phytotoxicity (leaf scorch and root damage) to a range of ornamental species, thus use is at grower's risk.

Monitoring methods

- All growers and consultants checked for live vine weevil larvae around the roots, in order to decide on the need for drench treatment if appropriate and to check for efficacy after treatment with a drench or (in ornamental crops only) a growing media-incorporated treatment.

Awareness of neonicotinoid restrictions

- All growers and consultants of ornamentals interviewed were aware of the recent neonicotinoid restrictions.

Problems with using pesticides successfully and gaps in knowledge

- Application of pesticides at night is not always practical or safe to operators. Some growers are aware of the initial laboratory results in SF/HNS 112 that indicated that spraying at night when weevils are active may not be required, and have flagged up the need for reliable information on whether spraying after dark is more effective than spraying during the day or evening before dusk.

- Growers of ornamentals are now very concerned that they have very limited chemical control options for effective control of vine weevil. Ornamental crops are now being rejected if live vine weevil larvae are found around the roots. Due to the neonicotinoid restrictions, problems with effective application of nematodes and lack of confidence in Met52, they urgently need a robust IPM solution.
- Growers of soft fruit only have the options of using pesticides against adults or in strawberry crops, a drench of chlorpyrifos after harvest. The latter control method is commonly used on soil-grown crops due to the impracticalities and reduced efficacy of using nematodes, compared with use in substrate-grown crops via drip irrigation. Although one grower commented that the loss of chlorpyrifos would result in 'big trouble', using chlorpyrifos is not popular with growers as it requires high volume drenching and presents operator exposure risks. An alternative, effective treatment for control of larvae in soft fruit crops would be beneficial.
- Growers of both ornamentals and soft fruit are aware of the side-effects of some pesticides used for adult control on biological control agents used in their IPDM programmes. There is interest in using more compatible pesticides for adult control and more research is needed to test the efficacy of Chess and Steward, building on initial research results in SF/HNS 112. One HNS grower suggested that the potential for Chess as a drench or growing media-incorporated treatment should be investigated, and one soft fruit grower commented that an IPM-safe pesticide to use through drip irrigation would be useful to supplement nematodes in severe infestations.
- A pesticide supplier agreed that there is a need to identify IPM-compatible pesticides for use against vine weevil adults and to confirm the robustness of treatments under different environmental conditions. It was suggested that further work on weevil movement and behaviour would help growers and advisers to time and target applications and use the most appropriate application methods.

Task 1.2. Systematicajlly retrieve relevant peer reviewed literature, Task 1.3. Retrieve 'grey' literature and identify key knowledge and gaps in our understanding of vine weevil biology and management techniques, Task 1.4 Collate and summarise key relevant information

The summary table in the Excel spreadsheet is attached in Appendix-1.

The comprehensive report on current understanding of vine weevil biology and components of an IPM strategy, together with key gaps in knowledge is given below in the following five sections:

- Vine weevil biology and behaviour
- Biological control with entomopathogenic nematodes
- Biological control with entomopathogenic fungi
- Other non-chemical control methods
- Management with chemical insecticides

Vine weevil biology and behaviour

This literature review has identified over 150 scientific papers and reports on various aspects of vine weevil biology. To date there have been three detailed reviews of vine weevil biology; Feytaud (1918), Smith (1932) and Moorhouse *et al.* (1992). Early literature referred to this pest as *Brachyrhinus sulcatus* while in the USA it was originally known as *Curculio apiculatus.* Common names include 'cyclamen borer', 'strawberry root-weevil', 'black vine weevil' and simply 'vine weevil', the accepted British common name.

Vine weevil is endemic to temperate regions of Europe but has, primarily through plant trade routes, colonised many parts of the world, including North America, Australia and Japan (Moorhouse *et al.*, 1992). Vine weevil has been of concern to growers since the 1830s when it emerged as a glasshouse pest in the UK, Germany and the USA. However, vine weevil has only been considered to be a serious pest in horticultural crops in the last 50 years or so. A number of modern horticultural practices have been associated with the increasing importance of this pest; these include use of growing media, plastic mulches, loss of persistent insecticides and a general expansion of horticultural production.

Vine weevil are all female and so reproduce asexually (parthenogenetically). In the only study of vine weevil population genetics, Lundmark (2010) concluded that there is very little genetic diversity but that vine weevil possess a 'general purpose' genotype that allows this species to feed on a wide range of host plants and to successfully colonise different environments.

Adult vine weevil are approximately 11 mm in length, dull back in colour but with tufts of orange hair on the wing cases (Smith, 1932). The wing cases are fused together and so vine weevil are unable to fly and instead walk from one area to another. Adults are nocturnal and seek refuge during the day in leaf litter, under pots and other suitable refuges. Adults feed at night, producing characteristic notches along leaf margins. This damage is usually not important in terms of plant health, although adult vine weevil have been reported to ring bark grape vines (Smith, 1932). However, the presence of adult vine weevil damage often leads to ornamental plants becoming unsaleable (Backhaus, 1996).

Vine weevil eggs, which are 0.8 mm in diameter, are typically laid at night in the soil. Eggs are initially white in colour but turn chestnut-brown after a few days (Moorhouse *et al.*, 1992a). The larvae that hatch from the eggs are generally white, although the colour may vary slightly depending on the host plant on which it feeds. Larvae are legless, often adopt a characteristic C-shape and have a chestnut-brown head. Young larvae tend to feed on finer roots while older larvae may burrow into roots, corms and rhizomes and can ‘girdle’ stem bases. Vine weevil larvae moult between four and nine times, the number of moults appears to be temperature-dependent (Masaki & Ohto, 1995), before pupating in an earthen cell (Moorhouse *et al.*, 1992a).

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Development rates

The length of time taken for a vine weevil to develop from an egg to a reproductively active adult is largely determined by temperature and to a lesser extent by relative humidity (e.g. Son & Lewis, 2005; La Lone & Clarke, 1981). The time taken for a newly emerged adult to become reproductively active may be as little 12 days but typically takes closer to 30-40 days (e.g. Casteels *et al.*, 1994; Moorhouse *et al.*, 1992). Development times of eggs, larvae and pupae decrease with increasing temperature. However, under constant temperature conditions above 24°C few larvae and pupae survive and above 27°C few eggs survive, although Son and Lewis (2005) did not record how quickly weevils, at each development stage, are killed at these temperatures. At 27°C the egg stage is completed in 9 days while at 24°C the larval stages are completed in 101 days and the pupal stage in 10 days (Son & Lewis, 2005). Development times determined by Son & Lewis (2005) are shown in Table 2. At lower temperatures, for example at 12°C, each development stage takes much longer to complete; 33 days for eggs, 231 days for larvae and 34 days for pupae (Son & Lewis, 2005, Table 2). At each developmental stage vine weevil require a minimum temperature in order to continue to develop. Development continues, albeit very slowly at low temperatures, if the temperature is above 6.32°C for eggs, 2.45°C for larvae, 6.09°C for pupae and 8.44°C for young adults to develop into reproductively active adults (Masaki & Ohto, 1995).

Table 2. Development times for vine weevil eggs, larvae and pupae at constant temperatures (adapted from Son & Lewis, 2005).

Temperature (°C)	Eggs	Larvae	Pupae
11	47	184	36
12	33	231	34
15	20	143	22
18	15	127	14
21	13	124	13
24	11	101	10
27	9	*	*
29	*	*	*

* Most or all vine weevil killed at this temperature

The link between temperature and speed of development means that vine weevil develop faster in protected crops. If temperatures in these crops maintained above those required for continued development then weevil development may continue throughout the year. Vine weevil do not require a winter diapause. In a polytunnel grown raspberry crop near Dundee, Scotland, Johnson *et al.* (2010) recorded average air temperatures to be 4°C warmer in tunnels than in a nearby field plantation. As a result newly emerged weevils achieved sexual maturity 8 days earlier than those emerging at the same time in the field.

Vine weevil are found throughout many temperate regions and as such are capable of surviving harsh winter conditions. Stenseth (1987) reports that larvae were better able to survive sub-zero temperatures than the adults. At a constant -3°C, 90% of adults died after 30 days, while 90% of larvae died after 90 days. At a constant -6°C, 90% of both adults and larvae had died after about 9 days.

Knowledge gap – the development rates of each stage of the vine weevil life cycle have been determined. However, this information only exists for constant temperatures. Crops grown in the field, polytunnel or glasshouse are subject to different degrees of temperature fluctuation. This is important in determining both the time taken for vine weevil to complete their development but also their ability to survive high or low temperature extremes. This knowledge might lead to opportunities to manipulate temperatures as a cultural control method and to predict reduction of vine weevil populations in seasons when lethal temperatures occur.

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Oviposition behaviour

Vine weevil typically lay eggs at varying depths below the soil surface (Garth & Shanks, 1978). However, eggs are also laid on the soil surface and in certain situations, such as in glasshouse environments, eggs may be laid onto leaves and stems of plants as well as into hollows and clefts of stems and corms (Smith, 1932). Indeed growers report larvae infesting the fleshy crowns of species such as Sedum and Heuchera (Bennison pers. comm.). Eggs laid in the soil are more likely to result in mature larvae than those laid on the soil surface (Cram, 1965). Buxton (1996) found that significantly more eggs were laid at the edges of pots rather than in the middle or inner areas around the stems of *Euonymus* plants. This may reflect the fact that young larvae feed on fine roots which are likely to be found away from the stem. It isn't known if vine weevil display similar egg laying behaviour in other crops. Buxton (1996) points out that although only applying controls to the central core of compost may help to reduce costs, this approach would probably lead to reduced levels of vine weevil control in cases such as this where the majority of eggs are laid around the edges of the pot.

Under optimum conditions vine weevil adults lay on average 1,600 eggs, although individuals may lay in excess of 3,000 eggs (Casteels *et al.*, 1994). Under field conditions the fecundity of adults is much lower at 270 to 450 eggs per adult (Cram, 1965). However, weevils infesting polytunnel grown crops may lay more eggs than weevils infesting field grown crops. In a polytunnel-grown raspberry crop, five week old adult vine weevil had laid 20 times as many eggs as similarly aged weevils in a field grown crop (Johnson *et al.*, 2010). The number of eggs laid per day varies widely but may be as many 17 under glasshouse conditions (Mason, 1960). Egg laying activity appears to cycle between periods of peak egg laying and periods where few or no eggs are laid. The causes of these cycles are not fully understood but may relate to the nitrogen content in the host plant and to temperature (Moorhouse *et al.*, 1992). There is conflicting information on the minimum temperature required for egg laying. Stenseth (1979) suggests that egg laying only occurs at temperatures above 12°C while Blackshaw (1992) reports egg laying at lower temperatures.

An important consideration when thinking about the management of vine weevil is the relative importance of overwintering adults and newly emerging adults in terms of the total number of eggs laid in a crop in a season. In this respect the importance of overwintering adults is often not fully appreciated. Overwintering adults start egg laying in May and June while newly emerging adults, emerging in June and July may not start to lay eggs until August (Blackshaw, 1996). In addition, overwintered adults may lay eggs at a faster rate than newly emerging adults (Cram, 1965). As a result, overwintering adults may contribute more than half of all the eggs laid in a season (Blackshaw, 1996), although this will depend on the severity of the preceding winter and the numbers of adults successfully overwintering.

Knowledge gap – understanding when the start and peak of adult feeding as well as the start, peak and end of egg laying occurs in field, polytunnel and glasshouse crops is important for the management of vine weevil. An easy, practical method(s) for growers to monitor adult feeding and egg laying need to be developed (see Monitoring section later in this review). The timing of these events is likely to differ from region to region, crop to crop and year to year. However, the relative importance of overwintering and newly emerging adults is an important factor to consider in this respect.

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Feeding behaviour

Vine weevil adults and larvae are polyphagous and are capable of feeding and successfully developing on a wide range of host plants including soft fruit and ornamental crops and various weed species (Smith, 1932, Masaki *et al.*, 1984, Buxton & Pope, 2011). Approximately 150 plant species have been identified as potential hosts for vine weevil (Moorhouse *et al.*, 1992). As the larvae are relatively immobile, feeding in the soil on plant roots, corms and tubers, the oviposition behaviour of the adults largely determines which plants the larvae feed on (Moorhouse *et al.*, 1992; Clarke *et al.*, 2011).

Adult vine weevil show preferences for certain plant species and this in turn may affect where eggs are laid (e.g. Cowles, 2004; Labuschagne *et al.*, 1997). For example, odours of yew (*Taxus baccata*) and *Euonymus fortunei* damaged by adult vine weevil are attractive to other adult vine weevil, but *Rhododendron* and strawberry (*Fragaria x ananassa*) are not (van Tol *et al.*, 2002). It isn't fully understood how vine weevil discriminate between the odours of potential host plants as weevils appear to detect and respond to plant volatiles that are common to many plant species (van Tol & Visser, 2002; van Tol *et al.*, 2012; Karley, 2012). Despite this, when given a simple choice between plants producing odours that are attractive to vine weevil and suitable host plants that do not produce odours attractive to weevils, adults often feed more readily and lay more eggs around plants producing the attractive odours. For example, when given a choice, vine weevil adults feed and lay more eggs around yew or *Euonymus* instead of *Rhododendron* (van Tol & Visser, 1998). However, when weevils were deprived of a choice between host plants they fed equally well on each of these plant species but still laid more eggs (up to 100% or more) when fed on yew.

A number of plant traits have been identified that are closely linked to host plant preferences of vine weevil adults. These include leaf nitrogen content, leaf hairiness in strawberry, leaf scales on certain *Rhododendron* species containing essential oils and leaf shape (Doss, 1984; Rivero-Lynch *et al.*, 1996; Cowles, 2004). Vine weevil feeding is known to be stimulated by the plant sterol sitosterol (Shanks & Doss, 1987). Feeding on strawberry leaves may be deterred when cinnamamide, a synthetic derivative of the naturally occurring plant secondary compound cinnamic acid, is applied to leaves (Mosson *et al.*, 1996). However, often while it may be possible to select for at least some degree of vine weevil resistance, for example in strawberry, the basis of the observed resistance is unknown (Doss *et al.*, 1991).

Several studies have considered the potential of exploiting genetic resources from other *Fragaria* species in order to breed vine weevil resistant strawberry varieties. Specifically, the potential of the beach strawberry (*F. chiloensis*) has been considered (Shanks *et al.*, 1984; Sherman, 1985; Shanks & Doss, 1986). In addition to conventional breeding, a small number of studies have investigated the potential of exploiting transgenic approaches (Watt *et al.*, 1999). These include the potential of the cowpea protease trypsin inhibitor (CpTi) gene, which has been shown to confer resistance to vine weevil larvae feeding on strawberry roots under glasshouse conditions (Graham *et al.*, 1997).

The environment in which the crop is grown may affect the nutritional status of plants, which in turn affects weevil feeding behaviour. For example, polytunnel-grown raspberry plants grew significantly faster (50% increase in height and 16% increase in leaf area) than nearby field-grown plants (Johnson *et al.*, 2010). However, the carbon/nitrogen ratio in leaves was higher in the tunnel-grown plants than in the field-grown plants. As a result, weevils consumed approximately twice as much raspberry foliage in tunnels as weevils in the field, suggesting compensatory feeding to counteract the lower leaf nitrogen concentrations.

In general the success of vine weevil larvae feeding on plant roots is closely related to the size of the root mass. Larval survival and performance is typically lower on plants with a smaller root mass and higher on plants with a larger root mass (Clarke *et al.*, 2011). Indeed, nutrition, physical factors and even cannibalism have all been reported as factors affecting larval survival (La Lone & Clarke, 1981). Roots are thought to be located by larvae using carbon dioxide gradients (Klinger, 1957). Interestingly, adults feeding on the leaves of raspberry plants may significantly reduce the size of the root mass and thus reduce larval survival. In addition, adults do not distinguish between plants already infested with larvae and those not yet infested in terms of egg laying. This means that the behaviour of the adult weevil does not always benefit the larvae developing from the eggs laid (Clarke *et al.*, 2011).

Interactions between host plants and vine weevil are likely to be influenced by climate change. Under elevated CO_2 levels the root growth of raspberry plants was reduced and this in turn reduced both the vine weevil population size and the body mass of larvae (Johnson *et al.*, 2011). However, these interactions are likely to be complex. Under ambient CO_2 levels plants attempt to respond to vine weevil feeding with the production of phenolic compounds that, contrary to expectations, actually improve weevil performance. By contrast, at elevated CO_2 levels plants were unable to mount a response to vine weevil attack.

As may be expected, damage caused by vine weevil larvae is often related to the number of viable eggs infesting a plant (Penman & Scott, 1976; Miller *et al.*, 2012). However, in some cases, such as vine weevil attack on *Rhododendron*, as few as three larvae are capable of killing a plant (La Lone & Clarke, 1981). Similarly, a single mature larva is capable of killing a Cyclamen plant (Moorhouse, 1990).

The age and relative position of vine weevil larvae within the root mass are important factors in determining the amount of damage to the plant. For example a larva feeding on the main root of a strawberry plant just below the soil surface is likely to cause much more damage to the plant than a similar larva feeding at the periphery of the root mass (Evenhuis, 1978). In addition, it is the older larvae that are more likely to be found feeding on the main roots or stem bases than younger larvae (La Lone & Clarke, 1981).

In general, plants with a larger root mass, or an enhanced ability to regenerate roots, are better able to tolerate attack by vine weevil larvae (Cram, 1978; Cowles, 2004). As such, young plants and cuttings and plants suffering stress from other factors are particularly susceptible to vine weevil damage. However, it is also worth remembering that while established crops may be better able to withstand vine weevil damage, these plants also support larger numbers of larvae (e.g. Blackshaw and Thompson, 1993). In addition, while a plant may be able to withstand attack by vine weevil larvae, the presence of any larvae in the substrate of ornamental crops will be sufficient for the crop to be rejected.

Other factors that may determine whether a plant is able to withstand attack by vine weevil larvae is the presence of arbuscular mycorrhiza. In strawberry the presence of arbuscular mycorrhiza (*Glomus mosseae* and/or *Glomus fasciculatum*) mitigated the effects of vine weevil larvae feeding on the roots of plants (Gange, 2001).

Knowledge gap – there is a clear link between feeding by vine weevil adults on a host plant and the number of eggs laid. Quantifying the relationship between numbers of adults and numbers of larvae per plant would help to determine the benefit of controlling adults in addition to larvae in IPM programmes. In addition, plants attacked by vine weevil may be more or less tolerant to attack depending on the size of the root system and the ability of the plant to regenerate its roots. Knowledge of these plant traits in currently grown varieties could be exploited by growers in selecting appropriate varieties depending on the likely risk of vine weevil damage.

There is evidence in the literature that certain chemicals can stimulate or inhibit feeding by vine weevil adults. These chemicals have great potential in reducing or diverting damage from the crop and indirectly reducing egg laying around crop plants. There has been little work on these chemicals to date and further investigation is warranted.

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Aggregation and dispersal

Several studies have reported aggregation behaviour and the presence of an aggregation pheromone produced by vine weevil adults (e.g. Pickett *et al.*, 1996; Kakizaki, 2001; Nakamuta *et al.*, 2005). Vine weevil appear to be attracted by the odour of other weevils (Nakamuta *et al.*, 2005) and specifically to the frass (droppings) produced by these weevils (van Tol *et al.*, 2004). As a result there is some evidence to suggest that weevils prefer refuges that have previously been used by other weevils and therefore contain weevil frass (Pickett *et al.*, 1996). However, Nakamuta *et al.* (2005) did not find weevils to be attracted to the odour from previously used refuges, while Karley (2012) found no evidence of attraction to frass, although the frass did increase weevil activity.

While an aggregation pheromone produced by vine weevil adults has not been identified, plant odours do play a role in the aggregation behaviour of these insects. Several plant volatiles have been identified from *Euonymus fortunei*, which are known to be detected by vine weevil (van Tol *et al.*, 2012). When released from a simple trap a combination of two plant volatiles, (*Z*)-2-pentenol and methyl eugenol, increased the numbers of weevils caught in the trap or found in surrounding plants compared to control traps. Similarly, Karley (2012) found plant derived cues, such as the plant volatile *E*-2-hexenol, was much more attractive to vine weevil than insect-derived cues.

There is relatively little available information on the dispersal behaviour of vine weevil. This is at least in part due to the difficulty in studying these nocturnal insects. Attempts to mark weevils in order to study their behaviour have been limited by the small numbers of weevils recaptured. Recapture rates range from 2-11% (Maier, 1978; Clarke *et al.*, 2012). Clarke *et al.* (2012) did not record the distance moved by each weevil, however, in an earlier study Maier (1978) used a marking technique to record the dispersal behaviour of adult vine weevil in an urban environment. Maier recaptured weevils 21, 35 or 57 days after release. During this time the weevils had travelled mean distances of 6.8, 17.2 and 31.2 m, respectively. Although most weevils were recovered less than 10 m from the release site a small number had dispersed over 70 m from the release site. Similarly, Rasmussen (1978) reported that vine weevil are capable of migrating up to 50 m to new strawberry plantations.

In order to overcome the limitations of traditional techniques for studying vine weevil dispersal behaviour electronic tracking systems have been developed. Brazee *et al.* (2005) developed a harmonic radar system while Pope *et al.* (2013) developed a radio frequency identification (RFID) tag system. Both systems allow the position of the weevil to be recorded within a crop without the need to recover the insect. Pope *et al.* (2013) recorded weevils to move up to 4.3 m in seven days in an outdoor grown strawberry crop but only up to 1.45 m over seven days in a glasshouse grown *Euonymus* crop. This difference in distance moved could have been partly due to the quality of the adult food source; the post-harvest strawberry crop was senescing whereas the *Euonymus* crop was young and healthy.

Living in the soil, vine weevil larvae have a limited ability to disperse. However, Masaki (2000) reports that middle instar larvae can move to a depth of 75 cm in seven days and horizontally 40 cm in 10 days. This movement is likely to be important for larvae to locate roots on which to feed, escape environmental extremes (temperature and soil moisture) and may be an important consideration in relation to controls, such as entomopathogenic nematode drenches, applied to field grown crops.

Knowledge gap – the movement of vine weevil larvae is given little consideration but the fact that larvae can reach depths of 75 cm may have important implications for controls that rely on drenching soil-grown crops. Understanding where vine weevil larvae can be found in the soil profile during the year may allow controls to be applied at times when they will be most effective.

Vine weevil adults typically move short distances, however, long distance movement is also reported. Triggers for this long distance movement are not known but are important if we are to prevent adult weevils moving from infested crops or non-crop plants e.g. hedges to newly planted crops.

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Monitoring

The characteristic leaf notching damage caused by adult vine weevil feeding on leaf margins is a reliable indicator of the presence of this pest (Buxton, 2003; Raffle, 2003). Often this leaf notching is the only sign of damage and can be used in a monitoring system where indicator plants that are particularly attractive to vine weevil (such as *Primula*) are placed within the crop. These plants can be checked regularly and any damage removed so that fresh damage is immediately obvious. Presence of the larvae feeding on the roots of plants becomes apparent when plants wilt as the main roots are severed and plants are easily lifted. In strawberry crops, larval damage can often be detected in the autumn months by the appearance of orange/red leaf colours, indicating symptoms of plant stress (Raffle, 2003). Unfortunately, detecting the presence of vine weevil larvae through evidence of plant stress means that there has already been significant damage to the crop before control measures can be applied.

Directly monitoring vine weevil adults can be done by simply searching through leaf litter, under pots or other suitable refuges during the day (Buxton, 2003; Raffle, 2003). After dusk the crop may be searched for the presence of adults. A torch may be used to search the crop, although care needs to be taken so as not to disturb the adults as they have a tendency to drop from the plant and feign death. Alternatively, raspberry crops may be 'beaten' to dislodge the adults, which may then be counted when they fall onto a white sheet under the plants (Gordon *et al.*, 2003)

Trapping of vine weevil adults is possible and a number of techniques have been developed. Grooved boards placed on the ground within crops (Li *et al.*, 1995; Gordon *et al.*, 2003) and corrugated cardboard wrapped around the stems of larger bushes or trees (Phillips, 1989) exploit the fact that vine weevil seek out refuges during the day. Similarly, simple plastic crawling insect traps are readily used as refuges by vine weevil (Pope *et al.*, 2013). Pitfall traps consisting of a plastic cup sunk into the ground so that the lip of the cup is at soil level may also be used to effectively monitor for the presence of vine weevil adults (e.g. Casteels *et al.*, 1995; Solomon, 2000; Buxton, 2003). Work by Karley (2012) and van Tol *et al.* (2012) suggests that the efficacy of each of these monitoring tools may be enhanced by the addition of attractants based on plant volatiles, however a commercial vine weevil lure has not yet been developed.

The start of egg laying is a key piece of information for the management of vine weevil. There are two ways in which this information can be gathered. Firstly, sharp sand can be added to the top of pots, this sand can then be removed at weekly intervals and the eggs, if present, can be sieved (500 μm mesh) or floated off in a saturated salt solution (Blackshaw, 1992; Buxton, 2003). An alternative approach is to place a small number of adult weevils, collecting from within the crop, in a ventilated plastic box together with fresh foliage. This box may be kept in a shaded position under ambient temperature conditions in the crop and checked for eggs laid in the box after a few days (Phillips, 1989). This process may be repeated at regular intervals, each time replacing the adults with newly caught individuals from the crop.

Monitoring for vine weevil larvae in pot grown crops can be done by simply knocking out the pot and examining the root ball (Buxton, 2003). Vine weevil larvae may be immediately apparent or it may be necessary to break open the root ball to find the larvae. The presence of vine weevil larvae feeding within the root ball can also be detected non-destructively by using highly sensitive sound recording equipment (Mankin *et al.*, 2000; Mankin & Fisher, 2002).

Knowledge gap – there is a clear need for improved early detection of vine weevil infestation. An easy, practical method for growers to monitor for eggs and larvae needs to be developed and validated in commercial outdoor and protected crops. Plant odours have been identified that attract vine weevil. These chemicals have a potential role in producing more reliable monitoring tools that detect vine weevil earlier and at lower population levels.

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Population forecasting

In outdoor-grown crops, such as blackcurrant, overwintered adults may be recorded from April and May and new-generation adults from mid-June onwards (Alford, 1996). The numbers of vine weevil successfully overwintering and the time that they become active the following year is thought to be determined by the severity of the winter (number of days with mean temperatures below 0°C) and cropping system e.g. open field, container or glasshouse (Casteels *et al.*, 2005; Blackshaw, 1996). This is at least in part because adult weevils often overwinter in exposed positions, such as the crowns of strawberry plants and in leaf litter. While the link between severity of the winter and the start of egg laying the following year has been made further work is required to develop this into a forecasting system.

Information on minimum temperature requirements as well as rates of development and survival at different temperatures exist for each stage of the vine weevil life-cycle (Masaki &

Ohto, 1995; Son & Lewis, 2005). This means that with appropriate meteorological and soil/substrate temperature data it should be possible to forecast vine weevil development within a crop. However, although vine weevil complete one generation per year in outdoor or polytunnel grown crops, there may be considerable overlap between different stages of development due to the extended egg laying period (Moorhouse *et al.*, 1992). Knowledge of the starting age structure of vine weevil populations may therefore be required in order to accurately forecast how populations are likely to develop. Despite these challenges, work by Morgan (1996) modelling vine weevil populations determined that the duration of immature stages and to a lesser extent mortality during these stages affected vine weevil population sizes while adult development rates and fecundity had little impact.

Knowledge gap – despite the understanding of vine weevil development and the clear link with temperature there is currently no working vine weevil population/activity forecasting system. As many growers already routinely monitor temperatures within crops a regional or grower-based forecasting could be developed.

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Vine weevil biology: knowledge gaps

There is a wealth of available information relating to many aspects of vine weevil biology. Much of this information has been supported by more than one study while in other cases researchers have reported contrasting results. This may simply reflect variation in conditions from study to study but may also reflect an incomplete understanding of some aspects of vine weevil biology. From the papers and reports reviewed a number of gaps or inconsistencies in knowledge have been identified that if addressed would help improve vine weevil management:

- The development rates of each stage of the vine weevil life cycle have been determined. However, this information only exists for constant temperatures. Crops grown in the field, polytunnel or glasshouse are subject to different degrees of temperature fluctuation. This is important in determining both the time taken for vine weevil to complete their development but also their ability to survive high or low temperature extremes, which may lead to opportunities to manipulate temperatures for cultural control, and to predict reduction of vine weevil populations in seasons when lethal temperatures occur.
- Understanding when the start and peak of adult feeding as well as the start, peak and end of egg laying occurs in field, polytunnel and glasshouse crops is important for the management of vine weevil. The timing of these events is likely to differ from region to region and crop to crop, therefore simple, practical method(s) for growers to monitor adult feeding and egg laying in their own crops needs developing and validating in both outdoor and protected crops. However, the relative importance of overwintering and newly emerging adults is an important factor to consider in this respect.
- There is a clear link between feeding by vine weevil adults on a host plant and the number of eggs laid. In addition, plants attacked by vine weevil may be more or less tolerant to attack depending on the size of the root system and the ability of the plant to regenerate its roots. Knowledge of these plant traits in currently grown varieties could be exploited by growers in selecting appropriate varieties depending on the likely risk of vine weevil damage. Quantifying the relationship between numbers of adults and numbers of larvae per plant would help to determine the benefit of controlling adults in addition to larvae in IPM programmes.
- There is evidence in the literature that certain chemicals can stimulate or inhibit feeding by vine weevil adults. These chemicals have great potential in reducing or diverting damage from the crop and indirectly reducing egg laying around crop plants. There has been little work on these chemicals to date and further investigation is warranted.

- The movement of vine weevil larvae is given little consideration but the fact that larvae can reach depths of 75 cm may have important implications for controls that rely on drenching soil grown crops. Understanding where vine weevil larvae can be found in the soil profile during the year may allow controls to be applied at times when they will be most effective.
- Vine weevil adults typically move short distances, however, long distance movement is also reported. Triggers for this long distance movement are not understood but are important if we are to prevent adult weevils moving from infested crops or other host plants e.g. hedges to newly planted crops.
- There is a clear need for improved early detection of vine weevil infestation. Plant odours have been identified that attract vine weevil. These chemicals have a potential role in producing more reliable monitoring tools that detect vine weevil earlier and at lower population levels.
- Despite the understanding of vine weevil development and the clear link with temperature there is currently no working vine weevil population/activity forecasting system. As many growers already routinely monitor temperatures within crops a regional or grower-based forecasting could be developed.

Management of vine weevil with entomopathogenic nematodes

Entomopathogenic nematodes have been used for the control of vine weevil in the UK since the 1980's and the species (and strains of species) commercially available has changed over the years as use of nematodes for control of various pests has developed. Currently available species and products for the control of vine weevil are shown in Table 3.

Table 3. Currently available nematode species and products available for the control of vine weevil.

Nematode species	Trade name	Producer/supplier
Steinernema kraussei	Nemasys L	BASF
S. kraussei	Exhibitline sk	Syngenta Bioline
Heterorhabditis bacteriophora	Exhibitline h	Syngenta Bioline
H. bacteriophora	Nemasys H	BASF
H. bacteriophora	Larvanem	Koppert
H. bacteriophora	Nematop	e-nema
A mix of *Steinernema carpocapsae, Steinernema feltiae* and either *H. bacteriophora* or *H. megidis*	SuperNemos	Flowering Plants Ltd

This literature review has identified over 146 published papers and reports on using entomopathogenic nematodes for control of vine weevil larvae and pupae. Many of the papers refer to the use other nematode species than those currently available in the UK (sometimes with the same trade name as currently available products), but give useful information on various aspects of using nematodes for optimum control of vine weevil and have thus been referred to in this review.

Nematode biology

Life cycle

Entomopathogenic nematodes are infective to certain insects and were initially identified for use as biological control agents as they occur naturally in soil, although their distribution varies (Bruck, 2004). Only one part of the nematode life cycle is infective to insects and this is the third instar infective juvenile (IJ) (Westerman, 1997). This stage does not feed but survives in the soil using only its lipid reserves (Westerman, 1997). The infective juveniles enter a host via natural openings (anus, mouth and spiracles (breathing holes)) and release a symbiotic bacteria which they carry in their gut. *Heterorhabditis* sp. carry bacteria of the genera *Photorhabdus* and *Steinernema* sp. carry *Xenorhabdus* (Campos-Herrera *et al.*, 2009). The bacteria then multiply within the host and kill it by septicaemia (Bathon, 1996). Within the host, the multiplying bacteria provide suitable conditions to support nematode reproduction (Gaugler, 1990). However, once resources become limited nematode reproduction stops and the nematodes only develop to the third IJ stage where they then ingest some of the surrounding bacteria and exit the dead insect (cadaver) in search of a new host (Campos-Herrera *et al.*, 2009). Westerman (1999) reported that 2.4 and 2.6 IJs per vine weevil larva of the *Heterorhabditis* sp. strain NL-H-F85 and UK-H-UK211 respectively are required at 20°C to kill 50% of the larvae.

Following infection of an insect a new generation of IJ nematodes can develop in approximately 15 days at 18-20°C (Simons, 1981). However, various studies have found that only a proportion of an IJ population are infective at any one time, e.g. Westerman (1998) observed that when they are applied to a host some do not penetrate and are assumed to be temporarily inactive or dead. When comparing the proportion of IJs penetrating vine weevil larvae and *Galleria mellonella* (wax moth) larvae, significantly fewer nematode juveniles were infective in the presence of vine weevil larvae. The study found that temperature and insect species influenced the proportion of infective nematodes. *G. mellonella* is very susceptible to entomopathogenic nematodes and is often used as the host insect in research studies. Another study by Fairbairn *et al*. (2000) supported these findings and concluded that the proportion of IJs that are infective is dynamic and that more become infective over time. The study also concluded that IJs respond to chemical cues produced by a nematode-infected host which inhibits subsequent infectivity (Fairbairn *et al*., 2000). However, this is unlikely to effect the control of vine weevil larvae and pupae by nematodes as only a few IJ are required to achieve host death.

Age of vine weevil larvae and pupae infected

Nematodes from the *Heterorhabditidae* and families are used commercially for the inundative control of vine weevil larvae. Species within these families are most effective against late larval and pupal stages of vine weevil, as larger larvae are more likely to encounter nematodes than smaller larvae, have larger openings which provide easier penetration and may also be more attractive (Simons, 1981; Georgis and Poinar, 1984; Kakouli, 1995; Lola-Luz *et al*., 2005; Morton and Del Pino, 2005). Although vine weevil pupae are highly susceptible to nematodes, application timed for the control of pupae is often not recommended by suppliers due to the small window of opportunity for control, as once temperatures are high enough in the spring for nematodes to work, many pupae may have started to, or have already emerged as adults (A. Dillon, personal communication). This was observed in a study by Booth *et al*. (2002) when applications of IJs were made in May to strawberry and cranberry sites in Washington. Nematodes are not generally considered to infect vine weevil adults, however, *Heterorhabditis heliothidis* was reported to infect low numbers of newly emerged vine weevil adults on grape, when of 131 infected vine weevils detected, eight were pupae, three were new adults and the rest were larvae (Bedding and Miller, 1981).

Early instar larvae can also be infected but *Heterorhabditi*s spp. have been observed to be more effective than *Steinernema* spp. which has been attributed to their ability to penetrate the cuticle of the host larvae using a sclerotized dorsal tooth, while *Steinernema* spp. can only enter through natural openings which may be limited in smaller larvae (Gaugler,1990; Georgis and Poinar, 1984). While penetration may be difficult in smaller larvae, a study has shown that only one *H. megidis* IJ per vine weevil larva was required to kill 65% of small and medium larvae whereas this infection rate killed only 10% of larger larvae (Boff *et al*., 2000). Therefore, if penetration is successful in small larvae, few nematodes are required to kill it. However, it has been reported that vine weevil larvae can encapsulate and melanize infective juveniles of *S. feltiae* and *S. kraussei* in their mid-gut but only one of 500 larvae tested survived, suggesting the nematodes expelled their bacteria before being melanised, thus resulting in larval death (Steiner, 1996b).

Movement and searching behaviour

Nematode species also vary in the searching strategies used to find their host. *Steinernema feltiae* is described as an ambusher remaining close to the soil surface while *Heterorhabditis bacteriophora* is an active forager moving deeper into the soil (Kaya *et al*., 1993). While searching for a host, IJs direct their searching via a range of cues including plants, hosts and their associated cues such as faeces and carbon dioxide (van Tol *et al*., 2001).

Most of the research on nematode movement in response to plant roots and insect larvae has been carried out on *H. megidis* on strawberry and *Thuja occidentalis*. The majority of studies have found that this nematode species was stimulated to move by the chemical cues from either roots or larvae, but a strong attraction was observed when these two factors were combined, although variation in the cues were observed between different plant species (Boff *et al*., 2001b; van Tol *et al*., 2001; Boff *et al*., 2002). A study by van Tol *et al*. (2001) found that weevil-damaged roots were more attractive than mechanically-damaged roots or undamaged roots, which suggests that a plant chemical is produced in response to larval feeding which attracts IJs. This was later confirmed by Riemens *et al*. (2003) who determined that *H. megidis* was attracted to water soluble plant volatiles (in addition to carbon dioxide) released by the *T. occidentalis* when *O. sulcatus* larvae were feeding. Other studies have suggested that there are also differences in the host finding strategies between strains of the same species, with a strain of *H. megidis* (NL-H-F85) having a random searching strategy and being unresponsive to the presence of plant roots in peat, while another strain (UK-H-211) was described as having a root searching strategy (van Tol *et al*., 1998). Characteristics such as these contribute to the success of nematodes in finding their hosts.

Nematode biology: key knowledge identified

- Vine weevil late larval stages and pupae are the most susceptible stages.
- Only a few nematode infective juveniles are required to kill a vine weevil larva.

Nematode species

Heterorhabditis species

Two Heterorhabditis species are commercially available for the control of vine weevil larvae in the UK; Heterorhabditis megidis and the currently more the commonly used *H. bacteriophora.* However various other *Heterorhabditis* species have also been identified which infect vine weevil larvae including *H. marelatus* (Berry *et al*., 1997), *H. heliothidis* (Bedding and Miller, 1981; Scherer, 1987) and *H. downesi* (Lola-Luz *et al*., 2005). When vine weevil larvae are infected by *Heterorhabditis* sp. IJs the larvae turns a red colour following infection which is a good indication of successful infection (Tillemans *et al*., 1990; Shearer, 1999). However, infected larvae disintegrate very quickly in the soil (Backhaus, 1994) and larvae infected with *Steinernema* spp. turn a less noticeable yellow-brown colour, so use of colour as an indicator of infection is dependent on nematode species used. Growers should use numbers of live larvae remaining a few weeks after treatment as a more reliable guide to level of control achieved.

Steinernema species

T*hree Steinernema species are commercially available for vine weevil control in the UK; S. kraussei*, *S. carpocapsae* and *S. feltiae*. *S. kraussei* is the most commonly used of the three species used as it is effective at lower temperatures (see temperature section below) and can thus be used earlier in the spring and later in the autumn than other *Steinernema* and *Heterorhabditis* species However, various other *Steinernema* species have been identified which also infect vine weevil, including *S. glaseri,* (Georgis *et al*., 1982; Georgis and Poinar, 1984; Jackson *et al*., 1985) *S. riobrave* (Bruck *et al*., 2005) and *S. bibionis* (Richardson, 1990; Godliman, 1991).

Species mixtures

Usually, nematodes are applied as single species but a relatively new product, SuperNemos contains a mix of *S. carpocapsae, S. feltiae* and either *H. bacteriophora* or *H. megidis*, aiming to attack a range of insect species in one application. An experiment by Bennison and Hough (2013) carried out on strawberries in peat grow-bags in a polytunnel found that *S. kraussei* (Nemasys L) and SuperNemos were equally effective, giving 94% and 90% control respectively of vine weevil larvae compared with untreated plants. In this experiment, SuperNemos was applied at half the application rate of Nemasys L. Syngenta

Bioline also supplies 'Exhibitline Mix' for the control of vine weevil which contains *H. bacteriophora* and *S. feltiae* but this product is not currently available to growers in the UK.

Comparison of species efficacy

Various studies have compared these different species at the same conditions for the control of vine weevil larvae and some studies report differences between species while others find little difference. For example a study comparing *H. heliothidis, Heterorhabditis* sp. (HP88 strain, species not reported) and *S. feltiae* against vine weevil larvae on hops showed they were all equally effective (Dorschner *et al*., 1989). Furthermore, when *H. marelatus* and *H. bacteriophora* were compared on infested *Impatiens walleriana* in the glasshouse (soil temperature 20°C) and outdoors (soil temperature 11.5°C) both provided nearly 100% control (Bruck *et al*., 2005). Despite this, other studies have demonstrated the efficacy differences between species. For example, *H. bacteriophora* gave better control of vine weevil larvae than *S. carpocapsae* on Euonymus (Kaya *et al*., 1993).

When evaluating the efficacy of species against vine weevil larvae, most studies have found that *Heterorhabditis* species within their optimum temperature range are usually more effective discussed (Bedding *et al*., 1983; Stimmann *et al*., 1985; van Tol, 1993b; Schirocki and Hague, 1994; van Tol, 1996; Schirocki and Hague, 1997). However, a study by Hough & Bennison (2014) and Hough *et al.* (in press) also showed that *S. kraussei* (Nemasys L) was significantly better than two *H. bacteriophora* products (Nematop and Nemasys H) in controlling vine weevil larvae on protected strawberry plants grown in a coir substrate.

Nematode species: knowledge gaps

- Few studies have been done comparing currently available nematode species and products against vine weevil.
- Recent ADAS work in project CP 89 showed that on substrate strawberry in a poly tunnel, the mix of *S. carpocapsae, S. feltiae* and either *H. bacteriophora* or *H. megidis* ('SuperNemos') was as effective as *S. kraussei* (Nemasys L) in 2012 and that Nemasys L was more effective than two *H. bacteriophora* products in 2013. Similar studies on HNS are needed, to provide growers with robust information on species efficacy in different crops, substrates and seasons.

Effects of temperature on efficacy

Temperature is critical for nematode activity and different nematode species and strains are only effective within certain temperature ranges. Thus the time of year which IJs are applied can significantly affect control. A study carried out in Germany found that control of vine weevil larvae following application in mid-April was delayed until warmer conditions occurred after a couple of weeks, while control from IJs applied on 2 May was observed sooner (Neubauer, 1996). Temperature can often explain the variations in control observed between different studies and it is important that soil/substrate temperatures are recorded in experiments so that comparisons can be made between studies. For example, in a trial using *H. megidis* (the species in the Larvanem product at the time), 89-99% control was reported in one year, but in the following year when the same trial was repeated in hotter, drier conditions only 74% control was reported (Łabanowska *et al*., 2004).

At the right temperature nematodes can find and penetrate a host very quickly. For example, two *Heterorhabditis* sp. strains (species not reported) were shown to control vine weevil larvae at 12°C, but not at 9°C. When these strains were kept in a controlled climate room at 12°C for six hours followed by 9°C until the end of the experiment (six weeks) they gave 80-100% control (van Tol, 1993b; van Tol, 1994). Van Tol (1996a) also confirmed that for *Heterorhabditis* sp. strains (Larvanem NI-H-F85 and Nemasys H UK-H-211 – species not reported but likely to be *H. megidis* as this was the species in these commercial products at the time) there was no control of vine weevil larvae below 12°C degrees, but only one day above 12°C was required for Larvanem to provide control while Nemasys H required a few days (van Tol, 1996). These studies show that only a short period of time is required at suitable temperatures for the nematodes to find the host and infect it. In these studies, it was suggested that low temperatures may inhibit nematode searching ability rather than their ability to penetrate and infect the larvae, as the two *Heterorhabditis* sp. strains UK-H-211 and NI-H-F85 used in these studies have been shown to penetrate and kill at 9 and 5-7°C respectively (van Tol, 1993b; van Tol, 1994). However, Backhaus (1994) reported that IJs can infect vine weevil larvae at 8°C but the symbiotic bacteria cannot multiply and kill the insect. It is currently regarded that it is the symbiotic bacteria that vary in their activities at low temperatures, e.g. *S. kraussei* and *S. carpocapsae* are associated with *Xenorhabdus bovienii* and *X. nematophila* respectively, whereas *Heterorhabditis* species are associated with *Photorhabdus* species, and it is thought that *X. bovienii* is active at lower temperatures than the other species (A. Dillon, personal communication).

While the literature shows that nematodes are very quick to act, it is important to provide the IJs with optimum temperatures for as long as possible to guarantee the best control. General recommendations to growers are that at least a month of suitable temperature is required for good control (Irving *et al*., 2012). Supplier leaflets provided with the products recommend only using the products within the specified temperature range:

- Nemasys L (*S. kraussei*): 5-30°C
- Exhibitline sk (*S. kraussei*): 5-30°C
- Nemasys H (*H. bacteriophora*): 12-30°C
- Larvanem (*H. bacteriophora*): 14-33°C
- Nematop *(H. bacteriophora*) does not provide a temperature range on its product leaflet but specifies that soil temperatures must be above 12°C for several hours a day.
- Exhibitline h (*H. bacteriophora*): 12-30°C
- SuperNemos (*S. carpocapsae, S. feltiae* and either *H. bacteriophora* or *H. megidis*): above 10°C.

Studies on different nematode species have demonstrated that optimum temperatures vary for infectivity. For example, *H. marelatus* was reported to be more virulent than *H. bacteriophora* at 14°C in laboratory experiments (Berry *et al*., 1997). When a temperature experiment was carried out on *S. carpocapsae* (strain S25) and *Heterorhabditis* sp, (species not reported, strain HF 85) on cyclamen, *Heterorhabditis sp.* provided complete control at 20°C after 12 days of infection, while *S. carpocapsae* performed best at 20-25°C but only gave 65% control (Miduturi *et al*., 1994b).

Differences in infectiveness of different strains of a species at different temperatures have also been observed and this explains why different nematode products with the same species vary in their recommendations for temperature ranges. Two strains of *H. heliothidis* showed significantly different infectivity at both 12 and 10°C (Bedding and Miller, 1981). At 18°C, Long *et al*. (2000) found that one strain of *S. kraussei* (L137) provided 80% control while a second strain (L017) gave only 41% control. Other studies have also observed variation between strains of the same *Heterorhabditis* species in their infectivity at different temperatures (van Tol, 1993a, b). It is vital that optimum temperatures and limits for the species and strains being used commercially are known and given in product recommendations in order to support successful application by growers.

Cold-active species

A cold-active entomopathogenic nematode is a valuable product for growers particularly in temperate countries such as the UK. The failure of nematodes to control vine weevil can often to be attributed to low temperatures. For example Oakley (1994) found that application of *Heterorhabditis* sp. (Fightagrub) to an infested strawberry field on 6 and 24 September in the UK provided little control of vine weevil. While the nematodes were confirmed to have invaded the larvae by February they had not caused mortality. Some species and strains show no mortality at lower temperatures (e.g. 5-10°C degrees) (Miduturi *et al*., 1994b).

S. kraussei is currently marketed and widely used in the UK due to its activity at cold temperatures (lower limit 5°C). Mráček *et al.* (1999) found that *S. kraussei* had an infection rate and host parasitism at 10°C similar to a heat active strain at 25°C. When *S. kraussei* (strain L137) was applied in early winter (4 December) to potted strawberry plants (Levingtons compost) partially buried in the field, a dose of 60,000 nematodes per pot resulted in up to 81% control of vine weevil larvae, while the same dose of *S. carpocapsae* did not cause any significant mortality (Willmott *et al*., 2002). The current recommended dose rate for *S. kraussei* products (Nemasys L and Exhibitline sk) on strawberry is 25,000 per plant. Using the Baermann funnel extraction method 44.3% of *S. kraussei* were recovered from the soil in the March following application in early December, showing that they survived winter conditions well (Willmott *et al*. 2002). Another study screened 12 strains of *S. kraussei,* six strains of *S. feltiae*, one *Steinernema* sp. (species not reported, strain CH-S-PIL91) and one *Heterorhabditis* sp. (species not reported, strain CH-H-FLU91) at 9°C (Steiner, 1996a). All these strains except for one commercially available *S. feltiae* species were collected from alpine regions. Higher mortalities of *G. mellonella* larvae were given by some of the *S. kraussei* strains and by *S. feltiae* in a choice experiment in a sand substrate. At 9°C, *S. kraussei* migrated the furthest and showed the highest host-finding ability in response to vine weevil larvae in the choice experiment, although all the larvae survived. In a study by Long *et al*. (2000), *S. kraussei* (strain L137) at 6°C gave 76 and 28% control of small larvae and pupae respectively and at 10°C provided 78 and 33% control of small larvae and pupae respectively . Interestingly none of the nematode species evaluated in this study were effective against large larvae at 6°C and 10°C, which contradicts other studies discussed previously, that both *Steinernema* and *Heterorhabditis* spp. are more effective against larger larvae and pupae than young larvae. All of these studies demonstrate that certain strains of *S. kraussei* do have the qualities required to be used in cold conditions.

All nematode species are regarded to be less effective in field soils than in substrate used in well-irrigated containerised crops, due to the less reliable soil moisture affecting nematode survival and movement and some soil types, such as heavy clay soils, inhibiting movement. In a strawberry field in Norway, *S. kraussei* (supplied by Becker Underwood now BASF) was applied as a late autumn drench to test its activity at cold temperatures in field soil. Control of vine weevil larvae only reached 50% when the mean daily soil temperatures were just above 10°C for 12 days after nematode application, followed by a period at 8 - 0.5°C (Haukeland and Lola-Luz, 2010). The soil type in this trial was silty clay loam (10-25% clay content) which may explain the poor control observed. Another study observed minimal infection of vine weevil larvae after spraying three cold-active strains of *Steinernema* spp. (species not reported) at the field application rate to soil at 8 and 12°C (Ingraham and Webster, 1991).

Researchers continue to search for cold active strains. For example, a strain of *S. kraussei* from soil in the Italian Alps gave 80-90% mortality of vine weevil larvae at 5°C (Ricci *et al*., 2004). When compared to a commercially available *S. feltiae* strain, the new strain was superior at 5°C and *S. feltiae* gave similar levels of control only at 20°C. A study evaluating a large number of nematode isolates at 9 and 12°C identified three isolates which provided almost 50% control at 9°C (Westerman and Zeeland, 1994). Interestingly one of these isolates had undergone breeding selection for improved infectivity at low temperatures for eight generations suggesting selection may be possible. Other methods of improving cold active ability has been investigated such as replacing the natural bacterial symbiont of a nematode which performs poorly at low temperatures, with the symbiont of a more cold active strain. However, this method did not improve cold activity indicating that the symbionts either do not a play a role in cold activity or the nematode was so poor under cold conditions it could not be improved to an acceptable level (Westerman and Zeeland, 1994).

In studies where the application of entomopathogenic nematodes have been unsuccessful, high temperatures can also be responsible if they exceed the upper temperature limit of the species and strain used. For example, when *S. carpocapsae* was applied to beds of *Euonymus fortunei* in an urban park (Philadephia) in July 1988 and 1989, no control of vine weevil was achieved (Owen *et al.,* 1991). This could be due to both high temperatures and dry soil conditions; in June 1988 temperatures exceeded 26.7°C throughout the trial. Temperature data for 1989 was not reported.

Various studies have delineated the temperature profiles for different species, however these can vary between each study, which is likely to be due to the differences in strains and experimental methods being used. The range of *S. carpocapsae* (strain unknown) was described between 15 and 33°C by Kakouli (1994), however other studies have suggested 13°C (Schirocki and Hague, 1997) and 14°C (Schirocki and Hague, 1994) as the lower limits. The latter two studies both investigated a strain originating from Wales but it cannot be confirmed that they were the same.

Effects of temperature on efficacy: **Gaps in knowledge and important points:**

- Only temperature data reported for specific commercial strains and current products should be relied on as data from the same species (but potentially a different strain) may not be representative of a current commercial product.
- When using the scientific literature to support decision making in vine weevil management, it important to use only the data from studies which have been carried out on vine weevil as other hosts such as *G. mellonella* are more susceptible to IJs.
- Research supports the recommendation for use of *S. kraussei* at low temperatures.
- There is contradictory evidence on the efficacy of nematodes against young and old vine weevil larvae. Although substrate temperature will guide product choice, when temperatures are within the range of all available nematode products, this aspect needs clarifying for current commercial products to aid optimum timing and product choice according to age of larvae.

Persistence after application

Most nematode current products claim to give four weeks protection against vine weevil larvae after application and warn that larvae hatching from eggs after this period may be poorly controlled (see for example, BASF website for information on Nemasys L (*S. kraussei*) and Syngenta Bioline website for product guides on Exhibit line sk (*S. kraussei*) and Exhibitline h (*H. bacteriophora)*).

Nematode persistence will be affected by factors including substrate or soil type and moisture, temperature and also by the density of vine weevil larvae present, as in high densities the nematodes will be able to continue the infection as they breed in the 'broth' which they produce in the dead host and the new IJs then infect other larvae. Therefore, reported persistence of nematodes in the soil following application varies between published studies. Persistence is usually determined by baiting the soil with greater wax moth larvae, *G. mellonella,* and presenting the results as the percentage which became infected.

A study by Haukeland and Lola-Luz (2010) on field-grown strawberries supports a four week persistence, where following one application at 30,000 IJs per plant in two separate trials, *S. kraussei* infected 83% and 67% of *G. mellonella* larvae respectively one month after application. However, persistence may vary between species. In the same study, *H. megidis* applied at 25,000 IJs per plant infected only 58.5 % of larvae compared to *S. kraussei* at 83% one month after application.

When *S. carpocapsae* and *H. megidis* were applied to field-grown strawberries on raised beds using a double line irrigation system in early May, the numbers of *S. carpocapsae* decreased by >90% within four weeks following application but did not reduce significantly more thereafter, while *H. megidis* continued to decline until week five when no IJs were recovered (Kakouli-Duarte *et al*., 1997). This study demonstrates the typical trend observed in other studies where the numbers of IJs start to decline shortly after application and then continue to persist in low numbers (Wilson *et al*., 1999).

Various studies have investigated the efficacy of these low numbers of persisting IJs. In a Cranberry bog persistence was found to last 10 months with *H. heliothidis* infecting 14, 20, 6, 16, 42, 32 and 2% of *G. mellonella* larvae 6, 12, 18, 23, 30, 36 and 42 weeks respectively after treatment with 160 IJs /cm^2 (Shanks and Agudelo-Silva, 1990). It was suggested that the lengthy persistence in this case was due to a combination of suitable moisture, temperature, soil type and host abundance. In less suitable conditions such as field-grown strawberries, the persistence of *H. megidis* two months following application of 50,000 IJs per plant in summer was 92%, but in the following spring only 5% were detected (Haukeland and Lola-Luz, 2010). Evidence of the lengthy persistence in the soil, although at lower numbers than at application, shows that nematodes will continue to recycle in the soil if conditions are suitable (Booth *et al*., 2002).

There is also evidence to suggest that recycling nematodes may be possible by re-using nematode-treated compost which is free from vine weevil larvae. When compost treated with *S. bibionis* was re-used to pot up Impatiens plants, the IJs continued to kill vine weevil larvae when they were re-infested with the pest for up to eight months following the initial application (Richardson, 1990). This method led to a loss of 17% of treated plants compared to 69% of untreated plants.

Persistence after application: **Gap in knowledge and key points:**

- The literature supports the suppliers' product information that in suitable conditions, nematodes persist in sufficient numbers to infect hosts for at least four weeks. However, most studies have been done using wax moth larvae rather than vine weevil larvae.
- The IJs will persist at much lower numbers for significantly longer than four weeks but will not continue to provide significant control of vine weevil larvae, e.g. those emerging from eggs hatching after four weeks following application.
- These results demonstrate the need for repeated applications of nematodes, for example, when adult vine weevil egg laying continues late into the autumn so that hatching larvae are not killed by nematodes applied more than four weeks earlier.
- This also highlights the need for a simple method for growers to monitor vine weevil egg laying on their own farms and nurseries, to guide nematode application timings.

Migration after application

Various studies have focused on the migration rate of IJs as this is an important factor contributing to host location and therefore control. This is a particularly important assessment when trying to identify a cold active nematode.

In trials carried out in a glasshouse on *Neoaplectana carpocapsae* (renamed *S. feltiae), Neoaplectana glaseri* (renamed *S. glaseri*) and *H. bacteriophora*, IJs were able to locate vine weevil larvae at a depth of 15 cm in pots (Georgis *et al*., 1982). However, similar studies comparing *H. heliothidis*, *N. carpocapsae* and *N. glaseri* found that *N. glaseri* when applied to the surface of pine seedlings in sandy loam soil provided the best control of vine weevil larvae at a depth of 20cm (Georgis and Poinar, 1984). This demonstrated that migration rate can vary significantly between species. The study also showed that application method can influence migration as when IJs were injected 5 cm into the soil the three species were equally effective at 5, 10 and 20cm. This method is not available to growers but illustrates the importance of following supplier's recommendations such as apply to moist substrate or soil and use the recommended volume of water for application, to allow the nematodes to move through the growing medium to find and infect the target hosts.

Usually the studies of IJ movement have been carried out in sand/soil columns. However these studies often do not represent the movement which occurs in the field where soil type/disturbance, root systems, temperature and moisture play a role (Westerman and Godthelp, 1990). For example, *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* infected vine weevil larvae at a 20cm depth in a soil column but in the field only *S. carpocapsae* moved to this depth, while *S. feltiae* moved to 17.5cm and *H. bacteriophora* did not infect any larvae lower than 12.5cm (Hanula, 1993).

Variation in migration has been observed between species, strains of the same species and batches of the same strain. For example, a Dutch *Heterorhabditis* strain was been found to have superior migration in a sand column compared to other *Heterorhabditis* and *Sterinernema* species and strains, with an average speed of 0.6-0.8 cm/hour in the absence of a host (Westerman and Godthelp, 1990). Westerman (1994) found that the efficacy and migration of IJs in separate experiments were different using IJs from the same batch. As the quality of a batch affects migration it was suggested that migration could be used as a tool to evaluate batch quality (Westerman, 1994). The quality of a nematode usually refers to its activity, viability, energy reserves, ability to migrate and the number of symbiotic bacteria it contains (Grunder *et al*., 2005).

In addition to movement through the substrate, IJs have also been recorded to migrate through capillary matting as observed in a cyclamen crop (Richardson, 1990). In this study, plants treated with *Steinernema* sp. were either placed on dishes to stop migration or placed on capillary matting amongst untreated plants on dishes or the matting. Nematodes were found in the compost of 42% of the untreated plants on the matting but none were found in compost in the untreated pots stood on plates. There were also 15% fewer vine weevil larvae found in untreated plants placed on the matting compared to in untreated plants on dishes. Control of vine weevil larvae has also been reported in untreated plots in other studies as a result of migration of the nematodes between plots (Simons, 1981; Shanks and Agudelo-Silva, 1990; Mráček *et al*., 1993).

Migration after application: **Key points:**

- Infective juveniles can migrate long distances but this varies depending on the conditions and species. As recommended by the suppliers, it is best to apply IJs as close to the root system as possible, particularly for *Steinernema* spp. which have been found to display an ambushing approach when searching for a host.
- Research supports suppliers' recommendations to apply nematodes to moist substrate or soil and use the recommended volume of water for application, to allow the nematodes to move through the growing medium to find and infect the target hosts.
- Infective juveniles can also migrate through capillary matting and move into pots of compost stood on the matting, where they can kill vine weevil larvae.

Storage and formulation carriers

Nematode carrier

Commercial IJs are provided in an inert carrier which is added to water and applied as a drench or through drip irrigation systems to control ground-dwelling pests including vine weevil. Studies have shown that the carrier itself can have an effect on nematode quality and viability. A study which compared IJs of *H. marelatus* carried on a sponge carrier with those carried on vermiculite has shown that the latter did not significantly reduce numbers of vine weevil larvae while the sponge-packed IJs did (Wilson *et al*., 1999). However, the sponge system for packing and distributing nematodes is no longer used and they are packaged in a carrier, the exact formulation of which is commercially sensitive. All currently available nematode products are subjected to quality control checks before dispatch and are distributed with cool packs to help maintain quality in transit.

Quality checking on receipt of product

When nematodes are received, checking for movement once the carrier has been added to water prior to application will give an indication that they are alive, however, a microscope is needed to do this. The nematodes can also be checked to see if the IJs look dense and whitish, rather than transparent, which shows they have a higher lipid content and will thus persist longer (Shearer, 1999; Fitters and Griffin, 2006). Supplier recommendations vary with regard to storage conditions prior to use but all products have a pack expiry date on the product. BASF (Nemasys L and H) and Syngenta Bioline (Exhibitline sk and h) recommend storage at 5°C, Flowering Plants Ltd (SuperNemos) recommend 4-6°C in a cool dry place out of direct sunlight, e-nema (Nematop) recommend 4-12°C and Koppert (Larvanem) recommend 2-6°C in a dark place.

Potential use of insect cadavers

In addition to the possible adverse effects of the carrier on nematode quality, studies have also shown that IJs are influenced by the condition in which they are reared, which impacts their virulence later in life (Boff *et al*., 2001a). For example, *H. megidis* IJs received direct from a supplier were compared with those also received from the supplier but allowed to infect *G .mellonella* once prior to use (Long *et al*., 2000). The results showed that those cultured in *G. mellonella* killed a higher percentage of vine weevil larvae (small and large) and pupae at 10°C and 18°C than those received direct from the supplier. At 10°C the IJs used directly following receipt from the supplier provided little control of larvae and pupae (1-3%) while those cultured in *G. mellonella* provided between 11-47% mortality. This suggests that nematodes reared in an insect have increased pathogenicity.

Research has been carried out to see if infected cadavers could be used as an alternative application method and significantly better control of vine weevil has been observed compared to using an aqueous suspension of IJs, although control mortality was high in this study (Shapiro-Ilan *et al*., 2003). A study by Bruck *et al*. (2005) also confirmed that when IJs were applied to potted Impatiens via infected cadavers, the numbers of IJs per pot was higher both in the glasshouse and outdoors. However, temperature did delay/reduce emergence of the nematodes which slowed the control process compared to an aqueous suspension (Bruck *et al*., 2005). Using cadavers for nematode application would also be a very expensive and time-consuming method.

Storage after mixing with water

Once IJs are mixed into water, suppliers recommend applying IJs straight away and not to store the diluted product. While this recommendation should always be followed by growers some studies have been carried out to determine the effect of storing suspensions of IJs.

Westerman (1992) found that storage in aerated water at 4-5°C led to a reduction in persistence and efficacy. After 25 days of storage, a *Heterorhabditis* sp, strain HFr86 (species unreported) gave 100% kill of mealworm (*Tenebrio molitor)* pupae for up to two weeks after application, and more than 50% kill after five weeks. However, after 234 days of storage the nematodes gave 100% mortality one week after application which was then reduced to 65% by week two and to 4% by week five. The author also commented on the variation in efficacy between IJs stored for the same length of time, suggesting other factors in addition to storage time and temperature were involved. In a second study, where nematodes were kept in unaerated water at 20°C, it was determined that their 50% survival time ranged from 5.6 to 12.5 weeks, with the variation being explained by differences in how quickly they used their energy reserves (lipid content) (Fitters and Griffin, 2006). Both of these studies indicate that mortality and efficacy declines with storage time. However, one interesting study observed a 27 fold increase in mortality of vine weevil larvae when a North West European strain of *H. megidis* was stored in water (shaken once a week to provide aeration) at 9°C for 12 weeks compared to using freshly produced IJs (Fitters *et al*., 2001a).

A model has been developed to describe the performance (migration rate, persistence and efficacy) of a *Heterorhabditis sp.* strain (species not reported) following storage in aerated water at 4-5°C. The model showed that; a) food reserves were an important factor in describing migration rate; b) percentage ensheathment (cuticle or skin which protects IJs from fungi and bacteria) was important in describing persistence; c) migration rate in the absence of a host was a good method of describing and predicting the efficacy and persistence of this particular strain of nematode (Westerman and Stapel, 1992). The model also suggested that mortality was not a good indicator of how the remaining live IJs would perform, and that efficacy was particularly affected by storage time, with a 10% decrease in efficacy occurring after 100 days followed by more than 30% decrease in the following 100 days. After 300 days of storage 90% efficacy will be lost.

When IJs are stored in different suspensions to water, efficacy and survival can often be unaffected by storage which is important for researchers working on IJs. For example, Klinger (1990) found that there was no significant decrease in the number of surviving IJs of a *Heterorhabditis* sp. found after 63 days of storage at 6°C in a culture flask containing a sponge holding a nutritive substance and there was also no reduction in infectivity.

Nematode storage: Key points and knowledge gaps

- To avoid losing quality and viability entomopathogenic nematodes should be stored before mixing according to supplier's recommendations and used before their use-by date
- Checking nematodes for movement on opening the pack can only be done using a microscope, with knowledge on the correct method to use. Some growers with microscopes wish to do this themselves. Training growers who have microscopes how to do this correctly would be useful for knowledge transfer.
- Once mixed with water, nematodes should be applied immediately and agitated during application

Effects of pesticides and fungicides on nematodes

Fungicides have been found to have little effect on entomopathogenic nematodes. When a range of fungicides (e.g. propamocarb, prochloraz, iprodione, benomyl and metalaxyl) were applied to Fuschia and *Taxus baccata* after treatment with *Heterorhabditis* sp. no detrimental effects were observed (Backhaus, 1994).

Other combinations which have been tested include combining *H. bacteriophora* and *S. carpocapsae* with *Bacillus thuringiensis* and a pesticidal soap with the aim of controlling soil and foliage pests in one application. It was found that combining entomopathogenic nematodes with these products was effective as long as the formulations were applied straight away as storage of these formulations adversely effected the IJs survival (Kaya *et al*., 1995).

Some commercial nematode supplier product leaflets provide information on compatibility of pesticides in the leaflets supplied with the nematode products. E-nema state that Nematop (*H. bacteriophora*) is compatible with most fertilizers, fungicides, pesticides and herbicides and a web link is provided so that growers can determine compatibility of a product. BASF ask growers to contact their technical service hot line for advice on compatibility of their nematode products and they have a database of compatibility. Syngenta ask growers to contact the supplier. Koppert provide information on compatibility of pesticides with all their biological control agents including nematode species in the side effects section on their website.

Effects of potting media/soil on efficacy and migration of IJs

A review of the literature identified only one comprehensive study which has been done to determine the effects of growing media (peat, bark, coir, and peat blended with 10 and 20% compost green waste (CWG) on the dispersal of entomopathogenic nematodes and efficacy against vine weevil (Ansari and Butt, 2011). The study found that growing media significantly affected both dispersal and efficacy of IJs in both laboratory bioassays and glasshouse experiments.

In the laboratory bioassays, three *Heterorhabditis* spp. caused 100% mortality of vine weevil larvae regardless of the media, however *Steinernema* sp. only achieved 100% mortality in the peat blended with 20% CGW. When evaluating the effect of growing media on the vertical dispersal of IJs in the presence of vine weevil larvae, *H. bacteriophora* (strain UWS1) had the highest dispersal in all media, while *S. feltiae* (Entonem) and *S. carpocapsae* (Millenium) dispersal was reduced and restricted to peat blended with 20% CGW and coir, respectively. The dispersal of each species evaluated varied depending on the growing media.

The study also reported on a glasshouse experiment using rooted cuttings of *Euonymus fortunei* in 2L pots. Two weeks after application, the efficacy of *H. bacteriophora* was 100% in peat, and peat blended with 10% and 20% CGW, but only 70% in bark and coir. These studies suggest that entomopathogenic nematodes work better in peat-based growing media for most of the species evaluated in this study. However, a separate study on *H. megidis* (the species that used to be in Nemasys H) and *S. carpocapsae* (Biosys 252) reported that results were slightly better in bark grow-bags compared to peat-based compost in pots when moisture content and temperature were the same (Kakouli-Duarte *et al*., 1997). Differences between these two studies could be due to differences between nematode species and strains, moisture content and temperatures of the growing different media

In the field, other studies have confirmed that nematode efficacy against vine weevil larvae is also limited by soil type. In Norway, an experiment on field grown strawberries determined that nematode efficacy was poorest in soils with a higher clay content such as silty loam sand (5-10%) and silty clay loam (10-25% clay) (Haukeland and Lola-Luz, 2010).

A grower guide to biological control in soft fruit produced by the HDC advises that nematodes are affected by soil type and migrate poorly through dry and heavy soils (Irving *et al*., 2012). Some nematode suppliers provide recommendations on measures that can be taken to improve efficacy in field soil, e.g. the Syngenta Bioline product guide for Exhibitline sk (*S. kraussei)* recommends that soil should be loose rather than compacted, soil crusts should be broken before application, scarifying between rows is recommended and soil should be kept moist for four weeks after application.

Effects of potting media/soil: Gaps in knowledge:

- Soil type can affect the control provided by entomopathogenic nematodes. Migration is reduced in dry and heavy soils such as clay. Differences also exist between growing media such as coir, bark and peat mixes but the results are not consistent. Research suggests that the migration rate and efficacy of different species can vary depending on the soil or growing media type. Information is needed on each commercial species and strain currently available, together with currently used growing media, as published data from other species, strains and growing media may not be representative.
- In the literature it is difficult to compare studies as strains are often not named. Furthermore, product names are often given, such as Nemasys H and the species is not confirmed. This makes comparing trials difficult as while the name of some products have remained the same over time the species used in the product have changed.

Application methods

Application method is key to successful control with entomopathogenic nematodes. All nematode suppliers provide clear recommendations on their product leaflets accompanying the packs, and on their websites. These recommendations include:

- Apply to moist soil or substrate, at the recommended temperature for the product
- Keep soil or substrate moist for four weeks after application
- Use clean application equipment
- Remove all fine filters from the system
- Once mixed with water, apply immediately
- Keep agitated during mixing and application to prevent nematodes settling out and to ensure even distribution

- If applied to foliage, irrigate after application to wash onto soil or substrate
- Guidance on recommended pressures and nozzle size
- Guidelines on using through dripline irrigation systems

BASF provide detailed illustrated protocols which are available through their distributors to advise growers on using a Dosatron, positioning drippers and storing and mixing nematodes (see Appendices).

Throughout the literature these recommendations are supported and accepted as standard practice when applying nematodes (Simons, 1981; Shearer, 1999; Buxton, 2003; Raffle, 2003). Another key point is to apply the IJs as close to the root zone as possible (Irving *et al*., 2012). Van Tol (1996) also recommended applying entomopathogenic nematodes in September to non-cropped areas of a nursery, including soil beneath hedges, where weevils were found in the summer, in addition to the infested crop plants.

Drenches

The most successful reports of using entomopathogenic nematodes are when they are applied as a drench, to the surface of individual potted plants in substrate, particularly in studies where temperature could be controlled. For example successful control has been reported on pine seedlings (5,000 IJs per plant at 22-25°C and 15,000 IJs per pot at 20°C) (Georgis and Poinar, 1984; Rutherford *et al*., 1987), *Taxus baccata* (154,000 IJs per plant at 18°C) (Backhaus, 1994), *Impatiens wallerana* (8,000 IJs per plant at 18°C) (Bruck *et al*., 2005), cyclamen (25,000 IJs per pot) (Godliman, 1991), and strawberry plants (5,000 IJs per plant at 20°C) (Rutherford *et al*., 1987).

Surface drenches by hand have also provided good results on field grown strawberries. In a field of strawberries heavily infested with vine weevil larvae, a drench of 240,000 *Heterorhabditis sp.* in 100ml per plant (soil temperature 13°C at application) gave 94% control after 21 days (Backhaus, 1994). However, this process is labour intensive and the presence of polythene mulches can make application difficult to target drenches in the planting holes. It has been suggested that straw mulches are less of an issue as providing there is sufficient irrigation following application to facilitate the movement of nematodes down to the soil, therefore the nematode drenches can be applied over the straw (Shearer, 1999; Wilson *et al*., 1999). However, in the UK most growers use polythene mulches on strawberry and straw is only used by some growers in the alleys.

It is also recognised that achieving control from drenches is often more successful in substrate in grow-bags or troughs than in field soil as maintaining a constant level of moisture which the nematodes require is more difficult in the field (Raffle, 2003).

Some studies have attempted to adapt the standard drenching method to see how the delivery of nematodes to the roots could be improved. In one experiment *H. bacteriophora* was stored in rubber foam and 25 cc of foam (approximately containing 300,000 IJs) was watered into the substrate of containerised rhododendrons during March (soil temp 16-24°C) using 500 ml per container (Barratt *et al*., 1989). While this method gave 93% control, it is difficult to determine whether this was due to the method used or solely to the high number of IJs applied. It is unknown whether this method of placing a nematode sponge on the surface of each plant could be combined with using overhead irrigation to drench them into the soil, as it would be less labour intensive compared to a standard surface drench to individual plants. A second study evaluated applying *S. feltiae* via an injection to the roots of the plant, however this was no more effective than a standard surface drench and would be more time consuming (Mráček *et al*., 1993).

Using a compression sprayer to apply nematodes is also an option which has been found to be successful. Between 90-100% control of vine weevil larvae was achieved under standard nursery conditions using *H. bacteriophora* at a rate of 66,000 nematodes per pot on *Bergenia cordifolia* (soil temperature at application 23°C) when applied to the substrate (mixture of peat moss, composted leaves, sand, and pine bark) (Gill *et al*. 2001). However, Godliman (1991) showed that when nematodes were sprayed over cyclamen plants the nematodes did not penetrate the leaf canopy in sufficient numbers and a drench of *S. bibionis* was significantly more effective. Therefore application methods which target the soil should be used where possible. This problem of achieving effective application to the substrate is commonly experienced by growers of containerised ornamental crops, particularly when the foliage is dense and the plant canopy covers the surface of the growing medium. In these circumstance, irrigation is recommended by suppliers to help to wash the nematodes from the foliage to the substrate, but the run-off often goes onto the floor or bench if the foliage overhangs the sides of the pots. Developing a specialised calibrated applicator for drenching containerised ornamentals more effectively would be useful.

Application via irrigation systems in soft fruit

For soft fruit growers the least labour intensive method of applying nematodes is to apply them via irrigation water using drippers onto the substrate or T-tape® trickle lines buried in the soil in raised beds. These systems are widely used by UK growers in substrate-grown soft fruit crops in grow bags, troughs or pots. Using sufficient drippers close to the root zone and ensuring that supplier recommendations are followed are key to success. Use of dripline systems in soil-grown crops is less successful than in substrate crops due to variable soil types, the larger root zone to target and inconsistent moisture levels.

Currently when using these systems growers are advised to remove filters to avoid the nematodes backing up (Irving *et al*., 2012). When *Steinernema carpocapsae* and *H. megidis* were passed through the system consisting of filters, lines and outlets, their viability was acceptable but numbers were reduced by 3% and 14% respectively (Kakouli-Duarte *et al*., 1997). This suggested that *H. megidis* was more susceptible and the authors proposed this was due to its larger size. The study then went on to evaluate the distribution of the IJs via single line and double line T-tape® systems. Kakouli-Duarte *et al*. (1997) monitored the distribution of *S. carpocapsae* following application through a single line T-Tape® at a 10cm depth beneath the soil (dose 4 billion nematodes per ha) using the fertigation unit feeding double row raised beds. Following nematode application, samples of soil were taken and baited with *G. mellonella* larvae. The results showed that the distribution of the nematodes was poor with nematodes failing to move towards the roots of the plants. Furthermore nematode numbers declined as the distance (100m length investigated) along the bed from the supply line increased, possibly due to low water pressure. It was also speculated that the nematodes were settling on the lower side of the T-tape which prevented the nematodes reaching the end of the beds.

When a double line T-Tape® system (*S. carpocapsae* used at 2.5 billion IJs ha-’m - soil type sandy loam) was evaluated the study again found that while the numbers of nematodes declined as the distance from the supply line increased (250 length investigated), the distribution of the nematodes in the bed was improved with the nematodes being effectively distributed along and across the beds. Variation was observed between beds and sampling positions which is likely due to the nematodes not being uniformly mixed in the irrigation water passing through the system.

A similar study evaluated the delivery of *H. heliothidis* using a trickle system at a dose of 48,000 and 80,000 IJs per plant in spring (predominantly <15°C) (Curran and Patel, 1988). Again this study found that there was variation in the distribution of the nematodes but only at the lower dose and as the water volume delivered was consistent it was attributed to non-uniform mixing in the water passing through the system. At the lower dose control of vine weevil larvae/pupae was 59% and at the higher dose, control was unexpectedly lower at 25%. The study also confirmed that <1% of the nematode were retained by filters suggesting they can be left in. Product leaflets of currently available nematode products usually advise removing fine filters; BASF recommends removing all filters of 18 mesh or finer from irrigation systems, Flowering Plants Ltd recommend cleaning and preferably removing filters, e-nema recommend removing all fine filters in tubing and nozzles, Koppert recommends removing all filters to avoid blockages and Syngenta Bioline recommend removing all filters including those in nozzles of the sprayer. Other recommendations to improve application through trickle irrigation systems are to calibrate the rate carefully, continuously agitate the supply tank when mixing and during application and use sufficient water pressure to maintain the flow rate throughout the lines. With dripper systems, dye can be added to the irrigation water or nematodes can be collected from a sample of drippers to check for even distribution and for any blockages.

Little published literature is available on using dripline irrigation methods for containerised ornamentals although one study found that applying *Heterorhabditis* spp. in the irrigation water (method not specified) gave 100% control of vine weevil infesting protected potted *Aralia* (Deseö and Costanzi, 1987). Although some suppliers recommend that dripline irrigation systems can be used in containerised ornamentals, setting these systems up is not cost-effective on pots smaller than 5 litres and they are not widely used by HNS growers (J. Atwood, personal communication).

Comparison of different application methods

When comparing a range of nematode application methods in a strawberry field on loamy sand soil, Curran (1992) found that using a back-pack sprayer to spray under the mulch and around the crown of the plant was the most effective method giving 86% control of vine weevil larvae at a dose of 100,000 *Heterorhabditis* sp. (species not reported) per plant.

Single and multiple injections gave 63% and 79% respectively and involved using a steel lance to inject nematodes at a 10cm depth. Using the trickle irrigation gave 65% control.

Studies have also been carried out on other fruit crops besides strawberry. For blackcurrant a high volume drench of an alginate gel formulation of *S. carpocapsae* applied via commercial spray equipment to the base of blackcurrant bushes on each side gave 34-66% control of vine weevil larvae when soil temperatures were above 12°C on a range of soil types (Sampson, 1994). The low level of control was attributed to the difficulty in applying nematodes to all of the root zone and the large root ball of the plant where the larvae could burrow and be less accessible to the nematodes.

A study carried out on cranberry, raspberry and strawberry sites compared the success of applying nematodes using a watering can, a portable pump sprayer and a temporary dripline using a Mini-Dos water powered injector (Booth *et al*., 2002). All of the methods used resulted in the delivery of viable nematodes and the dripline and portable sprayer gave uniform distribution. The water powered injector was slow but more compatible injectors could have been used.

Hayes *et al* (1999) evaluated a simple sprinkler irrigation system and boom sprayer to apply nematodes to cranberries. While there was no effect on nematode viability or infectivity of the IJs with these systems, the pattern of nematode distribution was uneven. The authors proposed that these results were due to low water pressure with the sprinkler not reaching the outer edges of the plot and the results with the boom sprayer being influenced by tractor speed and poor coverage by the selected nozzles.

In one study where nematode performance was reduced in the field compared to in containerised plants, antagonism in the soil from other microorganisms was suggested as a possible cause, however there was no reference to the irrigation regime (van Tol, 1993a).

Potential of pre-planting treatments

Growers of ornamentals commonly drench trays of either plugs or liners with nematode before potting up, if vine weevil larvae are found in the substrate of young plants, or as an ‘insurance’ treatment just in case undetected vine weevil infestations are present. Susurluk

& Ehlers (2008) evaluated a novel approach of dipping the roots of cold stored strawberry plants into a nematode suspension as a potential preventive method to avoid the need for drenches post planting. The nematode suspension used contained 1,500 *H. bacteriophora* IJs per ml supplemented with carboxy-methyl-cellulose which improved the attachment of the nematodes to the roots and prevented sedimentation. The study reported that each plant dipped into the suspension absorbed 4-5ml of the solution which is the equivalent of 6,000-7,500 IJs per plant. In pot trials in this study, vine weevil eggs were added monthly for three months and mortality of vine weevil larvae varied between 90 and 96% in two trials (controlled conditions 18-26°C). This study suggests that the addition of carboxy-methyl-cellulose supports nematode survival so that they can survive the time between application and planting by giving them sufficient moisture. However, both the infectivity of mealworm larvae used to monitor efficacy and the numbers of IJs decreased dramatically two weeks after application. Infectivity of mealworm larvae decreased from 75% after one day to approximately 20% after two weeks and to 10% after 12 weeks following application. The numbers of IJs recovered by baiting the soil with *G. mellonella* larvae dropped from 2,500 after one day to approximately 1,000 by week two and 400 by week 12. Despite this, 12 weeks following infestation of the vine weevil eggs in this study only 30% of the plants treated with nematodes died compared to 90% of the control plants. A similar study evaluated whether strawberry modules could be drenched 1-2 days before planting (early August). Following module drenches of either *S. feltiae, S. carpocapsae* and *Heterorhbditis* sp. (species not reported) there was no significant reduction in the number of vine weevil larvae compared to in the controls (N.B. in this experiment only a small proportion of the inoculated eggs survived) (Cross and Burgess, 1997). Currently, the only commercial nematode product leaflet which provides information on dipping frigo plants pre-planting is Larvanem (*H. bacteriophora*). This method is worthy of further discussion with suppliers and possible further development.

Application methods: key points and gaps in knowledge:

- Growers should follow all supplier's application recommendations carefully. Some growers are unaware of all the information available on optimum application techniques and this should be addressed in knowledge transfer activities.
- Application of nematodes to soft fruit crops via dripline irrigation is widely used by UK growers of soft fruit grown in substrate (bags, troughs or pots). Trickle irrigation using T-tape lines buried in raised beds in soil-grown strawberry are less effective, although using two lines per bed is better than using one.
- Drenches of nematodes to field-grown soft fruit crops is less effective and little-used.

- Nematodes are applied using drenches to ornamental crops. This is labour-intensive and can be inefficient and ineffective, when closely spaced plants with dense foliage are drenched and most of the nematodes can end up on the floor rather than in the substrate. Development of a specialised calibrated applicator for drenching containerised ornamentals more effectively would be useful, as dripline irrigation is not cost-effective in the production of most ornamental crops and is not widely used by HNS growers.
- Further discussion on the potential for pre-planting dipping of cold-stored strawberry plants in a nematode suspension or specialised formulation is worthy of further discussion with suppliers to investigate whether further development work is justified.

Timing and numbers of applications

Application of IJs must coincide with the presence of the susceptible life stages and when temperatures support infection for the particular nematode species or strain being used.

Most nematode species are recommended by suppliers and advisers to be applied in August to early September for vine weevil control, when the majority of eggs have been laid, larvae are present and temperatures are still 12°C (Buxton, 2003; Raffle, 2003; Irving *et al*., 2012). Control with a single application will not provide 100% control and repeat applications should be considered (Irving *et al*., 2012). A second application a few weeks after the first is thus often made against any surviving larvae if temperatures are still suitable. *S. kraussei* (Nemasys L, Exhibitline sk) can be used at temperatures as low as 5°C so can be used in October or even November for late applications. However, ideally, applications should not be delayed until then as this can allow larvae to burrow into the crown of plants where they cause severe plant damage and can also be protected from nematodes. Growers are also recommended to consider applying nematodes again in the spring against any surviving larvae which have overwintered but before pupae emerge as adults. Most nematode products are recommended to be used in a narrow window in April to early May although *S. kraussei* can be used as early as March if temperatures are above 5°C.

In glasshouses heated to suitable temperatures the timing of application is more flexible as IJs can be applied at any time when the susceptible stages are present. Studies looking at the timing of applications in heated glasshouses confirmed that the best results are obtained when nematode applications are timed at a specified time after egg hatch and not when vine weevil are laying eggs. For example, applying Nemasys H (*H. megidis* at the time) to cyclamen at 17,000 nematodes per pot six weeks after egg inoculation gave better control (mean of 1.3 larvae per pot) than application at potting on (mean of 9.1 larvae per pot) in a glasshouse at 25°C (Ellis and Emmett, 1993). On potted strawberry in a glasshouse (>18°C) the application of nematodes at 100 nematodes per cm^2 one to two weeks after egg infestation gave better results than 0 or 5 weeks after infestation (Simons, 1981). In a further experiment by Simon (1981), 50 and 100 nematodes per cm^2 applied three weeks after infestation gave good control on strawberry and cyclamen. In practice however, vine weevils lay eggs over an extended period so egg hatch is not synchronised as in these studies. For practical application by growers, timing can be aided by checking for larvae around the roots.

In a study by Lola-Luz and Downes (2007), single, double and triple applications of nematodes were evaluated in two experiments, one in a polytunnel and one in a glasshouse. Both studies showed that with each additional application the number of vine weevil larvae decreased further, for example in a polytunnel a single drench application of 25,000 *H. megidis* in 50ml of water per plant in mid-September, early October and March reduced numbers of larvae to 1.8 per 20 plants, 0.2 per 20 plants and 100% kill respectively (in March the controls had 8.2 larvae per 20 plants). These results support the multiple application of nematodes when cost-effective.

In the field the timing of nematode applications is far more complex. The first study on application of nematodes through dripline irrigation in the UK compared applications of nematodes to strawberry plants in double row raised beds via a double line T-tape® irrigation system in late summer (early September) and late spring (early May) (Kakouli-Duarte *et al*., 1997). A rate of 7.6 billion *S. carpocapsae* IJ per ha applied in early September gave 49.5% control of vine weevil larvae (soil temperature 18.8°C) and the early May application at a rate of 6.08 billion IJ per ha gave 65% mortality (soil temperature 13.1°C). The low mortality achieved in the early September trial was attributed to the high rate of IJs used which may have led to clumping in the system but also to the younger larvae being less susceptible to *S. carpocapsae*. The early May application is likely to have been better due to the lower dose of IJs, reduced clumping and the presence of the more susceptible late larval stage. This study also demonstrated that using a double line of T-tape® was more effective than a single line, giving better distribution of nematodes along and across raised beds and placing them close to the root zone where vine weevil larvae feed.

Many other trials have also evaluated the benefit of double and triple applications of nematodes and the most suitable timings.

In field-grown strawberry in Ireland, single drench applications of 25,000 *H. megidis* (strain UK211) per plant in 50ml of water to the crown of the plant in September and October were compared with a double application (September and October) and a triple application (September, October and April) (Lola-Luz and Downes, 2007). Temperatures were 17.7, 13.3 and 10.1°C on application dates in September, October and April respectively. In both the single nematode applications vine weevil larvae were reduced from four per 20 plants in the controls to approximately two per 20 plants. In the double application this was reduced further to 1.6/20 plants and the triple application reduced numbers to 0.4/20 plants. Little difference was observed between single and double applications in September and October but the spring application was significantly effective. It also appeared to make no difference whether the single application was applied in September or October which has also been confirmed in a study using the same strain of *H. megidis* by Fitters *et al*. (2001b).

Multiple applications (double in Norway and triple in Ireland) were also found to be most effective in a study comparing single nematode applications of *S. kraussei* and *H. megidis* with multiple applications at various timings (e.g. autumn, summer, summer+autumn or late autumn+spring) in strawberry fields in Ireland and Norway (Haukeland and Lola-Luz, 2010).

Further studies conducted, again on strawberries, but grown in 40 L peat grow-bags on raised beds naturally infested with vine weevil (Lola-Luz *et al*., 2005) were also evaluated for the benefits of multiple applications. Application of *H. megidis* (strain UK 211) at 25,000 nematodes in 55ml of water per plant were applied either as a single application (May), a double application (May and October) or a triple application (May, October and May). The results showed that *H. megidis* following a single, double or triple application gave 93.4%, 78.9% and 93.7% control respectively. Average soil temperatures in May 2001, October 2001 and May 2002 were 12.3, 11.9 and 12.5°C respectively. On blackberry, Sampson (1994) confirmed that in severe infestations there is a benefit to applying two treatments, one in autumn and another in the spring when soil temperatures exceed 12°C. Collectively these studies suggest that in the field more than one application of nematodes should be considered for improved control.

Recent advice given by one supplier to strawberry growers in Scotland has been to use a ‘little and often’ approach for nematode application through dripline irrigation for vine weevil control, after unreliable control had been given by growers applying the label-recommended dose at the ‘normal’ timings in early autumn and late spring (Syngenta Bioline, personal communication). See next section ‘Rate of application’ for further details.

Finally, late application of entomopathogenic nematodes in autumn when temperatures are below those suitable for infection should be avoided. Schirocki & Hague (1996) found that when vine weevil larvae infected with *S. carpocapsae* were stored at temperatures unsuitable for infection, over time mortality of the nematode IJs increased and when the vine weevil larvae were returned to warmer temperatures mortality never exceeded 50%.

***Nematode timing: Key points and* gaps in knowledge:**

- The literature supports current recommendations regarding the timing of application of entomopathogenic nematodes in the field (autumn and spring).
- However, see next section regarding recent use of a ‘little and often’ approach for control of vine weevil on substrate-grown strawberries in Scotland.
- Two consecutive applications in the autumn should be considered for improved control, but care should be taken to make applications when temperatures are suitable for the nematode species being used, and not to delay the second application until plant damage has occurred.
- When applying nematodes in the spring for control of overwintered larvae, careful timing is needed i.e. to wait until temperatures are suitable for the nematode species used but before pupae emerge as new adults.

Rate of application

In most of the research carried out on applying IJs, high doses have been used which would not be economically viable for growers. The literature available demonstrates that determining the optimum dose is difficult due to other influencing factors such as temperature, crop and soil type.

Field studies have found that using too high a rate can result in clumping of the nematodes in the sprayer or irrigation system. In field-grown blackcurrants and strawberries a rate of 5 billion *S. carpocapsae* per treated hectare gave the most consistent results against vine weevil when using a commercial sprayer, except at two sites which had sandy and silty soil respectively, where a rate of 2.5 billion per hectare gave comparable results (Sampson, 1994). Water volumes for strawberry were 100ml per plant and those for blackcurrants were 1500 -2000ml per plant. When deciding on a dose growers should therefore take into the account the soil type and whether it facilitates the movement of the nematodes. If the soil does not facilitate IJ movement it is likely that it will require a higher dose compared to that used on sandy soils.

When using a T-tape irrigation system, rates of 5 billion IJs per treated hectare also appear to be suitable. In a late spring application (early May) via a double T-tape irrigation system *S. carpocapsae* and *H. megidis* were compared and applied at a rate of 6.08 and 1.215 billion nematodes per ha respectively. *S. carpocapsae* caused 65% vine weevil larvae mortality while *H. megidis* caused only caused 26% mortality which is likely due to the differences in dose rate (Kakouli-Duarte *et al*., 1997). Rates of 0.5 and 1.0 million *H. heliothidis* per m2 were used in another study and reduced vine weevil larvae by 90% with a moist soil and >12°C soil temperature. However, details on soil type or application method were not available for comparison with the above studies (Scherer, 1987).

For currently available commercial nematode products, recommended rates are given in numbers per unit area or numbers per pot or plant. For vine weevil control on strawberry, Exhibitline sk, Nemasys L, Nemasys H and Nematop are all recommended at 25,000 IJs per plant. Other products give a recommended rate per unit area for vine weevil control on any crop - Larvanem is recommended at either 500,000/m^2 or 1,000,000/m^2 depending on the scale of the infestation, and SuperNemos is recommended at 500,000/$m^{2.}$ It would be useful for growers if the units that the recommended rates are expressed in were standardised between products to avoid confusion, particularly for use on substrate-grown strawberry, where numbers of plants per grow-bag and therefore per unit area are variable between growers. Research by Lola-Luz *et al*. (2005) supports a rate of 25,000 *H. megidis* per plant for strawberries grown in grow-bags, as applying surface drenches to each plant at this rate in May resulted in 93.4% control. Good control, with efficacy increasing with the number of applications, was also observed in further studies on field-grown strawberries at this rate (Lola-Luz and Downes, 2007). More recently a study was carried out by ADAS which compared the efficacy of currently available nematode products for the control of vine weevil (Hough and Bennison, 2014 and Hough *et al*., in press). At a drench rate of 25,000 IJs per plant Nemasys L (*S. kraussei*), Larvanem, Nematop and Nemasys H (all *H. bacteriophora*) significantly reduced the number of live vine weevil larvae per coir grow-bag to 1.5, 7.5, 16, and 20 respectively compared to the two controls which had 40 and 44 live larvae per grow-bag. Nemasys L and Larvanem were equally the best performing products. The above studies suggest that individual plant drenches of 25,000 per plant can provide acceptable levels of control. Other studies have tested higher rates such as 125,000 IJs per plant which were also successful (Fitters *et al*., 2001b).

For each nematode product available, one rate is recommended on the supplier leaflet for strawberry regardless of the application method. No information was found in the literature to determine whether the control achieved when using this single rate differs depending on whether the plants are drenched or whether irrigation systems are used for application. However, recent advice given by one supplier to strawberry growers in Scotland has been to use a 'little and often' approach for nematode application through dripline irrigation for vine weevil control, after unreliable control had been given by growers applying the label-recommended dose at the 'normal' timings in early autumn and late spring (Syngenta Bioline, personal communication). This strategy has been to apply *S. kraussei* at one fifth of the recommended rate (5,000 per plant) each month, through dripline irrigation to substrate-grown strawberry, although higher rates than this are sometimes used depending on the number of vine weevil larvae present and the time of year. Grower feedback has been that this strategy has given more reliable control of vine weevil, while maintaining the cost of nematodes at an acceptable level (see Task 1.1 in this report). The reason for the improved control could be that nematodes are maintained in the substrate throughout the year and thus give control of overlapping generations of vine weevil in polytunnels and glasshouses. Research is justified on validating this 'little and often' approach compared with application in autumn and spring at full recommended rate.

Studies have tested different doses of nematodes against vine weevil on container-grown ornamentals.

On yew, *Taxus baccata*, the dose of *Heterorhabditis* sp. was investigated at a soil temperature of 17°C. At a dose of 10,000 nematodes per plant (600,000 nematodes per

m^2) vine weevil larvae was adequately controlled and at higher doses than this, no significant differences in control was observed (Backhaus, 1994). Other studies have shown that rates of 10,000 IJs per plant have been effective on container grown azaleas using *S. carpocapsae* (68% control) and *H. bacteriophora* (83% control) (Cowles, 1997) and also rates of 15,000 per plant when using *S. feltiae* (100% control) (Mráček *et al*., 1993). These studies suggest that applications of IJs at around 10,000 per pot on these crops can give acceptable control.

A range of other doses have also been evaluated. In a greenhouse experiment (at 20°C) the doses of *S. carpocapsae* strain S25 (Exhibit) and *Heterorhabditis* sp strain HF85 (Optimaaltijes) were evaluated on cyclamen plants (in 250ml pots) (Miduturi *et al*., 1994a). The study found that as the dose of IJs increased so did vine weevil mortality. Control provided by *Heterorhabditis* sp. increased from 70 to 95% using nematode rates of from 1000 to 30,000 per pot after 10 days. A dose of 1000 nematodes per pot gave 100% mortality after 15 days. *S. carpocapsae* increased from 35 to 95% control using nematode rates from 1000 to 30,000 per pot after 10 days and a dose of 30,000 IJs per pot gave 95% control after 20 days. When the same species were tested in a field of cyclamen plants in clay loam soil (soil temperature between 10-15°C) at doses of 1000, 10,000 and 100,000 per plant mortality of large larvae was low and did not exceed 20% using *S. carpocapsae* and 40% using *Heterorhabditis* sp. after 15 days.

Another glasshouse study compared a high dose (30,000 per plant) of *S. feltiae* and *H. heliothidis* with a low dose (15,000 per plant). The study found that at a higher dose there was 71 and 75% control of vine weevil larvae on an unreported crop in two separate trials using *S. feltiae* and 85 and 89% control by *H. heliothidis*. A lower dose of 15,000 IJs per pot resulted in 71 and 75% control by *S. feltiae* in two trials and 64 and 90% control by *H. heliothidis* in two trials (Stimmann *et al*., 1985). Even lower rates of 5,000 *H. bacteriophora* (Cruiser) per pot have been evaluated on vine weevil-infested *Heuchera micrantha* (soil temperature at application 17°C) and *Epimedium x rubrum* (soil temperature at application 15°C) in a glasshouse which gave >90% control (Gill *et al*., 2001). These studies demonstrate that rates of 5,000-30,000 nematodes of various species per pot can give effective control of vine weevil.

For container grown plants kept outside, multiple applications of IJs gave acceptable results although this may not be a viable option for all growers, as most containerised hardy nursery stock are watered using overhead irrigation rather than by dripline irrigation, thus nematodes are usually applied using drenches, which are very time-consuming. A study looking into multiple applications found that when *H. bacteriophora* IJs were applied in mid-July to *Hucherella alba* in pots buried in the soil and infested with vine weevil eggs at the end of July and August, 96.5% control was achieved by repeating applications every two weeks for four applications at a rate of 2000 yd^2 (equivalent to 1672.3 m^2) (Swier *et al*., 1998).

For the current commercially available products the current rates recommended for pots and containers are; Nemasys L and Nemasys 0.5 million per m^2, Nematop 10,000 per pot, SuperNemos 500,000 per m^2 and Larvanem either 500,000/m^2 or 1,000,000/m^2 depending on the scale of the infestation. Some suppliers also provide numbers of nematodes per pot. Exhibitline SK (*S. kraussei*) is recommended at 7,692-20,000 per pot depending on pot size (1-5 litres) and Exhibitline H (*H. bacteriophora*) is recommended at 23,810-78,740 per pot depending on pot size (1-5 litres). Nematop (H. bacteriophora) is recommended at 8,000-50,000 per pot depending on pot size (0.8-5 litres). SuperNemos (mix of *S. carpocapsae, S. feltiae* and either *H. bacteriophora* or *H. megidis*) is recommended at one pack of 12,000,000 per 200 pot plants therefore 60,000 nematodes per pot.

Rates of application: Key points and gaps in knowledge:

- Effective nematode rates vary with species, soil or substrate type, crop and pot size.
- Rates used in different experiments in the literature do give indications of suitable rates for various nematode species (many not currently available) but it is difficult to use this information to represent current commercially available species.
- When using the literature to compare rates and timing information for currently available products it is important to remember that the name of the product has often remained the same, but the species in the product has often changed over time.
- Using nematodes at too high a rate (7.6 billion IJ per ha) via irrigation systems can result in poor control due to the IJs clumping together.
- Currently recommended rates on strawberry (25,000 per plant or 0.5-1 million per m^2 depending on product, are supported by research.
- However, the ‘little and often’ approach advised by Syngenta Bioline to strawberry growers in Scotland justifies validation compared with application at full rate in autumn and spring.

- Relatively few studies have been done on comparing dose rates in containerised ornamentals using currently available products and further research is justified. In addition, more practical and effective application techniques for use in containerised ornamentals are needed, as very few of these crops are irrigated using drip irrigation and thus application needs to be made using drenches which can be difficult, inefficient and time consuming (see Task 1.1, interviews with growers).

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Biological control with entomopathogenic fungi

Entomopathogenic fungi: biology, life cycle and use in biological control

Entomopathogenic (meaning “insect pathogenic”) fungi (EPF) are naturally widespread in the environment, particularly in the soil. About 750 species of EPF are known to science (Hawksworth *et al*., 1995), although evidence emerging from recent DNA diversity studies suggests that this is a significant under-estimate. Most of the EPF occur within the taxonomic phylum (group) Ascomycetes. Each EPF species in this phylum consists of thousands of genetically distinct strains, each with slightly different traits such as host range, temperature preference etc. They cause lethal infections in a range of insect and mite species and can be used as biological pest control agents.

The life cycle is basically the same for all EPF species. They produce spores that have the ability to adhere to, germinate on, and penetrate insect cuticle. Growing fungal hyphae then enter the insect haemocoel (the space between the organs) and overcome the insect immune system using a variety of enzymes, immuno-suppressors and other tactics. Insect death occurs between 3 – 10 days depending on the species of EPF and insect / mite host, the dose of spores applied, and the environmental conditions. Once the insect / mite has died, the fungus grows out through the body to produce a new generation of spores. The spores of most Ascomycete EPF can be produced on a factory scale using standard fungal culture technologies. The spores can then be formulated in a variety of ways including as granules, powders or liquid suspensions, and these are then applied to the crop for pest control. This application method is analogous to using a chemical pesticide, and for this reason EPF and other microbial natural enemies are often referred to as “biopesticides”. This term is a convenient label but it can detract from thinking about these agents as living organisms and give false expectations of chemical-like performance. At least 170 different EPF plant protection products have been developed in different regions of the world since the 1960s, and about 75% of these products are currently “active”, in the sense that they are commercially available, authorized or undergoing authorization for use (Faria & Wraight, 2007).

Although the ascomycete EPF are widespread in the natural environment in the UK, they generally do not cause natural disease outbreaks in pest populations. In the case of vine weevil, natural EPF infections are occasionally seen in individual larvae or adults, and EPF strains that are pathogenic to vine weevil larvae can be isolated easily from soil (e.g. Bruck, 2004). However we have not identified any cases where a natural fungal outbreak has killed an entire weevil population. The aim of the “biopesticide” approach is to apply much higher EPF spore concentrations than occur in nature and to use particular fungal strains that have characteristics suited for biocontrol, such as high virulence to the target pest. It has been found recently that some species / strains of EPF have potentially useful traits that have not been utilized much in biological control, including endophytism (the ability to grow inside plants) and rhizosphere competence (the ability to grow in the root zone, which usually involves some form of chemical signaling between the plant and the fungus), and ways are now being developed to incorporate EPF with these traits into biocontrol programmes (Vega *et al*., 2009).

Reviews on the safety of EPF show that they are not toxic to humans (Zimmermann, 2007) and they should qualify as low risk substances under updated EU pesticides legislation (Chandler *et al*., 2011). The use of EPF products for crop protection is regulated according to EU pesticides legislation which includes a detailed evaluation of their safety to people and the environment. The costs associated with this authorization can be a hurdle to getting new EPF biopesticides commercialized in the EU, which may help explain why Europe has lagged behind other regions of the world in the commercial development and use of microbial biopesticides (for example, only 12% of all fungal biopesticide products have been developed in Europe (Faria & Wraight, 2007)). However, new procedures have been introduced in the EU that are intended to make authorization of new products faster and less expensive (Chandler *et al*., 2011).

Fungal control of vine weevil

Our literature review identified 89 scientific articles on use of EPF for the biocontrol of vine weevil, including original research published in peer review journals, project reports (for example HDC reports) and reviews. These articles showed that EPF can be effective biological control agents of vine weevil. Vine weevil larvae have been shown to be more susceptible to fungal infection than the adults, and hence most of the research has been about targeting EPF for control of larvae. The research done to date has used five EPF species, *Metarhizium anisopliae* (also known as *Metarhizium brunneum*, see below), , *Beauveria bassiana*, *Beauveria brongniartii*, *Isaria farinosa*, and *Isaria fumosorosea* Of these, most of the research has used *M. anisopliae*. Different strains of these fungi vary in their ability to kill vine weevil, which is measured in terms of the speed of kill and also the amount of spores needed to cause death in the insects. A large number of the papers were concerned mainly with quantifying the effect of different EPF species / strains against vine weevil in laboratory, small-scale greenhouse or field plot experiments (e.g. Poprawski *et al*., 1985; Beck, 1992; Moorhouse *et al*., 1990, 1993a, b; Booth & Shanks, 1998; Booth *et al*., 2000). Some more detailed studies have also been done to develop particular EPF strains as practical control agents of vine weevil, for example measuring persistence of activity, effect of temperature on efficacy, or use in a potential IPM programme. The earliest work of this type was published by Moorhouse and colleagues in the early 1990s from the UK Glasshouse Crops Research Institute. At the same time as this, Bayer developed the first commercial EPF product for vine weevil control, BIO1020, which was based on *Metarhizium anisopliae* strain F52 (subsequently renamed *M. brunneum*, see below). BIO1020 was later withdrawn from sale, most probably because it could not compete on price with the chemical insecticides that were available at that time. However the same strain of fungus used in BIO1020 was later developed into the product Met52 which is now sold in the UK and elsewhere for vine weevil biocontrol. Detailed investigations on this fungal strain have been published by Denny Bruck and coworkers at the US Department of Agriculture Horticultural Crops Research Unit in Oregon USA, and by Tariq Butt and coworkers at the University of Swansea UK.

Use of *Metarhizium* strain F52 for vine weevil management

Met52 is produced in the USA by Novozymes BioAg Group and distributed in the UK by Fargro Ltd. It is based on the F52 fungal strain of *Metarhizium anisopliae*. The strain is also referred to in the scientific literature by other code numbers (Ma43, 275.86, ATCC90448, ARSEF1095). The F52 strain was found to be one of the most effective against vine weevil in screening programmes (e.g. Moorhouse *et al*., 1990) and it is also active against a range of other pest species such as thrips. Therefore, a lot of the more recent research published in the literature has tended to focus on this particular strain. Because Met52 is the only available product we have concentrated mainly on this for the rest of the review.

Metarhizium anisopliae has recently undergone a taxonomic revision, with a number of new species names being assigned (Bischoff *et al*., 2009). The *Metarhizium anisopliae* F52 strain used in Met52 has now been reclassified into a new species, called *Metarhizium brunneum*. Over time, the "correct" species name of *M. brunneum* for F52 is likely to become widespread but – at the present time - most people still refer to it as *M. anisopliae*. The F52 strain is referred to as both *M. anisopliae* and *M. brunneum* in the scientific and grower literature. We have used the term *M. brunneum* F52 for the rest of this review.

The Met52 product is available as a granular formulation. This is produced by growing the fungus on sterilized rice grains on an industrialized scale. The fungus mycelium colonizes the grains and produces copious amounts of spores on them. The resulting granules (comprised of fungal mycelium and spores on the rice grains, which develop the characteristic green colour of *Metarhizium* spores) are dried, pulverized, stabilized and packaged. They are applied by mixing into the plant growth medium for control of vine weevil larvae. Met52 can be bought either as the granules themselves (to be mixed into growing media by the grower) or as substrate that has had the granules already incorporated into it. Mixing into the compost causes spores to break off the rice grains and they become distributed through the growing medium. Spores adhere to the cuticle of vine weevil larvae and cause an infection. Larvae will die if they have acquired a sufficient number of spores, known as the "lethal dose". The fungus will continue to grow on the rice grains and produce more spores in the growing medium. The product contains a minimum of 9×10^{11} spores (measured as colony forming units, cfu) per kg. The recommended application rate is 0.5 kg granules per m^3 of growing media / soil. This is equivalent to a concentration of 4.5×10^5 spores per ml. For field crops, the recommended rate is 122 kg per ha, equivalent to 1.1 x 10^{14} spores per ha. These rates have been shown to be effective in peer review studies (see below).

Nearly all the research on using EPF for vine weevil control has targeted the larval stages for control, as these are more susceptible to fungal infection than the adults. All larval instars are susceptible to the fungus, with the smaller instars probably being the most vulnerable because of their smaller size. Early larval instars decay rapidly after death and so are usually not visible in the growing medium (Bruck and Donahue, 2007). Pope *et al*. (2013 and ongoing research) have investigated the potential to use the aggregation behavior of adult weevils during the daytime as a way of treating them with EPF, with the possibility of dispersal of spores throughout a weevil population as a result of weevil movement between refugia. In this research, eight strains of fungi from three different species (*B. bassiana*, *M. brunneum* and *M. anisopliae*) were evaluated in laboratory bioassays against adult vine weevils. All of the fungi were pathogenic (Pope *et al*., 2011) although the rate of death was slower than that observed with weevil larvae. In a current follow-up Defra project, *B. bassiana* and *M. brunneum* are being compared for use in refuge traps for adult vine weevil control in laboratory, semi-field and commercial soft fruit or hardy nursery stock conditions (Bennison *et al*, 2013).

Factors affecting the effectiveness of Met52

Met52 is a living organism, and its activity is dependent on the spores being alive and viable. Infection will only occur if vine weevil larvae are able to acquire spores on their cuticles from the growing media. The activity of the fungus is also dependent upon favourable environmental conditions, particularly temperature. The scientific evidence shows that, when these conditions are met, then Met52 can give excellent control of vine weevil larvae. However, because it is a living organism, it is unlikely to be as robust or as reliable in its effectiveness in varying environmental conditions as a broad spectrum synthetic chemical pesticide.

<u>Efficacy of Met52 on different plant species</u>

Experiments have shown *M. brunneum* F52 / Met52 to have good levels of efficacy against vine weevil larvae on strawberries and a range of protected and outdoor ornamental plants (e.g. Reinecke *et al*., 1990; Stenzel *et al.,* 1992; Stenzel, 1994; Ellis & Emmett, 1993; Moorhouse *et al*., 1993c; van Tol 1993a, b; Bruck, 2007; Fisher & Bruck, 2008; Ansari &

Butt, 2013). Moorhouse *et al*. (1993c) evaluated the effect of *M. brunneum* F52 as a drench application of a spore suspension, against vine weevil larvae on a range of ornamental pot plant species in a glasshouse (minimum temperature 15°C), and high levels of control were observed in all cases: Begonia, Coleus, Cyclamen, Dianthus, Gazania, Impatiens, Kalanchoe, Pelargonium, Primula, Sinningia. Differences were noted in the number of vine weevil larvae surviving on different plant types, with better larval survival on primula than on Impatiens, Cyclamen, Campanula and Begonia in the absence of a fungal treatment. Similar work was also done (Moorhouse *et al*., 1993d) evaluating *M. brunneum* F52 as a drench against vine weevil larvae on 27 different hardy nursery stock species grown outdoors. The fungus was used at a rate equivalent to that recommended today for Met52. These tests were done at two locations (Littlehampton and East Malling UK) and different plant species were evaluated at each location (with the exception of Fuschia and Pernettya, which were included at both locations). For this work, the fungus was applied in mid-July and assessments were done 17 – 28 weeks after treatment. The amount of control observed was variable, and depended on the plant species used, ranging from 0 to 96% control of larval numbers. The authors proposed that this variability may have been caused by factors such as the physical or chemical interaction of the plant with the fungus, for example the effect of the root architecture on the distribution of spores in the soil following the drench application (Moorhouse *et al*., 1993d).

<u>Persistence of efficacy</u>

The evidence shows that *M. brunneum* F52 spores persist well in growing media. Moorhouse *et al*. (1993e) observed no decline in the persistence of effect of M*. brunneum* F52 after 20 weeks when used against vine weevil larvae on glasshouse Impatiens (minimum temperature 15°C). Bruck & Donahue (2007) evaluated the granular formulation of the fungus at two rates of application (0.3 and 0.6 kg/m^3) applied into soilless potting media in April at six nurseries in Oregon USA growing outdoor ornamental crops. Persistence of the fungus in the media was measured over two growing seasons. This was done by taking samples of potting media at regular intervals and testing its effect on the survival of 6th instar vine weevil larvae. The percentage of larvae infected in these assessments declined from >90% three weeks after application to 40– 60% at 19 weeks after application. The larval infection then increased over the autumn to 80% infectivity before a slow decline over the second season. The increase in fungal concentration seen over the autumn may have been caused by infection of naturally occurring vine weevil larvae (Bruck & Donahue, 2007).

Effect of plant growth medium

Hartwig & Oehmig (1992) observed no effect on efficacy of BIO1020 (the early formulation of *M. brunneum* F52) on different types of growing media. In contrast, Moorhouse *et al*. (1992b) found that a drench application of *Metarhizium* spores on strawberry plants gave lower control on peat-based medium than with field soil, attributed to poor penetration of the drench into the peat-based medium (see below for more details). Research at the USDA in Oregon (Bruck, 2006) showed that the persistence of Met52 was not affected by different types of soilless potting medium (coir, bark, peat, perlite). The fungus persisted well for at least 133 days after application, although there was evidence that persistence could be reduced under fluctuating moisture conditions if the plant growth medium is allowed to dry out. Similarly Ansari and Butt (2008) observed no difference in the efficacy of Met52 against vine weevil larvae in different growing media (peat based, bark, coir, or peat blended with 10% or 20% composted green waste).

Metarhizium brunneum F52 has been shown to colonize the root zone of container grown *Picea abies*, to the extent that the fungal population in the root zone became significantly greater than in the surrounding bulk medium (Bruck, 2005). This could be a useful attribute for biological control. When vine weevil larvae were given a choice between (i) *P. abies* plants grown in media treated with Met52 and (ii) *P. abies* plants in untreated media, they were attracted to the plants in Met52-treated media (Keppler & Bruck, 2006). This might be an adaptation by the fungus to increase the chances of vine weevil larvae coming into contact with fungal spores and thereby becoming infected. This study was only conducted with larvae, no experiments were done in this study to see if vine weevil adults were attracted to Met52. However, in Defra funded research (Pope *et al*., 2011) a strain of *B. bassiana* was identified that looked to be repellent to vine weevil adults. It would be valuable to see if these behaviours (attraction of vine weevil larvae to Met52, repellency of *B. bassiana* to vine weevil adults) could be exploited to improve the effectiveness of vine weevil control. For example, it might be possible to apply Met52 in the plant growth substrate in such a way that vine weevil larvae are attracted away from plant roots in order to reduce plant damage.

Effect of chemical / microbial inputs on Met52 (pesticides, fertilizers, biocontrol agents)

In their studies of the persistence of *M. brunneum* F52, Bruck & Donahue (2007) used a slow release fertilizer as standard in experiments. Good survival of the fungus was observed over two growing seasons, from which we can infer that fertilizer application is unlikely to adversely affect the persistence of Met52.

Moorhouse *et al*. (1992c) evaluated 20 fungicides and insecticides (Table 4; only currently approved insecticides are shown) for their effect on the growth and germination of *M. brunneum* F52 on agar medium in a laboratory experiment, followed by a greenhouse experiment to investigate the effects of the pesticides on the effectiveness of *M. brunneum* F52 to control vine weevil larvae on Impatiens plants. While some of the pesticides reduced or prevented growth and germination of the fungus, there was no reduction in the efficacy of the fungus against vine weevil when the fungus was used as a prophylactic drench and the pesticides were applied seven days after weevil egg application.

Table 4: Fungicides and insecticides evaluated by Moorhouse *et al*. (1992c) for their effects on *M. brunneum* F52

Active ingredient	Product	Formulation
Fungicides		
Benomyl	Benlate	WP
Bupirimate	Nimrod	EC
Carbendazim	Bavistin	WP
Chlorothalonil	Repulse	SC
Etridiazole	Aaterra	WP
Fenarimol	Rubigan	WP
Furalaxyl	Fongarid	WP
Iprodione	Rovral	WP
Propamocarb	Filex	EC
Pyrazaphos	Afugan	EC
Quinomethionate	Morestan	WP
Triforine	Saprol	EC
Tolclofos-methyl	Basilex	WP
Zineb	Zineb	WP
Insecticides		
Cypermethrin	Ambush	EC
Primicarb	Pirimor	WP

EC emulsifiable concentrate, SC soluble concentrate, WP wettable powder

Bruck (2009) tested 17 different fungicide products used commonly on container-grown ornamentals in the USA for their effects on the growth and germination of *M. brunneum* F52 and on the persistence of the fungus in soil (Table 5). Although the fungicides affected fungal growth in the laboratory, there was no effect on persistence in the bulk soil. However captan and triflumizole did affect the population of *Metarhizium* in the rhizosphere. This was attributed to these fungicides having a short reapplication interval (Bruck, 2009). The technical notes for Met52 from Fargro states that the majority of fungicides that are used in growing media are thought to be safe to use with Met52 although some have potential to affect growth and germination, however if effects did occur, they could be circumvented by separating the timing of application of fungicides and Met52. The notes also state that fungicides based on propamocarb and metalaxyl-M are thought to be fully compatible.

Table 5: Fungicide products evaluated by Bruck (2009) for their effects on *M. brunneum* F52 / Met52 in Oregon USA

Active ingredient	Trade name	Formulation	Manufacturer
Azoxystrobin	Heritage	WG	Syngenta
Captan	Captan 50WP	WP	Agway Inc
Dimethomorph	Stature DM	WP	BASF
Etrodiazole	Terrazole 35WP	WP	Chemtura Corp
Fludiox + mefanox	Hurricane	WP	Syngenta
Fludioxanil	Medallion	WP	Syngenta
Fosetyl-Al	Alliette	WP	Bayer
Iprodione	Iprodione Pro 2SE	SE	BASF
Mafanoxam	Subdue MAXX	MC	Syngenta
Phosphorus acid /K salts	Agri-Fos	EC	Agrichem
Propamocarb	Banol	WP	Bayer
Pyraclostrobin	Insignia	WG	BASF
Quintozene	Terraclor 75WP	WP	Chemtura Corp
Thiophanate-methyl	Cleary's 3336F	F	Cleary Chemical
Thiophanate-methyl	Banrot 40WP	WP	Scotts Sierra Crop Protection
Triflozystrobin	Compass	WG	Bayer
Triflumizole	Terraguard 50WP	WP	Chemtura Corp

EC emulsifiable concentrate, F flowable, MC microemulsion concentrate, SC suspension concentrate, SE suspo-emulsion, WD water dispensable granule, WP wettable powder.

Little is known about the interaction of Met52 with other microbial control agents. It will be important to address this in the future, for example to find out if Met52 is compatible with antagonists of plant disease such as *Trichoderma*. A study has been published (Krauss *et al*., 2004) in which *M. anisopliae* was found to be very susceptible to the generalist mycoparasites *Trichoderma harzianum*, *Lecanicillium lecanii*, and *Clonostachys* spp. in a laboratory petri dish test. However, co-application of the mycoparasites with the *Metarhizium* did not affect the ability of the latter to kill insects in laboratory bioassays, including two weevil pests, the banana weevil *Cosmopolites sordidus* and the rice weevil *Sitophilus oryzae* (Krauss *et al*., 2004).

<u>Effect of temperature</u>

EPF are ectothermic organisms, meaning that their activity (growth, germination and infectivity) are all governed by temperature. For *M. brunneum* F52, the available evidence indicates that the fungus kills weevil larvae fastest at 25-20°C, and the speed at which it kills insects is reduced at temperatures below this. The fungus looks to have very low activity at 10°C. The speed of kill at 15°C appears to be significantly slower than at 20°C, but the fungus will cause the same total level of mortality in time. The technical notes for Met52 from Fargro state that the activity of the fungus is fastest between 20 and 30°C, and that control is delayed below 15°C as the fungus takes more time to kill the larvae

Moorhouse *et al*. (1994) measured the pathogenicity of six different *Metarhizium* strains (including *M. brunneum* F52) against vine weevil larvae in a laboratory bioassay at four different temperatures (10, 15, 20, 25°C). The speed of kill for F52 was fastest at 20 and 25°C, and was significantly reduced at 15 and 10°C. At 15°C, the speed of kill was 2.5x slower than at 20°C, and at 10°C it was 10x slower than at 20°C.

Hartwig & Oehmig (1992), working with *M. brunneum* F52 as the product Bio1020, evaluated the effect of four different temperatures (10,15,20,25°C) on fungal infectivity against the flour beetle *Tenebrio molitor*. This beetle is easy to culture and maintain in large numbers and has been used by some investigators as a surrogate for vine weevil. In this case, infectivity was reduced at lower temperatures. There was lower efficacy at 15°C compared to 20°C, but this could be compensated for by increasing the concentration of fungal spores used to treat the insects. At the lower end of the concentration range used in this study, there was a highly nonlinear relationship between fungal efficacy and the concentration of fungal spores at 15°C, which meant that small increases in spore concentration resulted in a large increase in efficacy. However, there was no infection at 10°C at any spore concentration used. It is not currently possible to say with precision how these results could relate to vine weevil control with Met52, because the two products have different formulations and application strategies.

Bruck (2007) evaluated a drench application of *M. brunneum* F52 against vine weevil larvae on container grown nursery plants in Oregon, USA (see below for more information about the use of a *Metarhizium* drench application). A drench was applied of 1.5 x 10^9 spores in 200ml water followed by additional 200ml water to aid spore movement into the potting media. The concentration was therefore c. 4 x 10^6 spores per ml of drench. Drenches were applied to hardy nursery stock, and the treated pots were maintained outdoors and compared to pots kept in a greenhouse at a constant 21°C. This experiment was done three times: once commencing spring 2004, then again in spring 2005 and finally in autumn 2006. In spring 2004, applications caused only a 30% reduction in larval populations. In this case, weevil mortality was assessed over a 14 day period and mean daily temperatures exceeded 15°C on only five days during this time. Spring applications in 2005 gave close to 100% control: in this case temperatures remained near or above 15°C and vine weevil mortality was assessed over a longer incubation time (28 days). Statistically significant control was observed in the autumn of 2006, but temperatures dropped from 15°C to 3°C over the 28 days of the trial, and weevil control was less effective as a result, giving about a 45% reduction in larval numbers. These broad findings were backed up by laboratory experiments of fungal growth and infectivity. It was found that temperatures below 20°C reduced fungal growth. In laboratory bioassays, it was found that lower temperatures reduced the speed at which larvae were killed by the fungus. After 14 days, there was no infection at 15°C or below, but 90 – 100% infection was obtained at 20°C and above. After 28 days, there was 77% infection at 15°C and 100% infection at 20°C and above.

Weevil infection and death can occur relatively quickly when the temperature is favourable (van Tol, 1993a). Therefore, the timing of application of fungal pathogens of vine weevil to take advantage of favourable temperatures is going to be important, particularly for crops grown without protection. Adult vine weevils lay eggs from June through to the end of September, with the peak of egg laying around mid-August (Moorhouse *et al*., 1992a). The early instar larvae – which are more vulnerable to infection than later instars (Moorhouse *et al*., 1993b) - should die rapidly if soil temperatures are at 15°C or above during this time. However, unseasonal cold temperatures after egg laying are likely to reduce fungal efficacy. The fungus persists very well in soil / growing media and therefore it should remain overwinter to infect larvae once temperatures become conducive to fungal development in the following spring, providing that spores have not been flushed out by excessive watering (see below). Larvae that have acquired infection during the autumn but have not died because of low temperatures should succumb to infection once temperatures increase in the spring. However, we do not yet understand the effects of temperature well enough to predict how fungal efficacy is likely to be affected by the typical fluctuating temperature conditions in different seasons. On ornamentals, depending on the crop and severity of infestation, delayed kill of vine weevil larvae may be too late to prevent plant damage or rejection of containerized ornamental plants due to the presence of live larvae in the growing medium at point of sale.

In the longer term there is potential to develop biopesticides based on fungal strains that are effective at lower temperatures. Different fungal strains vary in their pathogenicity at lower temperatures, with some strains able to perform significantly better than others against vine weevil larvae at 15°C (Soares *et al*., 1983). Cold active strains of EPF have been isolated in Norway (Hjeljord & Klingen, 2005) while a strain of *M. anisopliae* from Iceland is reported to have given good control of vine weevil larvae in tests on birch seedlings under typical field conditions in that country (Oddsdottir *et al*., 2010).

Effect of soil moisture

There is some evidence of adverse effects of very dry conditions on *Metarhizium* performance, but these are unlikely to occur under normal plant husbandry regimes. Hartwig & Oehmig (1992) investigated the effect of soil moisture content on the efficacy of BIO1020. This formulation consisted of granules of fungal mycelium grown in a fermenter tank. When added to soil, the fungus grew from the granules and produced spores which then infected weevil larvae. Very dry conditions (30% of soil maximum water capacity WC_{max}) inhibited the production of spore on the granules, but there was no effect at 60% or 90% of WC_{max}. These effects are unlikely to be a problem for the Met52 formulation as the spores are already formed on the granules.

Potential of other EPF species for vine weevil control

Although most of the work on fungal control of vine weevil has been done with M. brunneum F52, there are a range of other species and strains of EPF that are also able to infect and kill vine weevil. Moorhouse *et al*. (1993a) evaluated the susceptibility of vine weevil larvae to 19 different Metarhizium strains in a laboratory bioassay done under controlled temperature conditions (20°C). The strains originated from different geographic locations including Europe, Asia, South America and Australasia. Sixteen of the strains were pathogenic to larvae, although the amount of time required to cause death varied markedly. The M. brunneum F52 strain was included in this work and was identified as one of the most pathogenic strains. Elsewhere, Poprawski *et al*. (1985) evaluated five different EPF species (*B. bassiana*, *Isaria fumosorosea*, *Isaria farinosa*, *M. anisopliae*, *Metarhizium flavoviride*) against vine weevil larvae in a laboratory bioassay, which was done by spraying eggs with a spore suspension and then maintaining them at 20°C and monitoring the survival of the eggs or early instar larvae. All of the EPF species were pathogenic, although their effects depended on vine weevil life stage. For example, *B. bassiana* was very pathogenic to early instar larvae but was not pathogenic to eggs, whereas *M. flavoviride* was highly pathogenic to eggs but not to larvae. The other fungi were pathogenic to both eggs and larvae. Booth and Shanks (1998) evaluated a strain of *M. anisopliae*, *B. bassiana* and *I. farinosa* against early instar vine weevil larvae in greenhouse grown potted strawberry plants. The fungi were grown as a mycelium formulation on rice grains and applied to the surface layer of the plant growing medium. First instar vine weevil larvae were then added at different intervals up to 103 days after the application of the fungi. The three EPF all suppressed vine weevil larvae, with *B. bassiana* showing the greatest efficacy overall (Booth and Shanks, 1998). A related species, *Beauveria brongiartii*, has also been shown to be pathogenic to vine weevil larvae in laboratory experiments (Coremans-Pelseneer and Tillemans, 1991). This fungus has been used as a biocontrol agent in Switzerland for the control of cockchafer grubs affecting a range of tree species, with good levels of control (Keller *et al*., 1997).

Alternative application methods: using *Metarhizium* as a drench

At present, Met52 is only available as a granular formulation. However, grower feedback indicates that a liquid drench formulation would be useful, particularly for strawberry crops where such a product could be applied through drip irrigation systems. There has already been some valuable research on drench applications of Met52 for use on ornamental crops (done in the USA) and on tunnel grown strawberries (done in the UK by Tariq Butt and colleagues as part of HDC LINK research). These show that drench applications can be very effective.

The research done by Moorhouse and coworkers, described above, was normally based on drench applications of fungal spores to the surface of the growth medium. Moorhouse *et al*. (1993e), investigating spore drench applications on begonia plants, found that weevil control was affected by the timing of the drench application. Control was best when pots were drenched prior to the addition of weevil eggs, whereas applying the drench from 2 – 8 weeks after the application of eggs resulted in reduced control as follows: 2 wks = 92% control; 4 wks = 92%; 6 wks = 85%; 8 wks = 65% control. Poor control of vine weevil as part of a study on hardy ornamental nursery stock (Moorhouse *et al*., 1993d) was also attributed to a curative rather than a preventative application of a fungal drench.

As part of an HDC/ Horticulture LINK project (Butt, 2011) Ansari and Butt (2013) compared a granular formulation and a drench of Met52 against vine weevil larvae on polytunnel grown strawberries on commercial holdings. Different application methods were compared. These are different from the current way that Met52 is applied: (i) a premixed application was used, in which the granular formulation of Met52 was mixed in 1 litre of soil and then shaken to separate the spores from the rice grains. This was then added to the soil surface and mixed by hand into the top 5 cm of soil. (ii) A drench application consisted of spores washed from the rice grains and drenched around the base of each plant. (iii) A bare root treatment was used in which plant roots were dipped in a spore suspension before planting. These treatments were used at the manufacturer's recommended rate for field application (10^{14} cfu per ha) and at two lower doses, an "intermediate" rate (10^{13} cfu per ha) and a "low" rate (10^{12} cfu per ha). The amount of control depended on the application rate, but for each rate the three types of application all gave similar levels of control of vine weevil larvae: recommended rate = 71 – 96% control; intermediate rate = 40 – 75% control; low rate = 6 – 11%. The applications were applied in the late spring and assessments were done in the autumn. The soil temperatures ranged from 14 – 19°C. The authors of this study proposed that the drench application allowed a uniform distribution of fungal spores around the base of the plant, close to where vine weevil eggs were laid, which will provide a higher concentration of spores in the vicinity of eggs and early instar larvae. They also found that drench applications could result in percolation of the Met52 spores though the growth medium, resulting in up to 90% loss of inoculum in coir based medium, 80% in bark or 20% green waste, and 65% loss in a peat based medium (Butt, 2008). However, these results contrast with those of Moorhouse *et al*. (1990; 1992b) who found that a drench of *Metarhizium* spores against vine weevil larvae on strawberry plants gave significantly lower control on peat based medium than with field soil. This was found to be caused by poorer penetration of the spores into the peat compared to the field soil, with the highest concentration of spores being in the surface layer, which is likely to have resulted in fewer spores contacting weevil larvae lower down in the root zone. In field soil, the opposite effect was observed, in that the spore concentration increased with soil depth. These findings were found with four different *Metarhizium* strains. It is not known why the results of Moorhouse *et al*. (1990; 1992b) contrast with those of Butt (2008). If drench applications of Met52 were to become available, research would need to be done to resolve this conflict in findings. This should focus in particular on the percolation of spores when applied through drop irrigation onto coir, since this is now used by the majority of growers producing substrate-grown strawberry crops in the UK. Similar issues apply also for HNS growers, where peat replacement products – some of which have inferior water holding abilities and drain faster than peat – are being used in increasing amounts.

Alternative application methods: could *Metarhizium* colonize plant growth media?

As described previously, *M. brunneum* F52 was found to colonize the root zone of container grown *Picea abies* (Bruck, 2005). The fungus probably grew on soluble carbohydrates released by the plants as root exudates, possibly with additional chemical signaling from the plant to encourage fungal growth (Vega *et al*., 2009). Growth of *M. brunneum* F52 in the root zone might extend the persistence of the fungus in plant growth media. Moreover, if the fungus could be made to grow and multiply extensively, for example by amending the medium with sources of carbohydrate that the fungus can use, then this might result in better levels of control of vine weevil larvae (since the efficacy of the fungus is related to the amount of fungal inoculum present). This strategy was investigated recently in Defra funded proof-of-concept research by Fitzgerald and coworkers (Fitzgerald, 2013). The work was done in three parts. In the first part of the research, the growth of *M. brunneum* F52 and a strain of *B. bassiana* was investigated on ten different waste materials. Both fungi grew well on pasteurized spent mushroom compost while *M. brunneum* F52 also grew on pasteurized coffee grindings waste. This is important because the use of pasteurized material is cheaper than having to use a sterilized substrate. However, the investigators did not examine whether the fungi were able to grow on these waste substrates mixed into plant growth media. In the second part of the study, the persistence of the fungi on the substrates in cold storage (5°C) was examined. The results indicate that *M. brunneum* F52 grown on spent mushroom compost died after 50 days storage, while cold stored Met52 product declined over a thousand fold after 220 days. These results contrast with our own experience of working with entomopathogenic fungi, where we get very good cold storage of different fungal species and strains, including commercial products. Similarly Butt (2008) found that M. brunneum F52 mixed into plant growing media and stored outdoors for up to 20 months showed no deterioration in its ability to infect vine weevil larvae. We would be surprised to see a decline in the Met52 product in cold storage within 50 days, indeed the product has a guaranteed shelf life of one year at room temperature. In the final part of the study, a series of five laboratory bioassays was carried out in which spent mushroom compost inoculated with *B. bassiana* or *M. brunneum* F52 was incorporated into a plant growing medium (Bulrush professional multipurpose compost) at a rate of 10%. The resulting substrates were placed in pots to which vine weevil eggs or larvae were added and maintained at 20°C in the laboratory, and then the numbers of surviving larvae were recorded after a defined time. Unfortunately this part of the project produced no meaningful results. For three of the five bioassays, the fungi grown on the mushroom compost had been stored in the cold. Negligible levels of weevil control were observed, probably because the fungus had been accidentally depleted in storage. The remaining two bioassays used fungal material that had not been in cold storage. One of the bioassays tested the fungi against late instar larvae: only 30% control was observed when Met52 was used, and no control was observed in the media amended with fungus-inoculated waste. We would normally expect Met52 to give 100% control in such a bioassay. In the last bioassay, no viable fungal cells were detected following inoculation of the Bulrush compost for any treatment with the exception of *B. bassiana* and *M. brunneum* F52 inoculated onto spent mushroom compost plus brewery waste. None of the treatments resulted in any significant increase in weevil mortality. Although the overall concept of amending plant growing media to encourage Met52 growth looks attractive, there is unfortunately a lack of evidence that it could work in practice. Indeed, if an amendment caused *Metarhizium* spores to germinate and grow as mycelium in the plant growth medium rather than infect weevil larvae, this could reduce fungal efficacy.

Use of Met52 in an integrated pest management programme: interactions with entomopathogenic nematodes

Management of vine weevil larvae is now based on biological control, which at present comprises Met52 and entomopathogenic nematodes. There is no longer any “silver bullet” solution for vine weevil, and successful control will require a variety of methods in an integrated programme. Some highly effective IPM programmes have been developed against insect and mite pests in other sectors of horticulture by using combinations of different biological control agents, most notably in the protected salads industry. For vine weevil management, an important question is whether there are benefits to be had from integrating entomopathogenic nematodes and Met52 into a joint programme. This could be done, for example, by using a preventative application of Met52 incorporated into the growing medium at the time of planting, followed by a supplementary drench of nematodes later in the season.

Some valuable work has been done in this area by Butt and coworkers through HDC LINK funding. This was initiated as a set of laboratory and greenhouse experiments to investigate the effect on the survival of 3rd instar vine weevil larvae of applications of *M. brunneum* F52 combined with the entomopathogenic nematodes *Heterorhabditis* *bacteriophora*, *Steinernema feltiae* or *Steinernema kraussei* (Ansari *et al*., 2008). In laboratory bioassays done at 23°C, co-applications of *M. brunneum* F52 and each of the three nematode species resulted in either an additive (*S. feltiae*) or a synergistic (*H. bacteriophora*, *S. kraussei*) increase in vine weevil mortality. When nematodes were applied one or two weeks after the fungus, there was a synergistic increase in mortality for *H. bacteriophora* and *S. feltiae*, and an additive effect for *S. kraussei*. In a greenhouse experiment done as part of the same study, *M. brunneum* F52 was mixed as a liquid suspension of spores into dry compost, followed by a drench of nematodes (either *H. bacteriophora* or *S. feltiae*). Pots were planted with Euonymus cuttings and inoculated with vine weevil 3rd instar larvae, and larval mortality was evaluated after 1 week. The average temperature of the compost was 17°C. Combining *M. brunneum* F52 with *H. bacteriophora* resulted in a synergistic increase in larval mortality, while combining *M. brunneum* with *S. feltiae* resulted in an additive or synergistic increase depending on the concentration of the fungus and nematodes applied.

Ansari *et al*. (2010) also investigated interactions between *M. brunneum* F52 and *S. kraussei* against overwintering black vine weevil larvae in strawberry grow bags in unheated glasshouses done at different locations in Cambridgeshire and Swansea. Both the fungus and the nematodes were applied as a drench to strawberry plants that had been inoculated with 3rd instar vine weevil larvae. These trials were started in November and ran for 10 weeks. At Cambridgeshire, *M. brunneum* F52 on its own gave a mean of 50% control, while *S. kraussei* on its own gave 61% control, with no statistically significant difference in mortality between the two. But when combined together they gave 100% control, which was calculated to be a synergistic interaction. Note that the amount of fungus and nematodes used in these experiments were lower than the recommended rates and were chosen deliberately to give partial control, as this is necessary for determining the outcome of the combined application (synergistic or antagonistic interaction, or additive effect) with statistical accuracy. At the Swansea site, *M. brunneum* F52 on its own gave a mean of 88% control, which was statistically significantly greater than S*. kraussei* on its own (69% control). When used together, there was 100% weevil control which was calculated to have had an additive effect. The greenhouse temperature in Cambridgeshire at the start of the experiments was 15°C and average weekly maximum and minimum temperatures ranged from 19 to 13°C (max) and 3 to 0°C (min). At Swansea, the temperature was 14°C at the start of the experiment, and average weekly maximum and minimum temperatures ranged from 25 to 13°C (max) and 13 to 4°C (minimum).

In the final stage in this project (Butt, 2008; Ansari and Butt, 2013) the use of Met52 with reduced dose applications of *Heterhorhabditis bacteriophora* (Nematop, e-Nema Germany) or *Steinernema kraussei* (Nemasys L, Becker Underwood UK) were investigated against vine weevil larvae in polytunnel-grown strawberries on commercial farm sites. The trial was designed to use Met52 as a preventative treatment applied in spring ahead of the main vine weevil egg laying period in late summer, with the Met52 treatment supplemented with low doses of nematodes in the autumn for curative control of vine weevil larvae. Met52 was applied using a premixed application or as a drench at the recommended rate (10^{14} spores per ha) in early May. The plants were inoculated with vine weevil eggs in mid-August and then a single application of nematodes was applied in early-October) at a reduced rate. Plants were assessed for the numbers of vine weevil larvae six weeks after nematode application at the end of November. The soil temperatures observed for this experiment at different periods in the experiment were as follows: (i) time of Met52 application = 19°C; (ii) time of nematode application = 13°C; (iii) time of assessment = 8°C. The nematodes were applied at either 12,500 or 25,000 infective juveniles per plant. In this case, the two Met52 applications gave good levels of control on their own (94% for the pre-mix and 88% for the drench). Adding *S. kraussei* to the Met52 resulted in 100% vine weevil control but this was not statistically significantly greater than using Met52 on its own. The authors claimed in the paper that vine weevil control was greatly improved by using Met52 in combination with low dose applications of *S. kraussei*. This inference is probably not justified on the basis of the results, although it is certainly the case that the earlier studies (Ansari *et al*., 2008; 2010) showed that *M. brunneum* F52 and *S. kraussei* can interact synergistically when applied together. It is worth pointing out that these experiments involve a lot of work and require investigation over a number of seasons before the consistency of effect of the combined treatment can be ascertained.

In the ADAS IPM Fellowship project, funded by HDC, HTA and EMT, Hough & Bennison (2013) investigated the efficacies of four nematode products (three *Heterorhabditis bacteriophora* products and one *Steinernema kraussei* product and Met52 as an individual treatment or combined with each nematode product, for control of vine weevil on substrate-grown strawberry in a poly tunnel. All products were applied at recommended rates. All the nematode products and Met 52 in a coir substrate significantly reduced numbers of live vine weevil larvae when compared with untreated controls. Met52 in coir was as effective as the *H. bacteriophora* products but less effective than *S. kraussei*. Met52 in a peat substrate was ineffective. Combining nematodes with Met52 did not significantly improve the control of vine weevil larvae compared with using any of the nematode products alone. There would be merit in further investigating combined applications of Met52 and nematodes, particularly if a liquid formulation of Met52 is developed.

Evaluating the quality of fungal biopesticides

Because Met52 is based on a living fungus, it is essential that the spores are alive and viable in order for them to work. Spore viability can be adversely affected if the product is not treated with care according to the label instructions, for example if it is held under high temperatures in storage. The manufacturers of Met52 and other biopesticide products conduct quality assurance tests as routine, however there is also a place for growers conducting their own tests. Perhaps the simplest test is to measure the infectivity of the product to mealworms (*Tenebrio molitor*). These beetle larvae can be purchased from most pet stores, where they are sold as food for reptiles and amphibians. Mealworms can be added to a damp plant growing medium treated with Met52 and placed in a container (plastic petri dishes are ideal – these stop the mealworms from escaping and allow the growth medium to “breathe”). They can then be checked regularly for their time of death and for the presence of sporulating fungal mycelium on the surface. This simple test does not give information about the absolute level of viable spores in the growth medium, but it does provide a useful check of presence / absence of viable spores.

Fungal control of vine weevil: knowledge gaps

Putting biological control into commercial practice is not easy, particularly in the case of vine weevil, where growers using IPM are having to make a rapid transition from using persistent broad spectrum chemical pesticides to a full biological control programme. Growers need access to a range of control agents for vine weevil larvae in order to develop IPM programmes that meet their particular needs. The scientific evidence suggests that Met52 will be a useful biological control agent for growers, although our grower interviews show that results with Met52 have not been satisfactory for some ornamental growers and that in strawberry production, Met52 will not be widely used until a liquid formulation is developed. Our analysis of the scientific literature has indicated a number of gaps in knowledge that need to be addressed to give growers more confidence in using Met52 and to help improve vine weevil management. Here we make suggestions for addressing these gaps:

- The efficacy of Met52 is adversely affected as temperature is reduced. The work done to date on Met52-temperature interactions has been done at a small number of constant temperatures. There would be merit in developing an integrated model of fungal infectivity so that the performance of the fungus under fluctuating temperatures could be forecast. It may also be possible to compensate for the effects of low temperature by increasing the dose of fungus applied.

- Development of cold-active EPF strains is justified.
- Some of the early work with *M. brunneum* F52 showed that efficacy could vary significantly on different ornamental plant species. The cause for this was not known with certainty and may require investigation.
- It would be worthwhile investigating the distribution of vine weevil larvae on different plant species, with a view to developing smart application systems that concentrate Met52 spores in close vicinity to larvae. We also have a lack of knowledge about how many spores are acquired by vine weevil larvae, how many are needed for effective kill and the extent to which movement of the larvae is important for spore acquisition. There is evidence that larvae are attracted to the fungus, which may be an adaptation by the fungus to increasing its infectivity.
- There is a communications need to advise growers how to test for EPF infectivity in treated substrate using the mealworm test and to validate the effectiveness of the test under commercial conditions.
- A drench application has been shown to be effective and there is merit in developing this further, and for developing application through drip irrigation lines in soft fruit crops.
- Previous research indicates there can be synergistic or additive effects from combining Met52 with reduced rates of nematodes. There is merit in taking this forward in an IPM approach, although there are still knowledge gaps about how cost-effective such an approach would be and how it would perform under fluctuating temperature conditions, particularly in the autumn when periods of very low temperature may be experienced.

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Other non-chemical control methods

This literature review has identified over 60 papers and reports on other non-chemical control methods for vine weevil, including predators, bacteria and other microbial agents, plant extracts and botanical biopesticides and cultural control methods.

Predators

Naturally-occuring predatory beetles

Predatory carabid (ground) beetles such as *Harpalus rufipes*, *Nebria brevicollis* and *Pterostichus melanarius* commonly occur in soft fruit plantations (Crook, 1996). A similar range of ground beetle species and also staphylinid (rove) beetles occur in hardy nursery stock standing-out areas (Buxton 1996). These beetles are potential predators of different vine weevil life stages, but confirmation of this using dissection and examination of gut contents is difficult and laborious. In HDC-funded project (SF 15b), a monoclonal antibody technique was developed as a diagnostic tool for gut content analysis of potential predators of vine weevil (Crook *et al*., 1996). Subsequent gut analysis of predatory beetles collected from strawberry and blackcurrant plantations identified nine key carabid and staphylinid beetle species that were confirmed to have predated vine weevil eggs, larvae or adults (Table 6, Solomon 1997).

Table 6. Key predatory beetles confirmed to have predated on vine weevil eggs, larvae and adults.

Predators of eggs	Predators of larvae	Predators of adults
Notiophilus biguttatus	*Notiophilus biguttatus*	*Carabus violaceus*
Bembidion lampros	*Pterostichus madidus*	*Calathus fuscipes*
Ocypus olens	*Harpalus rufipes*	*Harpalus rufipes*

Further work in SF 15b showed that excluding carabid beetles from strawberry plants with polythene barriers sunk into the ground, led to a reduction in vine weevil numbers in low but not in high weevil densities (Solomon, 1997). It was concluded that predatory beetles should be encouraged in soft fruit crops susceptible to vine weevil damage, e.g. by avoiding the use of broad-spectrum pesticides such as pyrethroid and organophosphate insecticides, and by providing ground cover such as grass or other non-crop plants for day-time refuges (most of the predatory beetles are nocturnal). In a following project (SF 15c), there was evidence that a more open structure of plants, such as knotgrass and white clover, was preferred as refuges by predatory beetles than plain grass swards (Solomon 2000).

Subsequent work in the USA demonstrated that although predatory carabid beetles including *N. brevicollis* and *P. melanarius* ate vine weevil eggs, larvae and pupae placed on filter paper in laboratory studies, they did not reduce numbers of 'sentinel' larvae added to the roots of strawberry plants or of pupae buried two centimetres below ground near the strawberry plants (Lee & Edwards, 2012). It was concluded from this work that these adult ground beetle species have limited impact on vine weevil populations.

Although there is conflicting published evidence of the importance of naturally-occurring predators in vine weevil control in strawberry crops, particularly in high vine weevil densities, vine weevil is no longer a major pest in UK blackcurrant crops and this is thought to be due mainly to natural predation, although this might be by game birds in addition to invertebrate predators. Further investigation of why vine weevil is not currently a major pest in blackcurrants is justified, to determine if anything learned can be used to improve predation in other soft fruit crops and outdoor hardy nursery stock. Growers would be interested in faciliatating conservation of natural predators e.g. by providing suitable plant mixes in non-cropped areas.

Potential for release of commercially-available predatory beetles

In Defra-funded project PS2130, ADAS demonstrated that the commercially-available rove beetle, *Atheta coriaria* has potential for biological control of vine weevil larvae (Bennison, 2011). In laboratory bioassays, the beetles did not predate vine weevil eggs, possibly due to their spherical shape preventing handling and feeding. However, both *A. coriaria* adults and larvae predated young vine weevil larvae. Over a 3-day period, individual *A. coriaria* adults and second instar larvae predated means of 6.5 and 3.3 vine weevil larvae respectively when offered eight 1-4 day-old vine weevil larvae. The predators consume the body, leaving behind the head capsule (Figure 1). *Atheta coriaria* adults and larvae spend most of their time underground and are commercially available for the biological control of sciarid and shore fly eggs and larvae in protected crops. In HDC-funded project PC 239, *A. coriaria* adults and larvae were shown to reduce numbers of sciarid fly larvae in potted herbs such as parsley (Bennison, 2008). A grower system for mass rearing *A. coriaria* was developed, using turkey feed as an artificial food source in a coir-based substrate, and activity in crops can be monitored using 'bait pots' consisting of 9 cm plastic plant pots filled substrate and artificial feed (Bennison, 2010). Adults and larvae can readily be found at all depths in the bait pots, thus it is likely that they will predate on vine weevil larvae around the roots of host plants. Further research is justified on investigating the potential of *A. coriaria* as a predator of vine weevil larvae.

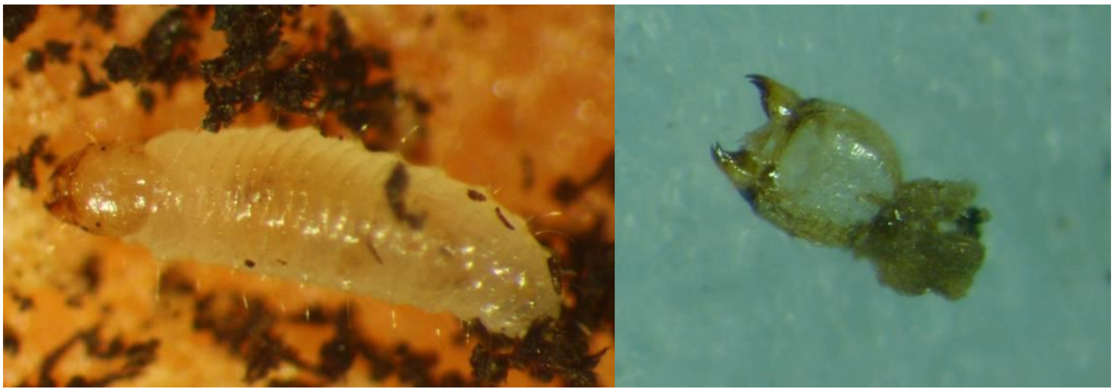

Figure 1. Healthy vine weevil larva (left) and head capsule of vine weevil larva (right), remaining after the body has been predated by *Atheta coriaria.*

Other predators and parasitoids

Other invertebrate natural enemies of vine weevil are earwigs and ants (Moorhouse *et al*., 1992) and the solitary digging wasp, *Cerceris arenaria* which paralyses vine weevil adults and buries them in their nests in the ground (Smith, 1932; Scott, 2012). The braconid parasitic wasp *Pandellia sexpunctata* has been reported as a major parasitoid of vine weevil adults and larvae in Germany (Smith, 1932). Other vine weevil predators include various birds such as songthrush, skylark, pheasant, partridge and chickens (Raffle, 2003), finches, flycatchers, warblers and tits (Moorhouse *et al*., 1992) and shrews and hedgehogs (Buxton 2003). Songthrushes will be encouraged by local woodland and game birds can be introduced to soft fruit plantations in co-operation with local gamekeepers. Hedgehogs can be encouraged by providing natural and artificial refuges.

Predators of vine weevil: Key knowledge gaps

- Most of the published research on predators and other natural enemies of vine weevil has focussed on naturally-occurring predatory beetles. Although these can be conserved as much as possible on HNS nurseries and in soft fruit crops by avoiding the use of broad-spectrum pesticides within an IPM programme, they are likely to contribute to vine weevil control only in low weevil population densities and this is difficult to quantify or predict.
- Growers would be interested in helping to conserve and augment natural predators e.g. in non-cropped areas by growing suitable plant mixes. Reference to current research in this area on arable and field vegetable crops is needed. Weed plants known to be hosts for vine weevil (Buxton & Pope, 2010) should be avoided.k

- Now that the native rove beetle *A. coriaria* is commercially available and a 'DIY' grower system for mass rearing the beetles has been developed, further work is justified to build on the ADAS laboratory work that demonstrated their potential for commercial release for the control of vine weevil larvae. In the current ADAS IPM Fellowship project, CP 89, a pilot experiment is planned during 2014 to quantify the predation of vine weevil larvae in containerised herbaceous nursery stock plants. This work will be reported in the annual Fellowship report due on 31 March 2015 and will be communicated to the industry before that date with the approval of HDC.

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Bacteria and other microbial control agents

Bacteria

The commercial strain of the bacteria *Bacillus thuringiensis* (*Bt*) subsp. *kurstaki*, used as a foliar spray for biological control of caterpillars, was compared with several insecticides for control of vine weevil larvae on *Primula* when used as a compost-incorporated treatment (Blackshaw, 1984). Although in an initial experiment, *Bt* gave significant (83%) kill of larvae compared with numbers in untreated plants, in a following experiment, five application rates of *Bt* were incorporated into the growing medium, including the rate used in the first experiment, but none gave significant control of larvae when compared with numbers in untreated control plants. An experimental strain of *Bt* subspecies *tenebrionis* was evaluated for control of vine weevil on protected and field-grown hardy ornamentals in Italy (Landi, 1990). The *Bt* strain was ineffective against adult vine weevils but led to 70% larval mortality at a constant temperature of 20°C, however, larval mortality was? only 28% in greenhouse conditions, when soil temperatures were below 12°C. Naturally-occurring entomopathogens including *Bt* were extracted from soils on hardy nursery stock sites in the United States but this strain of *Bt* was not pathogenic to vine weevil adults (Bruck, 2004).

A naturally-occurring bacteria, *Bacillus cereus* was identified from dead vine weevil larvae collected from strawberries in France (Marchal, 1977). Black, dead vine weevil larvae were found in a UK field-grown strawberry crop after application of entomopathogenic nematodes (see Grower survey section in this report). No infection with nematodes orentomopathogenic fungi were detected and it is possible that the larvae were infected with a bacteria (Bennison, Hough & Prince, unpublished).

Steinernema species of entomopathogenic nematodes are vectors for the symbiotic bacteria *Xenorhabdus nematophila*, which kills vine weevil larvae when the infective nematode juveniles enter the gut. Vine weevil larvae were killed by extracts of the bacteria and the bacteria persisted in soil for up to five months (Mahar *et al*, 2008). However, although entomopathogenic nematodes are currently exempt from pesticide regulations, the toxins produced by the entomopathogenic bacteria carried by nematodes would be classed as an insecticide if extracted and used independently from the nematodes, and would thus need to be approved for use as a biopesticide.

A range of naturally-occurring symbiotic bacteria, mainly *Rickettsia* spp. have been identified from *Otiorhyncus* species (Hirsch *et al*, 2012). Although these bacteria do not kill their weevil hosts, they may have potential in the future as biopesticides.

Other microbial control agents

Microsporidia are parasitic single-celled organisms that infect almost every group of insects. The infective stage is the spore, which needs to be ingested to infect the insect host. Some infections are benign, whereas others can lead to host mortality or to sub-lethal effects such as reduced longevity, reduced egg-laying or increased larval development times. A newly-discovered microsporidium was indentified from vine weevil in the United States (Bruck *et al,* 2008) and was provisionally placed in the genus *Canningia*. Vine weevil adults were orally exposed to the disease at various dose rates. The disease caused high levels of adult mortality and completely prevented egg-laying. Larval growth rate was reduced and larvae feeding on plant roots became infected when treated with a drench of the spores. As *Canningia* is an obligate pathogen, it is unlikely that it will have a future as a traditional microbial biopesticide i.e. as a formulation that can be applied as a drench. However, the disease can spread naturally within a vine weevil population and could potentially lead to high mortalities in confined infestations such as in a glasshouse or poly tunnel.

Bacteria and other microbial control agents: knowledge gaps

- Very little is known about the potential of bacteria and other microbial control agents of vine weevil. Bt subsp. *tenebrionis* has activity against vine weevil larvae but is not approved for use in the UK. If any further suspected natural bacterial infections of vine weevil are found in UK crops, the cause of infection should be identified.

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Plant extracts / botanical biopesticides

Neem / azadirachtin

Azadirachtin is a natural product derived from seed oils from the neem tree, and has recommendations for vine weevil control in the United States (Cowles, 2004). A neem product, NeemAzaal is approved in several European countries but is not currently approved in the UK. Vine weevil adults fed on azadirachtin-treated yew foliage laid fewer eggs than those fed on untreated foliage (Cowles, 2004). The reduction in egg-laying was dose-dependent, with high doses leading to 99% reduction and also to higher proportions of non-viability in any eggs laid. At the doses effective in reducing reproduction, azadirachtin did not act as an antifeedant and did not kill adult vine weevils. Cowles concluded that further research is required on the persistence of azadirachtin, optimum methods of application (persistence can be enhanced by systemic uptake by the roots) and the effect of reduced oviposition on vine weevil populations and on the impact on non-target organisms and on biological control agents used within IPM.

Neem seed cake is a by-product of neem oil production and can be used as a fertiliser. In Horticulture LINK project HL0171, neem seed cake powder, incorporated into a peat-based growing medium with or without the entomopathogenic fungus *Metarhizium anisopliae* was effective in reducing numbers of live vine weevil larvae in potted *Euonymus* (Shah *et al*, 2007). The effect of both treatments was dose-dependent and the combination was effective even with low rates of *M. anisopliae,* giving up to 97% control of larvae. The higher rates of neem led to increased *M. anisopliae* spore attachment. Neem treatment led to smaller vine weevil larvae and to slower larval development time. Neither treatment led to reduced adult vine weevil feeding or egg-laying; it is possible that azadirachtin levels are lower in neem seed cake than in the treatments used by Cowles (above). The authors suggested that neem could have stressed the vine weevil larvae, making them more susceptible to fungal infection, and thus the combined treatment may allow lower, more cost-effective rates of *M. anisopliae* to be used.

Polish products containing azadirachtin led to vine weevil larval development ceasing in laboratory studies and this led to high mortalities (Kowalska, 2008). Recent research in Germany showed that the commercial product NeemAzal, applied as a drench in three consecutive weeks to vine weevil-infested *Euonymus* plants led to 46% kill of larvae, compared with a single drench of thiacloprid (Calypso) which gave 90% kill (Reineke & Hauck, 2012). Three weekly foliar sprays of NeemAzal to *Euonymus* plants infested with vine weevil adults led to only 10% kill of adults, compared with 67% kill by a single spray of

Calypso. However, as reported by Cowles, significantly fewer eggs were laid by adults fed on neem-treated foliage than on untreated foliage. Reineke & Hauck concluded that a combination of thiacloprid, for its toxic effects, and neem, for its reduction in egg-laying, could lead to long-term reduction in vine weevil populations.

Isothiocyanates

Isothiocyanates are organic compounds derived from glucosinolates found in Brassica plants such as mustard and oilseed rape. Vine weevil eggs were killed by dipping them into acetone solutions of isothiocyanates (Borek *et al,* 1995). Soil amended with rapeseed meal or methyl isothiocyanate led to 50% kill of vine weevil larvae compared with no kill in untreated soil (Borek *et al*, 1997). Rapeseed meal applied as a mulch to potted *Rhododendron* and strawberry infested with vine weevil larvae reduced survival of larvae by up to 70%, depending on the dose rate and type of growing media (Elberson *et al,* 1997*). Rhododendron* growth was unaffected by treatment, but strawberry leaf margins became necrotic. Rapeseed meal and other *Brassica* spp. products have potential for use as biofumigants for vine weevil management.

Taxoids

Taxoid compounds extracted from yew foliage were shown to act as pyrethroid synergists, i.e. they improved the control of adult vine weevil by pyrethroid insecticides (Doss *et al.*, 1997). Although the use of pyrethroids is not compatible with IPM programmes due to their persistent effects on biological control agents used against other pests, this work demonstrates that plant extracts might significantly enhance the potency of other more IPM-compatible pesticides such as pymetrozine or indoxacarb, or of botanical biopesticides.

Other potential phytochemicals

In a Defra-funded review of the scope for using natural plant products to reduce reliance on conventional pesticides, ADAS reported that most or all reported plant species in the families Labiatae, Compositae and Scophulariacea were poor plant hosts for vine weevil larval feeding (Stafford, 2013). A further literature search of the chemistry of these plant species would identify further potential phytochemicals. In the same review, adult vine weevil egg-laying was reported to be lower on hop and rhododendron and this may be due to volatile compounds in the leaf oils of these species.

Plant extracts / botanical biopesticides: key knowledge gaps

- Relatively little is known about the potential of plant extracts or botanical biopesticides for vine weevil management. Further work is justified, particularly on botanical biopesticides that are available for use in other countries but not yet approved in the UK, and this would have strong grower support. Some work on novel biopesticides against vine weevil will be done in the current HDC-funded MOPS project during 2014 (Managing Ornamental Plants Sustainably) which may identify suitable candidates.

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Cultural control methods

Crop and nursery hygiene and cropping site selection

Hygiene measures for reducing vine weevil survival and breeding in fields and in or around glasshouses and polytunnels include prompt disposal of unsaleable infested plants, removal and careful disposal of crop debris and used grow bags and pots (Buxton, 2003; Raffle, 2003). Mowing off and removal of strawberry foliage after harvest led to 60% death of adult weevils, compared with 0-12% death in un-mowed plots in the United States (Garth & Shanks, 1997). Control of weeds known to be hosts of vine weevil is also recommended, these include dandelion, dock, knotweeds, plantain and rosebay willow herb (Buxton & Pope, 2010). As adult weevils can be taken from infested crops to 'clean' crops on machinery, containers and staff, 'clean' crops should be picked or worked on earlier in the day than known infested crops (Raffle, 2003). When planting a new soft fruit crop, ideally it should be sited well away from crops known to be infested with vine weevil, particularly crops that are older than first-year crops as these allow vine weevil populations to build up. Restricting strawberry crops to annual crops can reduce vine weevil populations and damage (Raffle, 2003).

Physical barriers

As vine weevil adults cannot fly, various physical barriers have been tested in order to prevent weevils from walking into uninfested crops. Aluminium flashing, used as 30 cm tall 'fences', dug 10 cm into the soil surface, excluded up to 75% of root weevil species (not including vine weevil) from plots of strawberry in Canada, and adding Teflon tape reduced weevil immigration further (Bomford & Vernon, 2005). Subsequent work reported that vine weevils could be excluded by barriers made of glass, plastic or aluminium treated with fluoropolymer, powdered talc or lithium grease, but under wet conditions the weevils were able to climb all treated surfaces (Bomford & Vernon, 2005a). In ADAS vine weevil trials in polytunnels, a sticky glue such as Insect Barrier Glue® has been applied to duct tape on the woven ground-cover matting on the polytunnel floor to exclude adult vine weevils and this has proved successful where the glue has been kept dry and free of plant debris. Such glue might also help to prevent adult vine weevils from crawling up the posts supporting table-top strawberries if used on new, uninfested plantings.

Mulches

The use of polythene mulches in soft fruit production is regarded as a major factor leading to an increase in vine weevil problems (Moorhouse *et al.*, 1992; Raffle, 2003). The polythene conserves soil moisture and provides warm, moist conditions for larval development, it also provides refuges for adult weevils during the day, and gives some protection from predation by birds and mammals. The mulch also restricts application methods for reaching the larvae with drenches of pesticides or biopesticides.

Physical damage to pupae

When ADAS Entomologists have reared vine weevil larvae through to adults for research purposes, it has been observed that if disturbed, pupae are very easily killed or damaged, with the survivors emerging as deformed adults that lay very few eggs (Saynor, personal communication). Investigation into developing a practical method for disturbing pupae in containerised ornamentals or soft fruit crops is justified as a cultural control method.

Varietal resistance/tolerance

Potential breeding for plant varieties with resistance to or tolerance of vine weevil damage has been discussed in the vine weevil biology section of this review, under 'Feeding behaviour'.

Cultural control: gaps in knowledge

- Cultural control is an important component of IPM programmes. Further work is justified on the potential of physical barriers such as glue applied to table-top supports to exclude adult vine weevil from new plantings.
- Investigation into developing a practical method for disturbing pupae in containerised ornamentals or soft fruit crops is justified as a cultural control method. Potential methods could include ultrasound technology.

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Management with chemical insecticides

The conventional method of controlling vine weevil has been with chemical insecticides. On containerised hardy nursery stock and on some potted protected ornamentals, this has included the incorporation of persistent insecticides into plant growing media for control of larvae, alongside some use of foliar sprays for control of adults. Media-incorporated insecticides have given good control of vine weevil larvae, but most of these have now been withdrawn for use on container-grown ornamentals, including chlorpyrifos (suSCon Green) and fipronil (Vi-Nil). Growers of containerised ornamentals currently only have the option of using the neonicotinoid insecticides imidacloprid (e.g. Imadasect 5GR) or thiacloprid (Exemptor) as an incorporated treatment against vine weevil larvae. The current EC restrictions on the use of neonicotinoid insecticides on ornamental crops now limit the use of imidacloprid to crops in glasshouses (see below). Chemical control on soft fruit is currently limited to foliar sprays against vine weevil adults, although a drench of chlorpyrifos can be used against larvae on strawberry crops after cropping.

Current EC restrictions on neonicotinoid product authorisations

- With effect from 1 December 2013, professional use of three neonicotinoid insecticides (clothianidin, imidacloprid and thiamethoxam) are no longer permitted for use on crops considered attractive to bees.
- Imidacloprid products can only be used (either as a growing media-incorporated treatment or as a drench) on ornamental plants in a glasshouse. These products are not permitted for use on ornamentals in a poly tunnel or outdoors.
- Plants treated with imidacloprid cannot be placed outside (or sold) until after they have finished flowering. Plants that do not flower can be moved outside following treatment in a glasshouse.
- Thiacloprid is a neonicotinoid insecticide but is not currently restricted under these regulations. Thus Exemptor may be used as a growing media-incorporated treatment for containerised ornamental plants in a glasshouse, poly tunnel or outdoors.

Our literature review identified 118 scientific articles on the use of insecticides to control vine weevil, including original research published in peer review journals, project reports and reviews. The first article published on chemical control was in 1932 with increasing frequency in the 1970s and 80s (20 and 31 articles respectively) and peaking in the 1990s with 42 articles. Since 2010 there have only been four published articles on chemical control of vine weevil. Due to product withdrawals, and replacement of “old” chemistry with new actives, most of the published information on chemical control is now obsolete. However, this literature does contain useful information on the effectiveness of different timings and methods of application that is relevant to the currently available insecticides. Therefore our review does include some reference to pesticides and application methods not currently approved in the UK.

Use of chemical insecticides against vine weevil larvae

Insecticides have been used extensively to control vine weevil larvae in ornamentals, accounting for 45% of the total insecticide-treated area larvae in container-grown hardy nursery stock in 2009 (Garthwaite *et al*., 2009). The insecticides used have mostly been slow-release formulations incorporated into growing media but have also included drenches. Chlorpyrifos drench is also still approved for use post-harvest on strawberry.

Growing media-incorporated insecticides

Growers of containerised ornamentals currently have access to slow-release products based on imidacloprid (e.g. Imidasect 5GR) and thiacloprid (Exemptor) for preventive control of vine weevil. However, use of imidacloprid is currently subject to the EC restrictions (see above) and both imidacloprid and thiacloprid products are currentlyk only recommended for incorporation into peat-based composts. Both of these products can give season-long control when incorporated into the compost (imidacloprid should give control of vine weevil for one year), although their effectiveness is dependent on being mixed in evenly and used at the correct rate (Buxton, 2003). Both the liner or plug and the potting on mix have to be treated to give acceptable control (Buxton, 1993), otherwise the liner or plug should be treated before potting on with a drench of either imidacloprid (Intercept 70 WG) or entomopathogenic nematodes.

Insecticide drenches

Imidacloprid (Intercept 70 WG) is still approved as a drench to containerised ornamentals but is subject to the current EC restrictions (see above). The drench can be used preventively e.g. to untreated plugs or liners before potting on into growing media with an incorporated insecticide, or for curative control when vine weevil larvae are detected. Unlike the imidacloprid formulations for incorporation into growing media which are persistent for one year against vine weevil, Intercept 70 WG is only persistent for up to six months. Imidacloprid drenches and sub-surface soil injections (not an approved method in the UK) were shown to reduce vine weevil larval numbers on field grown *Taxus* plants when applied either in the spring or autumn and gave season-long control (Reding & Persad, 2009). Reductions of 90% of 2nd – 4th instar larvae have recently been reported with a thiacloprid drench (Calypso) on Euonymus (Reineke & Hauck, 2012), but this method of application for thiacloprid is not currently approved in the UK.

In strawberry, a drench application of chlorpyrifos (e.g. Dursban WG) at the end of the cropping season can give good control (Buxton *et al.,* 1992; Łbanowska *et al*., 2004; Reding & Persad, 2009; Ansari & Butt, 2013). Łbanowska *et al*., (2004) showed that imidacloprid drenches also gave good control of vine weevil larvae in strawberries when applied after harvest, but imidacloprid is not approved for use on edible crops in the UK. Drench application of thiacloprid to strawberries after harvest (not approved in the UK) reduced weevil numbers by 59-61%, while an application in spring, two weeks before bloom, gave good control of overwintered larvae. Chlorpyrifos drenches also reduced larval numbers in this study but efficacy was reduced during hot and dry summer weather (Łbanowska *et al*., 2004).

Combined drenches of insecticides and entomopathogenic fungi

Imidacloprid and chlorpyrifos drenches have been shown to be compatible with applications of the entomopathogenic fungus, *Metarhizium brunneum* (used as the biopesticide Met52) (Shah *et al*., 2007; Butt, 2008; Ansari & Butt, 2013). Lower than recommended rates (1%) of an imidacloprid drench gave 78-92% and 95% control when applied in combination with Met52 applied as a drench or incorporated into the plant growing medium respectively (Shah *et al*., 2007). However, in strawberries, chlorpyrifos can have damaging effects on entomopathogenic nematodes (Raffle, 2003) and BASF recommend a 7-day interval between drenching chlorpyrifos and their entomopathogenic nematode products.

Timing of insecticide application

The timing of insecticide applications is important for good levels of efficacy. Preventive vine weevil control with growing media-incorporated treatments on containerised ornamentals will allow any larvae hatching from eggs laid into the growing media to be killed. As eggs can be laid by overwintered vine weevil adults as early as May, and adults resulting from overwintered larvae can lay eggs from July to September/October under protection (see vine weevil biology section), using a growing media-incorporated insecticide reduces the risk of sub-optimal timing. Applying insecticides in this way can maintain control of larvae in the original rooting area for a further growing season (May & Ellis, 1996), however this is not guaranteed; the suppliers of imidacloprid and thiacloprid state that compost incorporation will give vine weevil protection for one year and one season respectively.

The younger larval instars of vine weevil are the primary targets for control because they are more susceptible to insecticides than older larval instars (Buxton, 2003; Stimmann *et al*., 1985; Evenhuis & Alofs, 1982; Masaki *et al*., 1999; Rasmussen, 1977; Nielson *et al*., 1978). Chlorpyrifos will only control young larvae (May & Ellis, 1996). The control of the older larvae with insecticide drenches can also be reduced if they become established in the rootball, beneath the centre of the crown where insecticide drenches do not penetrate well (Nielson *et al*., 1978; Cross *et al*., 1995; Cross & Burgess, 1997).

Other factors affecting insecticide efficacy

Substrate type and moisture

To be effective, drenches have to be applied in high volumes of water and the plant growth substrate should be moist to aid the movement of the drench. Rainfall or irrigation after application can further improve its movement (Raffle, 2003). Insecticide molecules may also become absorbed into organic matter in some composted substrates which will reduce their effectiveness (Bogatko & Labanowski, 1993). Nielson and Boggs (1985) showed that the susceptibility of first instar larvae to insecticides was affected by the substrate organic matter composition (Nielsen & Boggs 1985). Although imidacloprid products for growing media incorporation are only recommended for use in peat-based composts, tests on different compost media (coir, bark, peat, 10% green waste blend) showed no reduction in weevil control of imidacloprid applied as a drench (Shah *et al.*, 2007).

Temperature and behaviour of weevil larvae

The effectiveness of insecticides can also be affected by environmental conditions. Growing media-incorporated insecticides kill young larvae, either when they come into contact with the insecticide in the compost, or when feeding on roots that have absorbed the insecticide (Buxton, 2003). In low temperatures, root uptake of insecticides is limited, also the movement and general activity of weevil larvae is reduced, both of which lead to lower insecticide efficacy due to reduced insecticide acquired (Buxton, 2003). In commercial practice, uneven mixing of the insecticide into the growing media could mean larvae move to places in the substrate with low insecticide concentrations, which might result in reduced control.

Effect of plant species

Differences in larval control with insecticides have also been found between different plant species (Buxton, 1997; Cowles, 2001). There is a limited of knowledge of the mechanisms responsible for these differences. In theory, larvae will develop rapidly on highly nutritious plants, resulting in larger later-instar larvae that will be more resistant to insecticides.

Larvae are also likely to move around less when feeding on good food quality roots, on roots containing higher concentrations of phagostimulants, or on plants with a high density of roots. This lack of movement could reduce the likelihood of larvae encountering insecticide residues (Cowles, 2001). However, the effectiveness of bifenthrin (not approved in the UK), incorporated into potting media, against vine weevil larvae was reported to be greater on Sedum plants, which are a good host for weevil larvae, compared to Mentha, which is a relatively poor quality host (Cowles, 2001). Similarly, plant species with woody roots restrict larval feeding to the cortex, which should increase their exposure to insecticide residues compared to non woody plants, where the presence of soft tissue can allow young larvae to burrow into the plant structure (root crown, tuber, and rhizome) and give protection from insecticide exposure. However, Cowles (2001) observed only a small influence of root architecture on the effectiveness of bifenthrin. This result is surprising, as growers can experience difficulties in controlling vine weevil larvae in herbaceous plants with fleshy crowns such as Sedum and Heuchera, as larvae tend to burrow into the crowns where the only effective insecticides are likely to be those with systemic activity such as imidacloprid or thiacloprid.

Use of chemical insecticides against vine weevil eggs

Several studies have reported insecticidal effects on vine weevil eggs (Sol, 1985; Verbruggen *et al*., 1985; Reineke & Hauck, 2012). The insect growth regulator diflubenzuron (Dimilin) slowed the hatching rate after the first treatment and inhibited hatching after the second treatment when sprayed on yew leaves and fed to adult weevils. Hatching rate returned to normal when adults were fed untreated yew (Sol, 1985). This research is worthy of further investigation, as Dimilin is approved for use as a foliar spray on ornamentals for caterpillar control and has an EAMU for use on some outdoor soft fruit crops including blueberry. Lufenuron (a chitin synthesis inhibitor) was also found to greatly reduce egg viability in laboratory tests (Jay & Cross, 2000). Reineke and Hauck (2012) showed that 34% of eggs sprayed with thiacloprid (Calypso) were killed.

Use of chemical insecticides against vine weevil adults

Use of insecticides to control adult vine weevils can supplement control measures used against larvae, but need to be carefully selected within IPM programmes to avoid adverse effects on biological control agents used for other pests. There are currently no insecticides approved specifically for control of vine weevil adults in the UK, but some insecticides with on or off-label approval for control of other pests can give incidental control of adult vine weevils.

The scientific literature contains a number of reports of insecticides that killed vine weevil adults. Many of these investigations were done using simple Petri dish experiments, where weevils were sprayed directly or fed with treated leaves. These Petri dish experiments have to be treated with caution as they do not replicate the more complex behaviours of vine weevil adults that occur on commercial crops and which affect the effectiveness of insecticide sprays.

Insecticide spray efficacy and time of day of application

Vine weevil adults feed on the foliage of susceptible crop plants at night and retreat to refuge areas in cryptic locations during the day. As most farms or nurseries will contain a very large number of suitable refuge locations (e,g, under pots, plant debris or polythene mulches, within plant crowns), it is unlikely that sprays of insecticides will effectively contact all the vine weevil adults in these refuges. For this reason, applications of insecticides against adult weevils are recommended to be sprayed after dusk, when adult weevils are active on the crop. Solomon (2000) reported that peak weevil activity occurs three hours after sunset, and advocated spray application at this time to ensure that adults come into direct contact with leaves before spray residues dry. However, no work was done in this study to determine adult control when pesticide application was made either during the day or three hours after sunset.

Not all individuals within a population are active every night, meaning that a single spray treatment on the crop is unlikely to directly contact all individuals present in the spray area. Nevertheless, there are some studies done in realistic settings that show that effective control of vine weevil adults is possible with insecticide sprays. In particular, research in semi-field conditions using caged Euonymus plants has shown that control of vine weevil adults can be obtained on ornamentals with lambda-cyhalothrin (Hallmark), pymetrozine (Chess) and indoxacarb (Steward) (Buxton, 2011) and on cranberries with indoxacarb (Avaunt) (Patten & Metzger, 2009). However, some inconsistency in activity against adults has been observed with Hallmark, with higher mortalities even in untreated controls when evening applications were made when temperatures were 25°C in a trial in 2010, than when evening temperatures were 18°C in a second trial in 2011 (Buxton, 2011). The results from this study suggest vine weevil susceptibility to higher temperatures, but the possibility of vine weevils developing resistance to pyrethroids cannot be excluded as a potential explanation for these results. Laboratory results in this study using detached Euonymus leaves also suggested that applications of Hallmark can remain active against adult weevils for up to 24 hours, which might allow sprays to be applied during the day (Buxton, 2011). Direct contact with Hallmark had little effect on weevil mortality. However, Hallmark residues up to 24 hours old significantly increased numbers of moribund weevils. Both Hallmark and Steward also had a noticeable adverse effect on the behaviour of surviving weevils, including abnormal walking, and this behaviour persisted for up to 14 days after application (T. Pope, personal communication). If these moribund or abnormally behaving weevils subsequently die or do not lay eggs as a result of picking up spray residues on leaves, then it may not be necessary to restrict applications to the evening when adults are active. This result is worthy of further investigation, focussing on more IPM-compatible insecticides than Hallmark including Chess and Steward, as spraying at night is not popular with growers due to practical difficulties.

Recent work in Germany has shown moderate control using two consecutive sprays of thiacloprid (Calypso) which killed 67% of the weevil population after 21 days (Reineke & Hauck, 2012). Buxton (2011) reported that a single application of Calypso killed 67% adults after 14 days in tests done in 2010 but killed only 25% after 15 days in 2011 and these mortality rates were not significantly higher than in untreated controls in either year. Use of combinations of different insecticides may increase the amount of control. For example, tank mixing of chlorpyrifos with lambda-cyhalothrin increased the speed of control of vine weevil adults by seven days (Buxton, 2011). Elsewhere, the insect growth regulator diflubenzuron (Dimilin) has often been often added to acephate (Orthene) sprays as it causes adults to stop laying eggs for long periods (Zepp *et al.*, 1979; van der Horst & van Tol, 1995; van Tol, 1996).

Timing spray applications according to weevil activity

The timing of insecticide spray applications is critical in determining their efficacy against vine weevil adults. Sprays are usually recommended to target adult weevils when they have emerged but before they start laying eggs (Buxton, 2003; Parrella & Keil, 1984; Fregonese & Zandigiacomo, 1992). Egg-laying usually starts 30-40 days after emergence (see vine weevil biology section). This application period would normally be early/mid June for protected crops in the UK, and mid-July for outdoor crops (Buxton, 2003; Raffle, 2003). However, on crops that have overwintered, earlier sprays may need to be considered for overwintered adults which can start laying eggs in May. If timing is delayed then the adult weevils may have already laid eggs, leading to later plant damage by larvae. Studies also suggest that repeated spray applications with high water volumes to ensure good coverage of foliage and soil are often needed to obtain acceptable levels of control (Nielsen *et al.*, 1978; Buxton, 2003; 2011). The number of repeated applications required for effective control ranged from two to 11 depending on the type of insecticide used (Buxton, 1997, 2003, 2011; Bene, 1984; Barratt *et al.,* 1989, Sacco *et al.,* 2001). Weather conditions can affect adult activity; they are more active on warm, still nights, when spraying may be more effective (Raffle, 2003).

Targetted spray applications

The distribution of vine weevils within the farm or nursery may be patchy, particularly if the infestation is new. If a primary infestation area can be identified quickly, treating that area alone may be sufficient to control the weevil population rather than treat the whole crop. Treating the entire nursery is only necessary when weevils are well established (Parrella & Keil, 1984). Furthermore, it may not be necessary to spray the entire plant to achieve control. In vineyards, trunk sprays of Sevimol (carbofuran) in early May gave good control resulting in 85% suppression of newly emerged adults (Phillips, 1989), while basal sprays of two different pyrethroids (Alert and Brigade) on raspberry canes worked as well as full sprays (Tanigoshi *et al*., 1997; Tanigoshi & Chamberlain 1998a,b)

Sub-lethal effects of insecticide sprays

As well as killing vine weevil adults, insecticides can have sub-lethal effects, such as reductions in feeding which may reduce adult egg-laying and cause the weevil population to decline over time. For example, vine weevil adults treated with the pyrethroids bifenthrin and esfenvalerate were knocked down initially but remained alive and moribund for many days with reduced feeding (Buxton, 1997). Vine weevil adults sprayed with lamda-cyhalothrin (Hallmark) and indoxacarb (Steward) showed behavioural effects including abnormally slow walking, while treatment with Steward caused expulsion of a liquid from the mouth (Buxton, 2011), see Figure 2. Emesis (vomiting) was also seen with weevils fed on rhodendron treated with oxamyl (Vydate) (Barrett & Ferguson, 1987). While Vydate only gave moderate levels of adult weevil mortality initially, it reduced leaf feeding by 68% compared with untreated controls and reduced the longer term survival of vine weevil adults, with only five out of 20 weevils living for 12 weeks (Barrett & Ferguson, 1987). There is a positive relationship between egg production by vine weevils and the amount of leaf material consumed by them (Shanks & Doss, 1986), meaning that a reduction in feeding caused by an insecticide treatment will also reduce the amount of eggs laid. Shanks and Doss (1986) found that reduced feeding also increased the length of the pre-oviposition period, which should therefore increase the time window available for killing adults with insecticide sprays.

Figure 2. Adult vine weevil treated with Steward producing a liquid from the mouth (HDC project SF HNS 112)

Effect of plant host on insecticide efficacy

Studies have shown that the efficacy of pyrethroid insecticides against adults can be affected by plant host species. Shanks and Chamberlain (1988) showed that permethrin killed more adult weevils on strawberry plants than on cranberry and was more effective on yew than either strawberry or cranberry. In addition, fenvalerate was ineffective on strawberry and cranberry plants but gave good control of vine weevil adults on yew plants. Adults fed on yew first were more susceptible to pyrethroids. Yew extracts were also shown to have a synergistic effect on fenvalerate (Doss *et al*., 1997).

Bait formulations of insecticides

As an alternative to foliar sprays for adult control some authors have had success with bait formulations (Tanigoshi & Chamberlain, 1999ab; Cowles, 1996; Patten & Metzger, 2009). Work in the USA showed that the same level of adult mortality could be achieved with cryolite baits with only 20% of the active ingredient of the foliar spray (Cowles, 1996). Success of the bait was dependent on formulation (Tanigoshi & Chamberlain 1999; Cowles, 1996; Patten & Metzger, 2009). Limitations of the baits studied included a dependency on moisture for effect, while baits applied in the evening were more successful than those applied in the morning (Smith, 1932; Cowles, 1996). Baits could be enhanced with attractants or phagostimulants to improve control. However, no commercial attractant for vine weevil adults has yet been developed (see vine weevil biology section).

Insecticide drenches for adult control

Insecticide drenches for vine weevil control are usually considered as targeting the larvae in the growing media. However, the application of systemic insecticides to the soil surface could also offer an alternative to foliar sprays for adult control and to prevent egg laying. Due to the long pre-oviposition period, carefully timed systemic insecticides would have several weeks to affect foliar-feeding adults before oviposition occurs. Reding and Ranger (2011) showed that the systemic activity of insecticides varied between plant species, probably as a result of different uptake and movement within the plants, but there was activity for up to 42 days in some plant species. Prolonged exposure to the neonicotinoids dinofluron and thiamethoxam suppressed feeding, caused high adult mortality and reduced the number of eggs laid by 97%. Feeding assays with plants drenched with the systemic neonicotinoids clothianidin and dinotefuran reduced adult feeding on Sedum for at least six weeks and adults died from starvation and/or toxicity (Reding & Persad, 2009).

Use of insecticides against vine weevil adults within an IPM programme

Organophosphate and synthetic pyrethroids have broad spectrum activity and are generally harmful to biocontrol agents used against other pests and on naturally-occurring beneficial insects such as ground beetles (e.g. *Pterostichus melanarius, P. madidus* and *Harpalus rufipes*) that predate on vine weevil adults (Solomon, 1997; Cross *et al*., 2001). Damaging effects can last 8-12 weeks following application (Raffle 2003). Integrated Pest Management (IPM)-compatible insecticides, indoxacarb (Steward, Avaunt) and pymetrozine (Chess), for control of adult vine weevil have shown promise (Patten & Metzger, 2009; Buxton, 2011). Using an IPM programme to control adult weevils resulted in a 40-50% reduction in pesticide usage against black vine weevil in one Dutch nursery growing outdoor nursery stock (van Tol, 1996). The IPM programme consisted of intensive monitoring for adult weevils throughout the spring and summer, using wooden boards and *Euonymus fortunei* bait plants. Spraying for adult control was only done where weevils were found or where feeding damage was observed on the bait plants. Only localised spraying was done, using acephate (Orthene), sometimes tank mixed with diflubenzuron (Dimilin) as this makes adult weevils sterile for long periods. No insecticides were incorporated into the growing medium, but local drenches of entomopathogenic nematodes were used where necessary.

Insecticide control: knowledge gaps

Insecticides still play an important role in some Integrated Pest Management programmes for vine weevil management on horticultural crops, particularly on hardy nursery stock. There are a number of knowledge gaps that if addressed, would improve the effectiveness of IPM programmes that include insecticides:

- Due to the current EC restrictions on using neonicotinoid insecticides on ornamental crops, growers will now have to rely more on thiacloprid than imidacloprid if they wish to use a persistent insecticide in the growing media. An additional pressure is that many major customers are demanding that growers of ornamentals stop using imidacloprid altogether, and some are even demanding that growers stop using all neonicotinoid insecticides, otherwise trade losses will occur. For those growers who are still able to use thiacloprid (Exemptor), although there is some information available on the effect of a thiacloprid drench on biological control agents used for control of other pests in IPM programmes, there is no information on the effect of the incorporated formulation, which has recently been given a higher recommended rate than previously for the control of vine weevil. This gap in knowledge should be addressed to give growers of ornamentals information on integrating Exemptor in their IPM programmes.

- There is little knowledge of the effect of insecticides on vine weevil egg laying or egg hatch, or on other sub-lethal effects on insecticides on vine weevil, including effects on behaviour and fitness. However the little available evidence suggests that sub-lethal effects could make an important contribution to vine weevil management. There is a need to determine what impact sub-lethal effects of IPM-compatible pesticides have on adult survival, feeding, egg-laying and egg hatch. Candidate pesticides include Chess, Steward and Dimilin. Chess is approved for use on protected and outdoor ornamental crops and has EAMUs for use on both ornamentals (at a higher rate than on the label) and on various protected soft fruit crops including blackberry, raspberry and strawberry. Another pymetrozine product, Plenum, has an EAMU for use on outdoor soft fruit crops. Steward is approved for use on protected ornamentals and has EAMUs for use on outdoor ornamentals and for outdoor and protected soft fruit crops. Dimilin (e.g. Dimilin Flo) is approved for use on ornamental crops and blackcurrant for caterpillar control.

- As highlighted in the vine weevil biology section, there is a need to determine the relationship between numbers of adult vine weevils, subsequent numbers of larvae and the amount of damage caused by larvae on plant roots. This would help to determine the benefit of controlling adults in addition to larvae in IPM programmes.

- There is a need to compare the efficacy of well-timed, single or multiple insecticide applications for control of adult vine weevil, to avoid unnecessary costs and impacts on IPM programmes.

- As highlighted in the vine weevil biology section, there is a need to develop a prediction and monitoring system for detection of emergence of adult weevils, the pre-oviposition period and the start and finish of the egg-laying period, which would inform decisions over choice of treatments to use in an IPM programme and more effective timing of any insecticide applications.

- There is a need to determine the most effective time of day or night to apply pesticides for control of adult vine weevil. Research results showing that Hallmark residues up to 24 hours old increased numbers of moribund adult weevils under controlled conditions, and that both Hallmark and Steward residues caused abnormal movement justify further research, focussing on IPM-compatible insecticides such as Chess and Steward. If moribund or abnormally moving weevils subsequently die or do not lay eggs, then it may not be necessary to restrict insecticide application to the evening, in order to contact active weevils.

- There is a need to determine the potential effects of temperature and insecticide efficacy against adult vine weevil.

- There is justification for determining if insecticide resistance is developing in UK vine weevil populations.

- As highlighted in the vine weevil biology section, little information is available on the movement of vine weevil larvae and how this might impact efficacy of insecticides applied as drenches, particularly those applied in field soils. Movement of larvae could also impact efficacy of biopesticides used as drenches or mulches to large containers.

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Objective 2. Identify opportunities for the delivery of existing knowledge to support implementation

***Task 2.1 Review and collate previously and currently used methods for communication of current knowledge on vine weevil management and consult growers on the effectiveness of these knowledge transfer methods*.**

HDC communications – research projects

The HDC has commissioned a large number of research projects focused on vine weevil biology and control. Reports from these projects along with other HDC publications, including factsheets, have been used in the review of key information on vine weevil biology and control (see Task 1.4).

Reviewing currently available information on the HDC website has identified 20 final reports or on-going HDC-funded research projects (Table 7). Of these projects, 10 were completed in the 1990s, three were completed in the 2000s and seven were completed or are on-going since 2010. Considered by topic, these projects have or are focussing on larval and adult controls as well as aspects of vine weevil biology. In some projects, vine weevil was one of a number of pests studied. Projects in the 1990s can be characterised by considerable interest in insecticides, mainly suSCon Green, to control vine weevil larvae. Since 2000 the focus has switched to biopesticides and specifically the entomopathogenic fungus *Metarhizium anisopliae* (also known as *Metarhizium brunneum*). In contrast, there has been little HDC-funded research on the use of entomopathogenic nematodes other than that completed in SF 15a and that currently underway in CP 089.

Each HDC-funded project is available from the HDC website as a report (typically as a final report), which includes a Grower Summary (up to six pages in length). The grower summary also typically includes a number of headlines and action points for growers.

Table 7. HDC reports or on-going projects.

Project No.	Project Title	Date of final report
CP 124	Managing Ornamental Plants Sustainably (MOPS) – Developing Integrated Plant Protection Strategies	On-going
CP 089	Maintaining the expertise for developing and communicating practical Integrated Pest Management (IPM) solutions for horticulture (EMT/HDC/HTA Fellowship)	On-going
FV 389	Combining biopesticides and other treatments to increase pest control	2013
SF/HNS 127	Characterising vine weevil aggregation pheromone for use in traps at soft fruit and nursery sites	2012
HNS 185	Understanding and managing crop protection through Integrated Crop Management	2012
SF/HNS 112	Evaluation of insecticides for control of adult vine weevil under controlled conditions	2011
SF 103	Evaluation of *Metarhizium anisopliae* for control of black vine weevil in field grown strawberries	2011
SF 104	Biopesticide product gap analysis and evaluation to support development policy for biopesticides for use in integrated soft fruit production	2009
HNS 133	Development of the entomogenous fungus, *Metarhizium anisopliae*, for control of vine weevil and thrips in horticultural growing media	2008
SF 15c	Biology and biocontrol of vine weevil (in soft fruit plantations)	2000
HNS 15d	Evaluation of reduced rates of suSCon Green and Intercept 5GR when potting on treated liners	1999
HNS 61	Vine weevil: evaluation of insecticides for control of adult weevils, under controlled conditions	1997
SF 15b	Predation on vine weevil in soft fruit plantations	1997
HNS 62	Determination of vine weevil oviposition sites in nursery stock	1995
HNS 15c	Hardy nursery stock: efficacy and persistence of suSCon Green against vine weevil in different growing media	1995
HNS 15b	Vine weevil: phytotoxicity screening of suSCon Green in different growing media	1994
SF 15a	Strawberries – control of vine-weevil with suSCon Green, insect-parasitic nematodes and insect-pathogenic fungi	1994
HNS 15a	Hardy ornamental nursery stock: chemical control of vine weevil	1993
HNS 15	Influence of fungicides and insecticides on the entomogenous fungus *Metarhizium anisopliae*, a pathogen of the vine weevil, *Otiorhynchus sulcatus*	1992
SF/15/87	Chemical treatments applied to module/pot – raised strawberry runners	1990

HDC communications – HDC News

All HDC research projects now require an article to be published in HDC News, summarising the key results and implications for growers. There is no index available for HDC News articles, but recent articles on vine weevil projects are given in Table 8.

Table 8. HDC News articles.

Title	Project	Date of publication
Agents of their own destruction	CRD project PS2140	February 2014
New researchers earn their wings	CP 89	April 2013
Vine weevils run out of places to hide	CRD project PS2134	March 2012

HDC communications - factsheets

In addition to HDC-funded research projects, the HDC has also published a number of factsheets, available from the HDC website Table 9). Two of these factsheets provide information on vine weevil biology and control in hardy nursery stock (02/03) and soft fruit crops (01/03). A third factsheet provides information on the host-plant range of this pest (18/10). Several other factsheets cover a range of pests, including sections that provide information on vine weevil biology and control. Each of these factsheets, which range in length from five to 13 pages, is available from the HDC website.

Table 9. HDC factsheets.

Factsheet No.	Factsheet Title	Date of publication
07/11	Beetle and weevil pests of cane fruit crops	2011
18/10	Host plant range of vine weevil	2011
24/11	Successful bed re-planting	2011
06/08	A guide to best practice in handling bought-in plants	2008
14/06	Guidelines and best practice for pesticide spray application in protected ornamental crops	2007
02/03	Vine weevil control in hardy nursery stock	2003
01/03	Vine weevil control in soft fruit crops	2003

HDC communications – other publications

Complementing the HDC factsheets are a range of other HDC publications (Table 10). These include five Crop Walkers' Guides (see Table 10) that provide concise information required to successfully identify a vine weevil infestation. In addition, there are grower guides to Soft Fruit, Biocontrol in Soft Fruit and Production of Container Grown Plants, which are available on the HDC website. As with the HDC factsheets these printed publications are liable to become out of date, particularly in terms of the controls that may be used. In this respect the Herb Best Practice Guide, which is an online resource on the HDC website, provides concise information on vine weevil and other pests and their control within a best practice IPM programme and this is regularly updated, making this is valuable up-to-date resource for growers.

Table 10. Other HDC publications.

Title	Date of publication
Common pests and diseases of hardy nursery stock (poster)	2013
Crop Walkers' Guide – strawberry	2013
Biocontrol in soft fruit - a grower guide	2012
A grower guide – soft fruit	2012
Crop Walkers' Guide – hardy nursery stock	2012
Herbaceous perennials: A guide to the production of container grown plants	2010
Crop Walkers' Guide – pot and bedding plants	2008
Crop Walkers' Guide – cane fruit	2008
Crop Walkers' Guide – bush fruit	2008
Protected herbs Best Practice Integrated Pest and Disease Management Guide (http://herbs.hdc.org.uk/)	current

Grower workshops and discussion groups

There have been no HDC-funded seminars specifically on vine weevil, however vine weevil biology and management have been discussed together with that of other pests, in various Integrated Pest and Disease Management (IPDM) workshops for ornamentals and herbs growers over the past 20 years, funded by either or both HDC and Defra. The most recent of these were delivered by ADAS and funded by the Rural Development Programme for England (RDPE) for which Defra is the Managing Authority, part funded by the European Agricultural Fund for Rural Development: Europe investing in rural areas (Table 11).

Table 11. RDPE workshops 2013/2014

Title	**Location**	**Date**
IPDM workshop ornamentals	Palmstead Nursery, Kent	12 Sep 2013
IPDM workshop ornamentals	Growtrain and Walberton Nursery, Kent	3 Oct 2013
IPDM workshop ornamentals	Wyevale Nursery, Hereford	24 Oct 2013
Understanding raspberry production	Rectory Farm, Oxford	31 Oct and 14 Nov 2013
IPDM workshop soft fruit	EMR	5 Nov 2013
IPDM workshop soft fruit	ADAS Drayton	7 Nov 2013
Christmas trees	High Wycombe	4 Feb 2014
IPDM workshop herbs	Stoneleigh Park	27 Feb 2014

Jude Bennison and Gemma Hough (ADAS) presented an update on vine weevil control and recent and current research at the HDC Herbaceous Perennial Perennials Technical Discussion Group on 18 February 2014.

Technical information provided by suppliers of insecticides and biological control agents for vine weevil management

Information is given by suppliers of insecticides and biological control agents for the control of vine weevil on product labels and leaflets, supplied with the products and also available on the suppliers' websites (Table 12).

Table 12. Links to websites of suppliers of insecticides, entomopathogenic nematodes and entomopathogenic fungi for control of vine weevil.

Product (active ingredient)	**Marketing company**	**Information**	**Web page**
Intercept 70 WG (imidacloprid)	Everris	Label	http://www.everris.com/uk/Home/Ornamental-horticulture/Products/Product.aspx/Plant-ProtectionOH/InsecticidesOH/_/_/P50020
Intercept 5GR (imidacloprid)	Everris	Label	http://www.everris.com/uk/Home/Ornamental-Horticulture/Products/Product.aspx/Plant-ProtectionOH/InsecticidesOH/_/_/013283
Imidasect 5GR (imidacloprid)	Fargro	Label	http://www.fargro.co.uk/products/agrochemicals/imidasect_5gr/imidasect_5gr.asp
Exemptor (thiacloprid)	Everris	Label	http://www.everris.com/uk/Home/Ornamental-Horticulture/Products/Product.aspx/Plant-ProtectionOH/InsecticidesOH/_/_/P50038
Example products: Dursban WG and Equity (chlorpyrifos)	Dow AgroSciences	Labels	http://uk.dowagro.com/products/dursban-wg/ http://uk.dowagro.com/products/equity/
Nemasys L (*Steinernema kraussei*)	BASF	Technical sheet	http://www.agricentre.basf.co.uk/agroportal/uk/en/products/products_a_z/product_files_419.html
Exhibitline sk (*Steinernema kraussei*)	Syngenta Bioline	Technical sheet	http://www.syngenta.com/global/Bioline/en/products/allproducts/Pages/Exhibitlinesk.aspx
Nemasys H (*Heterorhabditis bacteriophora*)	BASF	Technical sheet	http://www.agricentre.basf.co.uk/agroportal/uk/en/products/products_a_z/product_files_419.html
Exhibitline H (*Heterorhabditis bacteriophora*)	Syngenta Bioline	Technical sheet	http://www.syngenta.com/global/Bioline/en/products/allproducts/Pages/Exhibitlineh.aspx
Larvanem (*Heterorhabditis bacteriophora*)	Koppert	Technical sheet	http://www.koppert.com/products/products-pests-diseases/products/detail/larvanem-3/
Nematop (*Heterorhabditis bacteriophora*)	e-nema	Technical sheet	
SuperNemos (mix of *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* or *H. megidis*)	Flowering Plants Ltd	Application information	http://www.supernemos.com/how-to-apply/
Met52 (*Metarhizium anisopliae* also known as *Metarhizium brunneum*)	Fargro	Technical update/notes and product manual	http://www.fargro.co.uk/products/agrochemicals/met52/met52.asp

In June 2014, Fargro produced a technical leaflet 'A Practical Guide to controlling vine weevil on ornamental nurseries, which is available from Fargro and is on their website, http://www.fargro.co.uk

In addition, BASF supply the following illustrated technical leaflets on application methods for entomopathogenic nematodes, which are available from their distributors and these are attached in Appendix-2, 3 and 4:

- Storing and mixing entomopathogenic nematodes (Appendix-2)
- Positioning of drippers for application of Nemasys®L (Appendix-3)
- Step-by-step guide to apply nematodes using a Dosatron (Appendix-4)

Agrovista also supply the following illustrated technical leaflet on the key features of Nemasys L and Nemasys H and when they should be used including best practice.

- Black Vine Weevil (*Otiorhynchus sulcatus*) Control Using Nematodes (Appendix-5)

Consultancy visits and back-up

Many growers use consultants for advice including biological control suppliers and distributors, ADAS and other private consultants. Feedback on the value of consultancy visits and telephone/email back-up is given under Task 2.2 below.

Task 2.2 Identify more effective knowledge transfer methods

When growers and consultants were interviewed about their current vine weevil management strategies in Task 1.1, they were also asked to comment on the effectiveness of the various knowledge transfer methods, which ones they prefer and which they would find most helpful in supporting the implementation of vine weevil control strategies. They were also asked to highlight any gaps in supply of information and to suggest potential improvements. The industry responses are summarised below.

HDC reports

- Most growers said that they read the Grower Summaries but that the full reports are too long and more suitable for advisers.
- Some growers said that reports are very good and effective for the keen grower but not the most effective for the average grower.

- One grower commented that he reads reports if HDC News highlighted something of interest.
- Consultants commented that reports are sometimes too technical for most growers and that they do not hear of many growers reading them. One commented that reports would be better circulated as a pdf rather than published on the HDC website.
- A supplier agreed that reports were an excellent tool but not read as widely by growers as one might wish. It was suggested that more succinct summaries should be provided, possibly just one page of bullet points / actions. Reports should highlight additional benefits to financial ones e.g. benefits to compliance, resistance management and sustainability.

HDC website

- Growers fell into the 'for' and 'against' camps. Some thought it was very good, easy to navigate and useful to find reports and old publications, factsheets and news articles. Others said they don't use it, they sometimes struggle to log in or navigate the site, and that it was more useful for businesses with younger generation staff.
- Most consultants found the website useful if they needed to refer to a report but agreed with growers and suppliers that the search facility needs improving.
- Suppliers found the website very useful but thought it would only reach a proportion of growers. Some found the site easy to navigate but agreed with other users that searching for information can be difficult.

HDC News

- Most growers said that HDC News was good, giving useful summaries of research, and more suitable for growers who are not comfortable with computer technology.
- Consultants agreed that HDC News is good for awareness of current research and that growers have commented on some of the articles. An example of an article that growers gave favourable feedback on was the one on using UV to track the movement of adult weevils (this article, in March 2012, summarised the CRD-funded project PS2134 on using natural weevil aggregation behaviour to pick up and disseminate entomopathogenic fungi).
- Suppliers thought HDC News was good, written in an informal journalistic style and often encourages growers to look at project reports of interest.

HDC Factsheets

- Most growers felt that Factsheets were good, well used reference documents and an effective method of communication.
- Growers commented that they were good for the average grower, easy to read and absorb, giving useful summaries which can be distributed to staff.
- Some growers commented that Factsheets can quickly get out of date with regard to approved pesticides and that they need to be regularly updated.
- Most consultants felt that Factsheets were very good and useful reference documents.
- Suppliers agreed that Factsheets are very good, succinct and easy to read, but those for priority issues such as vine weevil should be updated more frequently.

Other HDC/other funder Communications, workshops and seminars

- The soft fruit biological control handbook was found to be very useful by soft fruit growers.
- The Crop Walkers' Guides were described as 'fantastic' by one supplier.
- Reminders sent out to soft fruit growers by Scott Raffle in the weekly emails were reported to be useful, e.g. alerting growers that vine weevil adult activity had started. A grower commented that similar reminders should be sent to HNS growers.
- Most growers felt that specific workshops and seminars are very effective, and possibly the best way to engage with growers, particularly if carried out on a nursery or farm with grower participation in order to include experience from other growers.
- A few growers said they find it difficult to get to events so would prefer written information to be available.
- Consultants and suppliers agreed that workshops and seminars are an effective communication method if they include grower interaction, but commented that they are not always effective in reaching a large audience. A soft fruit consultant commented that events would be welcomed and well attended in Scotland.

Consultancy visits

- Those growers that used consultancy visits from ADAS, biological control suppliers and other HNS or soft fruit consultancies found them to be very useful, many growers commenting that advisers were their main source of technical information.
- Suppliers commented that consultancy visits play a vital role in disseminating information to growers on best practice, up to date research results and industry trends.

- Consultants agreed that visits and technical back-up from an experienced adviser is key to successful pest management and uptake of new technology.

ADAS Fruit Notes / Nursery Stock notes / other consultancy notes

- Those growers who receive ADAS Notes reported them to be very useful, giving topical technical information and notes on legislation.
- Suppliers and consultants also found ADAS Notes to be very good and useful.
- Soft fruit growers commented that other consultancy notes or leaflets e.g. the DLV technical booklet, Agrovista leaflets and FAST Notes are helpful and practical.
- A supplier comments that John Adlam's HNS notes in Horticulture Week are useful and that technical notes from all sources play a vital role in maintaining the relationship between the crop protection industry, consultancy services and growers

Product labels / leaflets

- Growers reported product labels and leaflets gave essential information.
- Some growers said that they consult an adviser or technical notes/books rather than the product leaflets.
- Several growers felt that clear best-practice application methods for using entomopathogenic nematodes was not always given on product leaflets inside the packs and that this was needed. However some growers also wanted essential information to be brief and simple.
- Suppliers reported that growers may not read the information provided with products as they frequently get asked for information that is given in the product label or leaflet.

Supplier websites / newsletters / events e.g. product launches

- Suppliers felt that websites are a standard market requirement and that they recognised the need for information to be easily found, accurate and up to date.
- Some growers use supplier websites and news articles/updates and found them useful, others reported that they do not use them.
- Angus Soft Fruit reported that they have done joint workshops with suppliers that were well-received by growers.
- Suppliers felt that product launches and having stands at trade events were important opportunities for interaction with growers.
- Some growers felt that product launches and supplier presence at industry events tended to have a strong marketing slant.

Other sources of information

- Growers reported using Google and overseas visits to other growers and events to source information and keep up to date.

Grower suggestions for communication methods for vine weevil management information

- Many growers felt that a vine weevil seminar/workshop/open forum with growers, with a practical session and a handout would be useful and that they would attend one if it was well-located and timed e.g. late January / early February.
- One HNS grower suggested that it would be useful to hold a short vine weevil seminar at a well-attended event such as Four Oaks, which most growers of ornamentals attend.
- Growers in Scotland said that events in Scotland would be supported and well-attended, and a grower in Wales suggested an event organised through the Welsh Assembly.
- Many growers thought that a demonstration of practical application on a commercial nursery / farm would give them confidence in specific products or methods.
- Most growers supported one or more Factsheets that are regularly updated.
- Some growers supported the idea of a vine weevil section on the HDC website, including videos of vine weevil behaviour, action points summaries and practical tips.
- One grower suggested an online interactive tool, where growers and advisers could input monitoring information, planned treatments and products, possibly for use only by members to flag up early warnings and seasonal trends.
- One growers suggested timely texts to growers at key times of year as reminders of vine weevil action points.
- Some growers suggested a combination of several communication methods in order to reach the maximum number of growers.
- Consultants supported an updated factsheet, interactive seminar and/or workshops with hands-on sessions, a vine weevil section on the HDC website, articles in HDC News, and emailing HDC members with a factsheet pdf attached.

Objective 3. Design 'best-practice' IPM protocols suitable for implementation on susceptible crops in each relevant horticultural sector

Task 3.1 Draft protocols

Using the information on vine weevil biology and control collated in Objective 1, two flow charts were produced, one for containerised ornamentals and one for soft fruit, summarising key decisions and options for vine weevil management within an IPM programme. HDC and the industry representatives were consulted before confirmation of the charts.

Task 3.2 Confirm protocols after consultation with key industry representative

Following consultation with the HDC and the industry representatives, the flow charts were confirmed. Each chart is presented in two parts, one for early season and the other for mid-late season (see Figures 1a and 1b (ornamentals) and 2a and 2b (soft fruit). Options for the various components of the IPM programmes are summarised in Table 1.

Table 1. Summary of components of IPM programmes for containerised ornamentals and soft fruit

IPM component	Containerised ornamentals	Soft fruit
Monitoring	Check around roots for larvae March-November, check again 2-4 weeks after nematode application to guide repeat applications Check for adult activity and damage April-October	
Cultural control	Dispose of badly infested plants and growing media, keep weeds controlled and maintain nursery hygiene	As for ornamentals, also consider removing polythene mulch, and using barrier glue on table-top legs
Entomopathogenic nematodes - timing	Apply as drench in April if live overwintered larvae found, repeat in August-November to control larvae hatching from summer and autumn-laid eggs if temperatures suitable (2 applications may be needed)	In substrate crops, apply by drip-irrigation in April if live larvae found and temperatures suitable, repeat in August-September (2 applications may be needed). Or consider the 'little and often' approach (low rates applied monthly April-October). Research is justified to validate this approach.
Entomopathogenic nematodes - temperatures	*Steinernema kraussei* (Nemasys L, Exhibitline sk) 5-30°C *Heterorhabditis bacteriophora* (Nemasys H, Exhibitline h) 12-30°C *H. bacteriophora* (Larvanem) 14-33°C *H. bacteriophora* (Nematop) minimum 12°C Mix of *Steinernema carpocapsae, S. feltiae* and either *H. bacteriophora* or *H. megidis* (SuperNemos) minimum 10°C	
Met52	Consider incorporation in growing media for spring/summer pottings. Minimum temperature for activity against larvae 15°C. Unlikely to be effective against larvae hatching September-November from late-laid eggs	Consider EAMU 1997/2011 for use in a mulch, e.g. to plants in large pots
Chemical control - adults	Consider foliar spray(s) against adults in April-May (overwintered adults) or June/July (new adults). Chess WG (EAMU 2834/2008 for protected ornamentals) or Steward (EAMU 2905/2008 for outdoor ornamentals) are more IPM-compatible than other pesticides and showed promise in HDC semi-field trial. (Lower, on-label or other EAMU application rates than those in the above EAUMUs have not been tested). Efficacy in commercial conditions needs validation.	Timing as for ornamentals. Chess WG (EAMU 2834/2008 for protected crops) or Steward (EAMU 2905/2008) on outdoor, uncropped soft fruit where a 1-year harvest interval is possible i.e. plants in propagation) are more IPM-compatible than other pesticides. Comments on efficacy at rates in other EAMUs as for ornamentals.
Chemical control - larvae	Consider thiacloprid (Exemptor) incorporation into peat-based growing media. Imidacloprid (Imidasect 5GR or Intercept 5GR only in peat-based growing media in glasshouses, do not move outside until after flowering).	Consider chlorpyrifos drench to strawberry after cropping if sufficient soil moisture and temperatures above 5°C

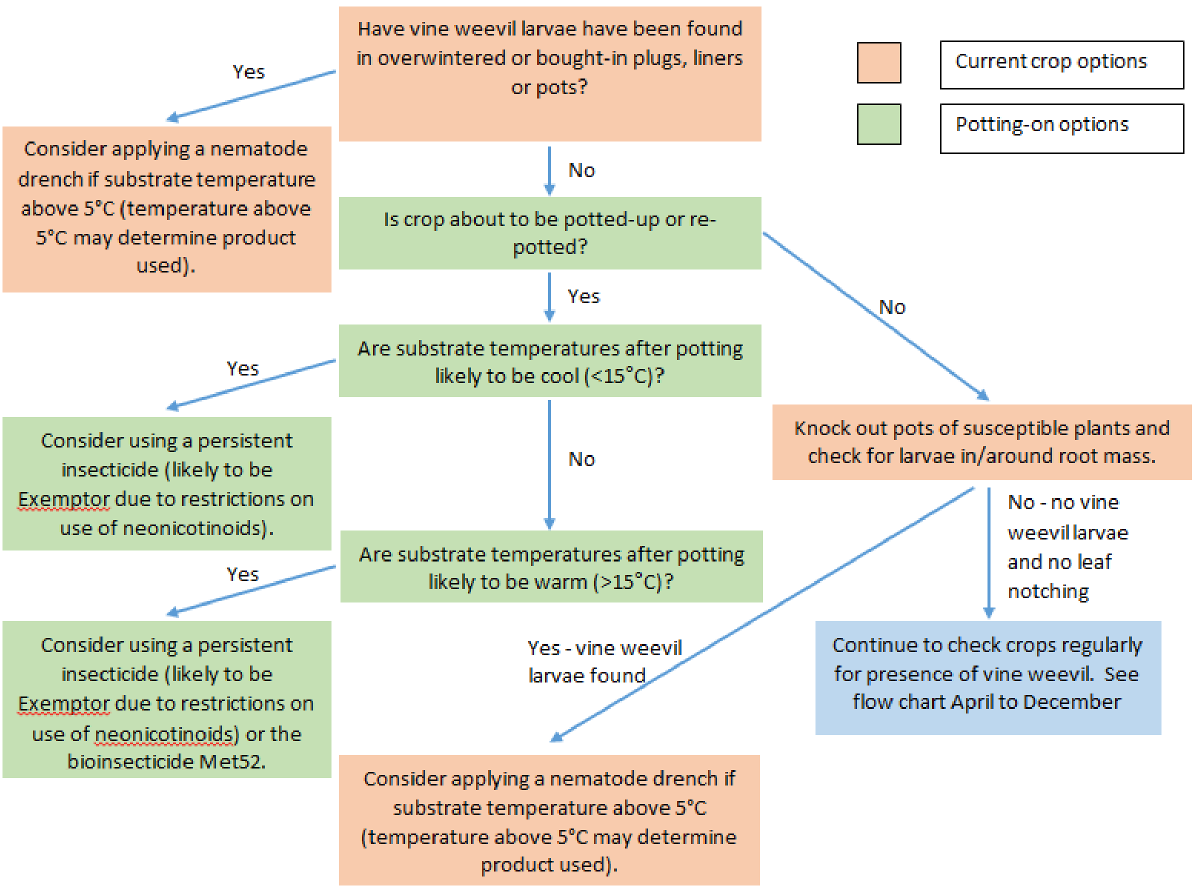

Figure 1a. Early season (January to April) decisions in vine weevil management on susceptible containerised ornamentals.

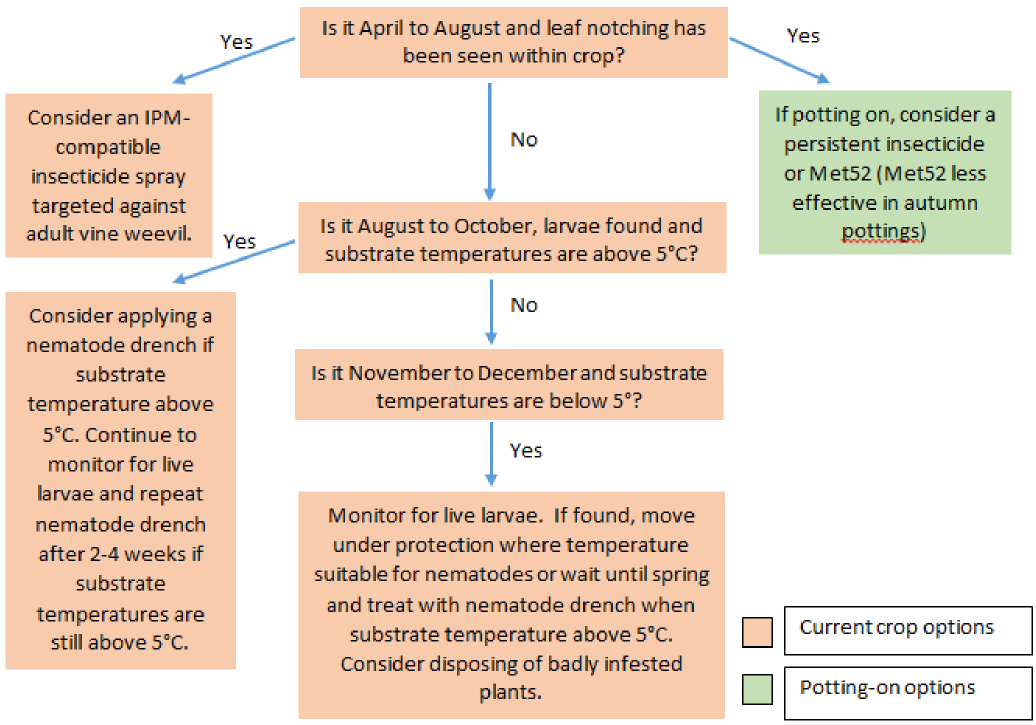

Figure 1b. Mid to late season (April to December) decisions in vine weevil management on susceptible containerised ornamentals.

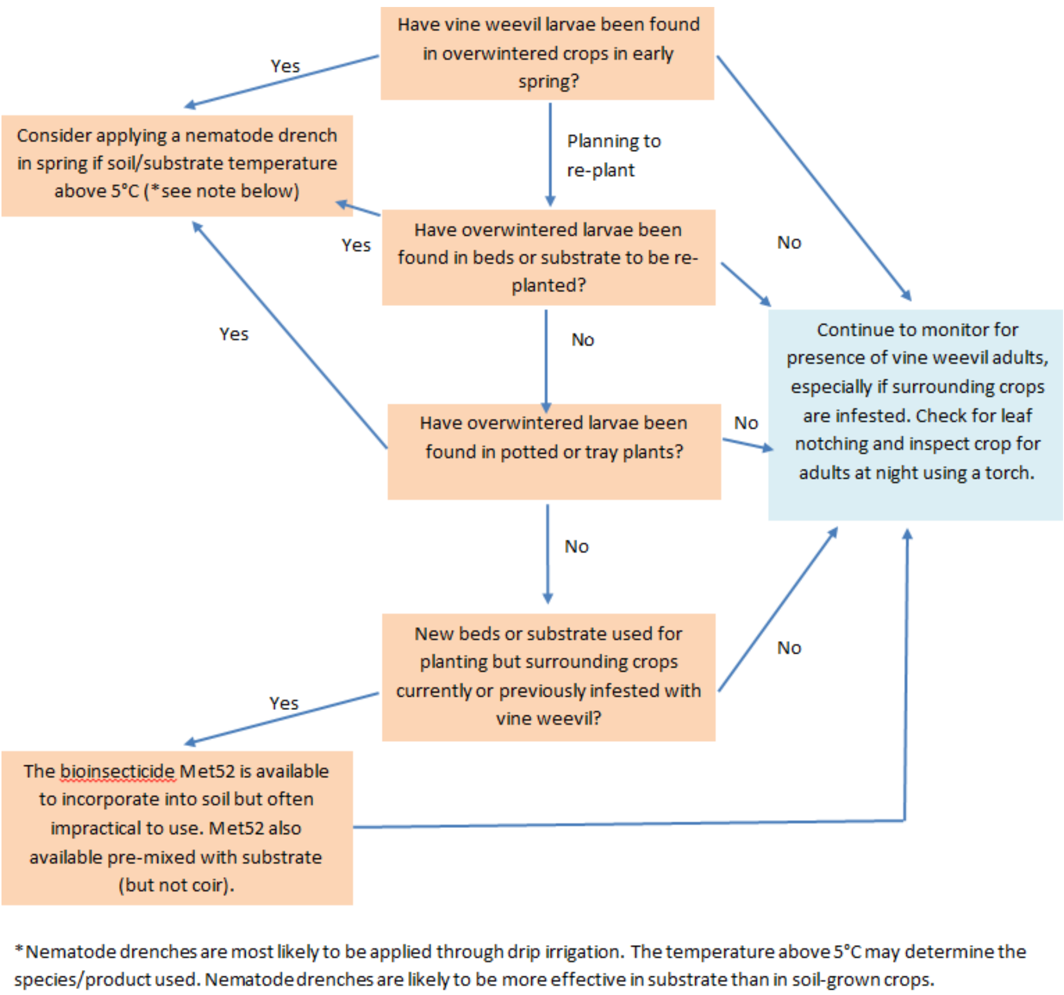

Figure 2a. Early season (February to April) decisions in vine weevil management on soft fruit crops.

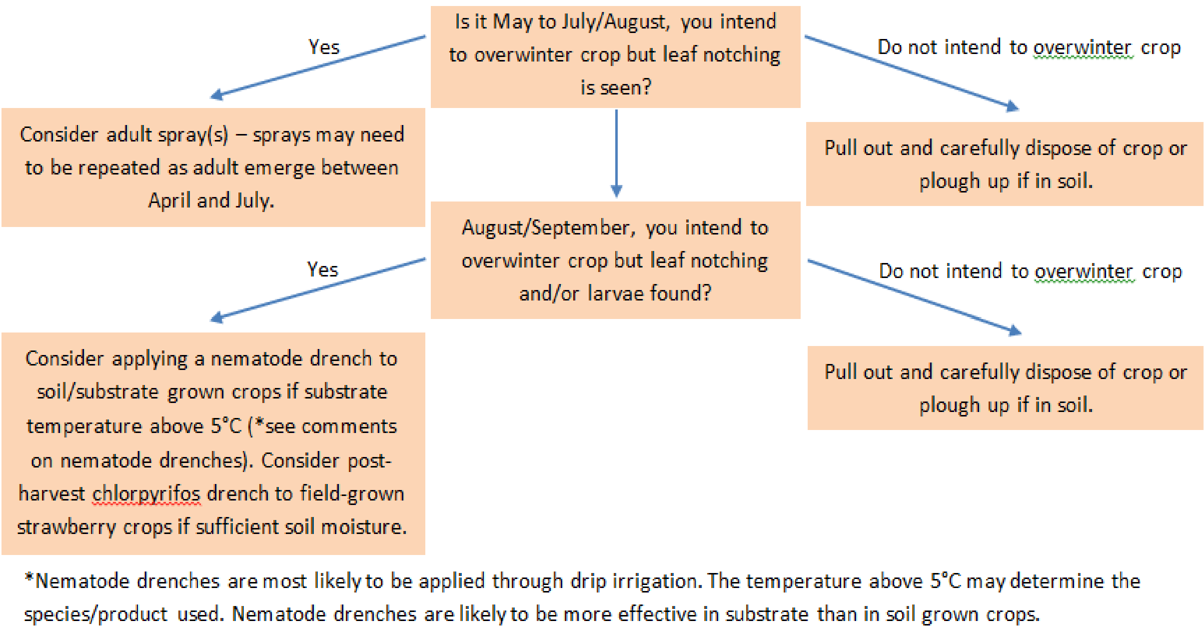

Figure 2b. Mid to late season decisions (May to September/October) in vine weevil management on soft fruit crops.

Objective 4. Provide plans for communicating relevant knowledge and IPM protocols to growers in each relevant sector

Task 4.1 Draft communication plans

4.1.1. Communication methods delivered within this project

Communication methods delivered within this project are as follows:

- Final project report to HDC
- Two HDC News articles, one on control of adult vine weevils and an update on the neonicotinoid restrictions, in June 2014. Another on vine weevil biology and control with biological control agents in July 2014.

4.1.2 Additional suggested communication methods

Following completion of this project, additional communication methods on vine weevil biology and management are suggested below, taking into account feedback and suggestions from growers, consultants and suppliers:

- HDC have invited the project leader to give a presentation on vine weevil management on containerised ornamentals at the HDC Herbaceous Perennials Technical Discussion Group on 9 July 2014, focussing on the IPM protocol and gaps in knowledge identified in the project.
- Vine weevil seminars or workshops to be held in England, Scotland and Wales, at locations and times agreed with HDC and growers. Events to include presentations and interactive demonstrations by the project team, vine weevil control suppliers and other researchers and consultants.
- Factsheets to be updated for both soft fruit and HNS/protected ornamentals
- Vine weevil section on the HDC website, carefully designed to allow easy navigation and access to key information. Section to include key knowledge of vine weevil biology, IPM protocols, biological and chemical control options, seasonal action points and practical tips.
- Emails/texts to growers with seasonal vine weevil alerts and action points
- Practical demonstration of current best-practice application methods for vine weevil control on a soft fruit farm and HNS nursery.

Task 4.2 Confirm communication plans

The draft communication plans listed in 4.1.2 above will be confirmed after discussions with HDC, the industry representatives and selected growers and other industry members in relevant sectors. After submission of this report, the project team will meet with key HDC staff, the industry representatives and other industry members to discuss the conclusions of the project and research and communication priorities to fill gaps in knowledge.

Knowledge and Technology Transfer

Knowledge transfer activities within this project included:

- Final project report to HDC
- Two HDC News articles, one on control of adult vine weevils and an update on the neonicotinoid restrictions, in June 2014. Another on vine weevil biology and control with biological control agents in July 2014.

Appendices (available separately)

Appendix 1. Literature review database

Appendix 2. BASF technical note on storing and mixing entomopathogenic nematodes

Appendix 3. BASF technical note on positioning of drippers for application of Nemasys L to pots

Appendix 4. BASF technical note on applying entomopathogenic nematodes using a Dosetron

Appendix 5. Agrovista technical note on vine weevil control using nematodes

