SCEPTREPLUS

Final Review Report

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Title:	**Glasshouse mealybug (*Pseudococcus viburni*): a review to identify potential IPM-compatible control measures in protected edibles**
Crop	Protected Edibles
Target	*Pseudococcus viburni* (PSECOB)
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I the undersigned, hereby declare that the work was performed according to the procedures herein described and that this report is an accurate and faithful record of the results obtained

16.12.2019 J. A. Banfield-Zanin

Date Authors signature

Review Summary

Introduction

The glasshouse (or obscure) mealybug (*Pseudococcus viburni*) is an important, and often chronic, pest of many protected edible crops. Infestations can impose considerable pressure on production in the UK, as the pest is capable of causing substantial damage to plants through direct feeding damage and by causing visual fouling of crop product.

Although certain control options have shown potential against individual mealybugs under laboratory conditions, at a commercial scale populations have proven extremely difficult to manage due to a combination of their behaviour and morphology, with remedial control particularly challenging to achieve. Female and late-instar mealybugs are covered in white, waxy filaments that, combined with their concealed habit and cryptic nature, protect them from, and make them resilient to, many products that would have been expected to be effective treatments. Under suitable conditions, mealybugs have a high reproductive rate and this fecundity allows rapid resurgence of populations after treatments. Finally, they have been shown to survive between crops on inorganic surfaces such as roof support bases, dwarf walls or irrigation drippers, and readily move to new areas on infested plant and packing material or equipment, such as irrigation drippers, if these have not been thoroughly cleaned and sterilised beforehand.

Several on- and off-label plant protection products are available, but these often have limited efficacy and are, broadly, often not compatible with organic systems or are challenging to effectively and safely utilise within existing IPM programmes. Control measures utilising natural enemies or other soft chemicals often prove inadequate, especially when infestation is severe. Physical control methods can be effective, especially when used as means to clean down and sterilise between crops, but these options are often labour intensive and thus expensive.

A series of previous AHDB (then HDC) projects, PC 161 ("Protected tomato: Integrated control of mealybugs"), PC 215 ("Tomato: Further development of sustainable mealybug control strategies") and PC 240 ("Organic tomato: Development and implementation of a robust IPM programme"), focused on integrated, IPM programmes and physical means of minimising the number of mealybugs surviving between crops and infesting newly-planted crops, particularly within organic tomatoes.

It remains of continued importance that UK growers have access to IPM-compatible control measures against *P. viburni* in protected edible cropping systems. Since the last AHDB-funded work was published in 2009, the list of potential and approved actives, and the regulatory environment, have changed, and with this in mind the overall objective of this review is to explore understanding of current and potential options for control and monitoring of *P. viburni*. In the preparation of this current review, particular attention has been paid to developments in terms of monitoring populations, biological control agents, biopesticides, chemical insecticides and basic substances.

Summary

Monitoring

Monitoring efforts for glasshouse mealybug are a key component in any management strategy for their control. Monitoring remains primarily reliant on laborious, time-consuming visual examination and sampling of plant material for mealybug presence, complicated by their cryptic habits and tendency to settle in protected areas of plants. Such monitoring can also result in inaccurate estimates of pest density in a crop, particularly early in a growing season. The sex pheromone for glasshouse mealybug was identified and synthesised in 2005, and work since has shown pheromone-baited traps to hold potential for deployment as a potentially more cost-effective monitoring tool in outdoor crops when combined with sticky traps. The pheromone, however, remains relatively expensive, though research to optimise lure dose rates may encourage and support commercial development. Any such system would need to be optimised for use under glass (in terms of lure dose rates and distribution throughout a crop), but should glasshouse mealybug-specific lures become commercially and readily available this would likely warrant investigation as a cost-effective means of monitoring populations, with the potential to then inform further management strategies and treatments within a growing season. Recent work on optical spectroscopy has also shown some promise but is still relatively early in terms of development and requires considerable early R&D at the time of writing.

Cultural control and management

Cultural management of glasshouse mealybug continues to play an important role and is particularly important in limiting the number of introductions or transfers of mealybugs to uninfested areas or crops, and in minimising the numbers of invading crawlers colonising plants early in the growing season. Practices are well-established and applicable across a range of crops, and there does not appear to be any significant advance in such practices mentioned in the literature at time of writing. Broadly speaking, cultural management focuses on good plant and equipment hygiene, careful cleaning of equipment and glasshouse facilities between crops, removal of 'green bridges' and refuges (such as volunteer crop seedlings) across which mealybug crawlers can walk to access new plants, rigorous implementation of strategies to manage potential mealybug introductions across different areas and quarantining areas of existing infestation. The efficacy of such measures, and development of a protocol to minimise survival and colonisation of plants were assessed as part of previous AHDB trials PC 161, PC 215 and PC 240.

Natural enemies

A variety of mealybug natural enemies are currently available commercially in the UK. The most well-known of these is the mealybug ladybird, *Cryptolaemus montrouzieri*, a relatively generalist predator. Lacewings can be released as a biocontrol agent for control of mealybug, and larvae, in particular, are good predators of smaller mealybug life stages. Various species of parasitoid wasp are also available, and in particular *Leptomastix epona* is used for control of *P. viburni*. Efficacy of natural enemies and, in particular, parasitoid wasps can be variable and limited, given the cryptic nature of the pest and defensive behaviours deployed by mealybugs when attacked. The glasshouse mealybug, in particular, appears to engage in relatively higher rates of such defensive behaviours in response to natural enemies, which may explain why attempts at control can lead to variable results. Several other generalist predator species, such as *Orius* and some predatory flies, have been reported to attack mealybug populations in field environments, and may provide some additional limited control in protected edibles. There is also interest, particularly in South Africa, in the use of entomopathogenic nematodes for control of mealybug pests. Although such research highlights the importance of nematode strain for efficacy, nematode application may nonetheless provide some level of

control in the UK protected crops industry, especially when combined with the use of adjuvants to improve nematode efficacy. Control could only be achieved, however, with careful and judicious selection and use of application technology, given the challenges of achieving good foliar application of a natural enemy that is typically targeted at soil pests.

Basic substances

A broad range of physically acting 'biorational' products are available, although these show variable efficacy when used against mealybug pests. Such products are typically targeted at smaller, soft-bodied insect pests and often rely on direct contact with the pest and good coverage for efficacy, which can be impeded by the tendency of glasshouse mealybug to hide in sheltered areas. Furthermore, although many such products usually have limited residual activity, they are typically broad-spectrum and require repeat, short-interval applications to achieve efficacy, posing a risk to IPM programmes. Such products show little promise as a stand-alone solution for mealybug control, but there is evidence to suggest that they could act as useful components of a control programme. They may also improve the efficacy of other insecticidal products against mealybugs, particularly when these are used in combination with basic substances which result in dewaxing of the mealybugs, improving contact by the following treatments. Considered evaluation of these products could be undertaken within the context of overall glasshouse IPM programmes, with particular care given to their impact on natural enemy releases and crop safety.

Biopesticides

Of the different classes of biopesticides, botanically-derived products may hold particular promise against *P. viburni*, though further work is needed to confirm efficacy at a commercial scale. In addition, control may not be complete where these are used in isolation. Although the literature suggests varied results and levels of control, these have been improved with judicious selection of adjuvants, with mortality levels determined by type of adjuvant depending on crop and mealybug species. The increasing number of such botanical products making their into the UK market, however, suggests that further investigations may well be warranted, though care must be taken to ensure commercially-relevant methodologies are deployed in trials, as the practicalities of field use (and application technology selection) may be more limiting to efficacy than the activity observed under laboratory conditions. Entomopathogenic fungi, particularly when applied with adjuvants, may also provide a useful tool, though again they are unlikely to achieve acceptable levels of control or complete control. Further evaluations, as with botanically-derived biopesticides, would need to be carried out within a commercially relevant setting.

Chemical insecticides

A range of different chemistries are currently available on- or off-label for control of mealybug pests in protected edibles, dependent on crop. Many of these have variable or limited efficacy when used against mealybugs, relying on good coverage and contact with the pest for maximal control. Studies have also shown that systemic insecticides, particularly when applied through irrigation, do not meet expectations, and it has been suggested that this is due to differences in mealybug feeding behaviours and locations when compared to other soft-bodied hemipteran pests such as aphids and whiteflies. Nevertheless, a number of actives remain that could provide relatively good efficacy against *P. viburni*, though consideration must be given in evaluations of these to harvest intervals and any potential implications or

impacts on existing IPM strategies. Several such products are either already available for use in protected crops, though against other target pests, or are approved in the EU. A number have also been shown to have greater efficacy against immature life stages of mealybug pests or to result in sub-lethal effects to mealybug reproduction, so targeted applications as part of an integrated strategy, with monitoring of the pest lifecycle to inform spray timings, may be the best approach.

Next Steps

- A number of biopesticidal products, particularly of botanical origin, have been identified with potential for good efficacy against *P. viburni*. Trials evaluating products, that are already on the market or are close to market, for efficacy may be useful to industry, though careful consideration of application methods and timings will be essential to maximise control, particularly in high-wire and layered crop systems.
- Several conventional insecticides, particularly relatively novel chemistries, have also shown potential for control of *P. viburni*. Validation of their full potential in full-scale glasshouse trials may be useful, but timing of application, in terms of targeted life stages, and harvest intervals will need to be incorporated, as will consideration of application method for appropriate coverage, particularly within high-wire and layered crop systems.
- Monitoring potential developments in terms of commercial availability of *P. viburni* sex pheromone lures should be undertaken, with a view to developing a monitoring strategy should these become available, in order to inform management and spray programmes.

Take home message(s)

- Obscure mealybug, and mealybugs more generally, remain a challenging and chronic pest for the UK protected edible sector. Remedial control is particularly challenging due to the concealed nature of the pest, the protection given by the waxy coating that develops as individuals move through their lifecycle, and high reproductive rates that allow rapid resurgence of populations.
- Monitoring and cultural management remain an important aspect of control programmes. Thorough hygiene and quarantine protocols that are strictly adhered to remain an effective means of limiting spread to uninfested areas. Careful cleaning down between crops remains important in limiting the survival of obscure mealybug between crops and reducing the numbers invading a new crop, though challenges arise in hydroponic and layered systems.
- Natural enemies which are commercially available remain the most effective means of biocontrol, but should be used as part of a control strategy and in combination.
- A range of on- and off-label products are available for mealybug control, though these often have limited or variable efficacy. There is potential to develop IPM programmes further based on relatively new biopesticidal and chemical options, but efficacy under commercial glasshouse conditions still needs to be validated.
- Immature stages of the lifecycle are often the most strongly affected by product applications. Monitoring of the crop to ensure targeting of the motile crawlers may help improve efficacy. Adjuvants or dewaxing agents can also help improve control, though results can be varied.

Review

Introduction

The glasshouse (or obscure) mealybug, *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae), is a polyphagous pest of a broad range of protected edible and ornamental horticultural crops, capable of causing substantial damage to plants and thus can present a significant economic burden following infestation. Although unable to spread widely by its own means, *P. viburni* has nonetheless become widespread globally, having been transported and spread across long distances very effectively on infested plant material from an early date. It is a resilient species, having adapted to a broad range of ecological conditions, and, in higher latitudes where it cannot survive winter conditions due to frost intolerance, it thrives in the protected conditions encountered in glasshouses. Currently believed to originate from South America (Charles, 2011), the species is also known under several synonyms (including *Dactylopius viburni* (Signoret), *D. affinis* (Maskell), *P. affinis* (Maskell), *P. obscurus* (Essig), *P. nicotianae* (Leonardi) and *P. longispinus latipes*, to name a few), and was confused with several other *Pseudococcus* species (such as *P. maritimus* (Erhorn) and *P. longispinus* Targioni Tozzetti, among others) until around 1970. Differentiation from *P. maritimus* remains challenging owing to the high morphological variability in the species, and it is possible that *P. viburni* as presently recognized may comprise several cryptic species identifiable through molecular analyses only (Charles, 2011).

Pseudococcus viburni imposes an important pressure on protected edible production in the UK, particularly in organic tomato crops and peppers, but increasingly in other crops (such as aubergine or cucumber). Although control options have shown considerable potential against individual mealybugs under laboratory conditions, at a commercial scale populations have proven extremely difficult to manage due to a combination of the pest's behaviour and morphology. Female and late-instar mealybugs are covered in white, waxy filaments that, combined with their concealed habit and cryptic nature, protect them from, and make them resilient to, many products that would have been expected to be effective treatments. Under suitable conditions, mealybugs have a high reproductive rate and this fecundity allows rapid resurgence of populations after treatments. Finally, they have been shown to survive between crops on inorganic surfaces such as roof support bases, dwarf walls or irrigation drippers, and readily move to new areas on infested plant and packing material or equipment, such as irrigation drippers, if these have not been thoroughly cleaned and sterilised beforehand. Remedial control of infestations is particularly challenging. Several on- and off-label plant protection products are available, for example deltamethrin, flonicamid and pyrethrins, but these often have limited efficacy, and are, broadly, not compatible with organic systems. Furthermore, a number of these are not particularly IPM-compatible, exhibiting high toxicity to natural enemies or requiring great care to ensure applications are carried out with the release of biological control agents in mind. Control measures utilising natural enemies (such as parasitic wasps or nematodes) or soft chemicals (such as maltodextrin or fatty acids) often prove inadequate, especially when the infestation is severe. Physical control methods can be effective, especially when used as a means to clean down and sterilise between crops, but these options are often labour intensive and thus expensive.

In light of the challenges and impact that *P. viburni* can have on crops and UK growers, AHDB (then HDC) has commissioned several pieces of work preceding this current review (e.g. PC 161, Jacobson & Croft, 2002; PC 215, Croft & Jacobson,

2007), with the most recent completed and reported in 2009 (PC 240, Jacobson & Morley, 2009). These projects focused on integrated, IPM programmes, particularly within organic tomatoes, with PC 240 placing emphasis on physical means of minimising the number of mealybugs surviving between crops that are then available to colonise new plantings. This built on the findings of PC 161 and PC 215.

It continues to be important that UK growers have access to IPM-compatible control measures against *P. viburni* in protected edible systems. Since the last work was published, the list of potential and approved actives, and the regulatory environment, have changed, and with this in mind the overall objective of this review is to explore current and potential options for control and monitoring of *P. viburni*.

Target Description and Life-cycle

Pseudococcus viburni is a soft-bodied, mobile pest with piercing, sucking mouthparts. Adult females are wingless and approximately 2.5-5mm long, with an oval-shaped body comprised of a fused head, thorax and abdomen and coated in a white, powdery wax layer (Koppert Biological Systems, 2017). Legs and antennae are short and yellow in colour, while the body is darker, pinkish in colour. The margin of the body has 17 pairs of waxy filaments, the back-most, or 'tail', of which are longer than the preceding by some 20-50% of body size (CABI, 2018). Adult males are diminutive in comparison to their female counterparts, approximately 1mm long, with well-developed legs, one pair of wings and long antennae (Koppert Biological Systems, 2017; CABI, 2018). They lack mouthparts and thus do not feed, and have a short adult lifespan in which they seek out females with which to mate.

In order to produce viable eggs, *P. viburni* must mate (da Silva *et al.*, 2010; Waterworth *et al*., 2011b). Yellow eggs are laid in batches of up to several hundred into egg sacs made of white, waxy filaments secreted by adult females, after which the ovipositing female dies (Koppert Biological Systems, 2017; CABI, 2018). There are three immature stages for females of the species, and four for males. First instar nymphs hatching from the eggs are highly mobile and known as 'crawlers'. At this stage, male and female nymphs are not distinguishable, and both actively feed (CABI, 2018), though some females may possess short wax threads that are absent in males (Koppert Biological Systems, 2017).

The two remaining immature female instars are similar to the adult females, though smaller in size. They begin to settle on the plant host in the second instar, secreting wax and moulting into the third instar and then into mature adult females (CABI, 2018). Immature males feed and secrete cocoons of filamentous wax in their second instar, in which they moult into a non-feeding pre-pupa with small wing buds (CABI, 2018). This in turn moults into a pupa and then an adult male, which rests in the cocoon, secreting two long wax tail filaments, before emerging to seek females (CABI, 2018).

Pseudococcus viburni does not have a true diapause, however eggs will not hatch when conditions are too cold (UC IPM, 2015) and the species is frost sensitive (Koppert Biological Systems, 2017). There are multiple, typically overlapping generations in a year (UC IPM, 2015; CABI, 2018). They typically survive cold periods in the ground or tucked into crevices, or hidden in glasshouses. In outdoor conditions, *P. viburni* typically overwinter either in egg sacs or as early instar nymphs, though mortality is high (CABI, 2018). However, under protected, glasshouse conditions, it is suggested that *P. viburni* is able to overwinter in any stage, though usually in either the late (third or fourth) instars (Koppert Biological Systems, 2017) or as female adults (Karamaouna & Copland, 2009a).

Population development

Despite the economic importance of *P. viburni* to protected edible and fruit production, knowledge of life-history parameters is considered to be relatively fragmented (Waterworth *et al*., 2011b; da Silva *et al*., 2017). This may, in part, be due to the difficulty in differentiating between mealybug species (e.g. between *P. viburni* and *P. maritimus*), and historic confusion therein caused.

The development time, fecundity and fitness of *P. viburni* are driven, primarily, by temperature. Heidari (1989) observed development times of 50 days at 21°C and 30 days at 26°C, with moderate temperatures of up to 28°C recorded to be most favourable. Koppert Biological Systems (2017), state that laboratory studies showed a development time of 132 days at 18°C, decreasing to 48 days at 25°C, and 69 days at 27°C, which are comparable to glasshouse conditions as reported in studies by Karamaouna & Copland (2009a). Koppert Biological Systems (2017) observed a maximum of approximately 240 eggs oviposited per female at 25°C, and estimated an optimal development temperature of 24.7°C.

Host plant has been shown to affect development, reproduction and survivorship of *P. viburni*. In laboratory trials comparing these aspects on apple, persimmon and grapevine leaves, da Silva *et al*. (2017) observed development times for female mealybugs ranging from 35 days on persimmon and grape to 41 days on apple leaves, with fecundity decreasing from an average of 88 eggs per female on persimmon and grape to 30 eggs on apple leaves. The lengths of the pre-oviposition and reproductive periods were also affected by host, as was longevity.

Symptoms and Identification

Pseudococcus viburni can feed from all aerial parts of a plant and can therefore be located on any of these, but are often located at the foot of the stalk or, in overhead wire systems of cultivation, along horizontal parts of the stem (Koppert Biological Systems, 2017). Common locations can also differ by crop; for example, Croft & Jacobson (2007) report that, in tomatoes, *P. viburni* are typically situated on the lower stems, while in peppers they are usually found beneath the fruit calyx. They are typically found feeding in groups. As is the case with all hemipteran pests, *P. viburni* feeds by inserting its mouthparts into plant tissue and drinking up sugar-rich phloem, reducing plant vigour. At sufficiently high population levels, they can trigger considerable water and carbohydrate stress in crop plants causing symptoms such as leaf wilting or defoliation. In order to extract sufficient protein and amino acids, the mealybugs must ingest large quantities of sap, excreting the excess sugars as honeydew. This fouls the surface of the plants and fruits, promoting the growth of dark sooty moulds (*Cladosporium* spp.) that, in addition to decreasing photosynthetic levels, with consequent impact on flower and fruit production, also reduce product marketability. Secondary infection by pathogens, such as *Botrytis* spp, can also occur (Jacobson & Croft, 2002; Canário *et al.*, 2017). Furthermore, extraction of the sap can stunt plant growth and cause deformation or yellowing of leaves (or, indeed, drop of flowers of fruit where these are concerned), again reducing photosynthesis and consequently yield. Direct feeding on fruits causes visual damage. The simple presence of mealybugs, and the residues of the white, waxy filaments (often found in combination with sooty moulds), can also render product unfit for sale.

Monitoring

Monitoring for the presence of *P. viburni*, and mealybugs more broadly, is mostly reliant on laborious and time-consuming direct visual examination and sampling of plant material for pest presence. This is complicated, in part, by their cryptic habits and tendency to settle in protected areas of plants. Such monitoring can also result in inaccurate estimates of pest density and status within a crop, particularly early in growing seasons (Franco *et al.*, 2004; Mudavanhu *et al.*, 2011). Frequent crop walking and examination of key plant areas, with additional attention given to spotting evidence of sooty moulds and marking of locations/plants at which mealybug have been spotted, is labour-intensive and therefore costly, on top of the potential costs for control and losses caused by reductions to yield or from unmarketable product.

Pheromone trapping

The use of pheromone-baited trapping can provide a more efficient and cost-effective means of monitoring population densities, particularly at low infestation densities early in the season, than visual sampling. Where capture levels are found to correlate well with infestation or damage levels, they can also help to inform management strategies and control programmes. Lures are deployed with traps, in the case of mealybugs in combination with yellow or red Delta traps and sticky cards, as pheromone 'bait' (Zaviezo *et al.*, 2007; Mudavanhu *et al.*, 2011; Waterworth *et al.*, 2011a; Charles *et al.*, 2015).

In order to attract winged males for mating, the sedentary female *P. viburni* produce a sex pheromone. This pheromone has been identified and synthesised (Millar *et al.*, 2005) and consists of (1*R**,2*R**,3*S**)-(2,3,4,4-tetramethylcyclopentyl)methyl acetate, an irregular and unique monoterpenoid structure, with 2'-2 and 3'-4 isoprenoid linkages. Chemicals with similar structures have not been reported in any host plant species, suggesting that the mealybug is able to synthesise the pheromone outright, rather than modifying or deriving it from compounds sequestered from the host (Millar *et al.*, 2005). Mealybugs are typically insensitive to stereoisomers or structural analogs of their pheromones. In this they differ from other closely related groups of insects, which are often sensitive to structural analogs of their pheromones. Millar *et al.* (2005) suggest that this may be due to the ability of mealybugs to create unique pheromone communication channels, resistant to interference, by biosynthesising species-specific chemistry, rather than creating species-specific pheromones through blends of different ratios and subsets of compounds, that are common to the members of a closely-related group of insects (as, for example, may be observed in some beetles, moths, or pentatomid bugs).

Pheromone-baited traps for *P. viburni* have been used successfully under field conditions in several crops. Zaviezo *et al*. (2007) described field use in Chilean table grape vineyards. Mudavanhu *et al.* (2011) deployed pheromone-baited traps in pome fruit orchards in South Africa, and, in addition to finding a correlation between male capture levels and fruit infestation levels, observed in some instances that the traps had captured males when visual monitoring procedures had not detected female mealybugs. This suggests that pheromone-trapping could have sufficient sensitivity to provide an early warning of infestation. Other studies have successfully deployed pheromone-baited traps in apple orchards in New Zealand (Charles *et al.*, 2011), and in Californian nurseries growing hardy nursery stock (such as azaleas and other woody shrubs) or crops for the cut-flower industry (Waterworth *et al.*, 2011a). Much research has been conducted on the use of pheromone-baited traps for the monitoring and management of other mealybug species, for example *Planococcus citri* (Risso) and *P. ficus* (Signoret). The breadth of research on the topic, the robustness of results and broad applicability across crops and mealybug species supports the use of pheromone-baited trapping for monitoring of *P. viburni*

populations in any location, although research on this particular species appears focused in outdoor orchard or containerized systems. It should, however, be possible to deploy pheromone-baited traps within glasshouse systems, though evaluation of optimal placement of traps within the crop and glasshouse area, as well as validation of a trapping programme on the whole, may be of benefit to support effective use in the protected edible sector.

A potential barrier to uptake lies, unfortunately, in cost. The sex pheromone of *P. viburni* is currently relatively expensive, though it is hoped that by optimising lure dose, potential economic gains in production may encourage commercial development of lures (Charles *et al.*, 2011). Preliminary field tests in Californian vineyards during the identification of the sex pheromone structure utilised lures loaded with a 100µg dose (Millar *et al.*, 2005). Waterworth *et al.* (2011a) optimised operational parameters for pheromone-baited traps in ornamental nurseries in California. Their work showed that pheromone doses ranging from 1µg to 320µg were attractive to males, with fewest males captured at the lowest (1µg) dose. However, there was no statistically significant difference between the numbers of male *P. viburni* captured in traps baited with lures ranging from 10µg to 320µg, suggesting that high doses would not necessarily attract higher numbers of males (Waterworth *et al.*, 2011a). Furthermore, lures loaded with 25µg of pheromone were recorded to have good field longevity, with no differences in the number of male *P. viburni* captured through using lures aged for 1, 2, 4, 8, or 12 weeks. This suggests field lifetimes of at least 12 weeks (Waterworth *et al.*, 2011a).

Charles *et al.* (2011) also investigated operational parameters to optimise lures for use in New Zealand apple orchards. Similarly to Waterworth *et al.* (2011a), Charles *et al.* (2011) observed a dose response in caught male *P. viburni*, although in their study this was observed to plateau at a pheromone dose of 1µg per lure, and an age-dependent efficacy of lure (where 'aged' lures captured more males than 'new' lures over a 62-day inspection period). A mathematical model was devised, and validated, which suggested a lure half-life of approximately 7.4 days and a maximal attractiveness with a dose of 0.19µg, with dose rate increasing from this to 5.41µg per lure as deployment time increased from 0 to 9 weeks. On the basis of this model, Charles *et al.* (2011) suggested that, at an initial pheromone load of 4µg, lure efficacy would remain at or better than 55% over an 8-week period (with peak efficacy after approximately 5 weeks), and concluded that this would provide an appropriate level for practical monitoring in a New Zealand summer. The pheromone load suggested by Charles *et al.* (2011) is six times lower than that used in Waterworth *et al.* (2011a), allowing 250,000 lures with a load of 4µg to be produced compared with 40,000 lures with a load of 25µg, indicating the economic gains anticipated to encourage commercial development. Again, these trials were conducted in outdoor conditions and further development would be required to optimise a deployment programme for enclosed, glasshouse systems in the UK. Such trials would be reliant on the commercial development of lures to ensure economic viability for growers.

Optical spectroscopy

In a recent study, Canário *et al*. (2017) used optical spectroscopy to detect presence of *P. viburni* on tomato plants at an early stage in infestation. Potted plants, maintained under field conditions, were either kept without mealybugs or inoculated with three egg masses, after which reflectance of marked leaves (using a spectrometer across a 400-1,000nm wavelength band), plant height, leaf size, mealybug density (and presence/density of any other incidental pests) was recorded weekly for five weeks. They were able to identify a difference in leaf reflectance between control and infested plants at 57 days in the near infrared region. The paper

notes that their results focused on detection of stress symptoms, as opposed to direct detection of mealybugs themselves. This is, of course, a limitation, as stress could be induced by other pests. Regardless, optical spectroscopy could, eventually, and perhaps in combination with technologies such as e-noses, be a useful tool for early detection of *P. viburni* infestation (and mealybugs, more generally), though considerable further R&D is essential before this could be considered a viable means of non-invasive monitoring. The technique remains far from market at time of writing.

Insect Models

The importance of timely identification and treatment of *P. viburni* infestations is essential to achieving adequate control of populations, though owing to the cryptic nature of the species this can be challenging. Subsequent to the identification and synthesis of the sex pheromone for *P. viburni*, Mudavanhu *et al*. (2011) developed a monitoring method utilising pheromone-baited traps for use in fruit orchards in the Western Cape Province of South Africa, correlating catches of males in these with fruit infestation data to determine an action threshold for insecticide application (currently the primary means of control in South African orchards). This is particularly important early in a season as early control can reduce outbreaks in the ensuing season, reducing the need for or frequency of further insecticide application.

Over the course of two growing seasons, Mudavanhu *et al*. (2011) assessed male catch numbers biweekly for pheromone-baited traps spaced evenly across a number of orchards in three fruit-growing regions. Visual monitoring and assessment of fruits was conducted fortnightly for the duration of each fruit season, by picking three fruits per tree, dissecting them and noting mealybug infestation. By correlating the pheromone-trap catch data and fruit infestation data, they observed a positive correlation between the numbers of males caught and fruit infestation, and calculated an action threshold of 2.5 male *P. viburni* caught per trap per fortnight at an economic threshold of 2% fruit infestation.

Although the study was conducted in outdoor fruit orchards, it shows the potential of pheromone-trapping as a useful tool to aid grower decision making with regards to management of *P. viburni*. The traps were suggested to be more sensitive and to provide early warning of infestation, while also being less labour intensive, more accurate, and faster than the visual monitoring methods employed at the time in South African fruit orchards (Mudavanhu *et al*., 2011). As such, development of a similar approach, using pheromone-baited traps and establishment of action thresholds, could be investigated within the parameters experienced in protected glasshouse systems to support IPM programme decisions (such as when to introduce natural enemies) before infestations become significant enough to require remedial chemical actions to be undertaken.

Cultural Control and Management

Cultural management is currently an important, albeit time-intensive and expensive, aspect of *P. viburni* control programmes. It is particularly important in limiting introductions or transfer of mealybugs to uninfested areas or crops, and in minimising the numbers of invading crawlers colonising plants early in the growing season. Practices are well-established and applicable across a range of crops, and there does not appear to be any significant advance in such practices mentioned in the literature at time of writing. Broadly speaking, cultural management focuses on good plant and equipment hygiene. The efficacy of such measures, and development of a protocol to minimise mealybug survival and colonisation of plants, were assessed as

part of AHDB (then HDC) trials PC 161 (Jacobson & Croft, 2002), PC 215 (Croft & Jacobson, 2007), and PC 240 (Jacobson & Morley, 2009).

Good crop management is an important aspect in limiting optimal outbreak conditions, though this is not surprising. Overwatering and over-fertilising of crop plants are to be avoided; excess nitrogen, for example, has been shown to increase female mealybug size and the number of eggs per egg sac (Daane *et al.*, 2012). Pruning of plant material to reduce infestations and to remove 'green bridges' across which mealybugs can walk to infest new plants is also suggested (CABI, 2018). Pruning to avoid plant canopies or foliage touching neighbours may not always be feasible in protected edible crops, particularly in high-wire systems; however, the removal of lower leaves and foliage brushing the ground can at least help reduce the number of mobile crawlers invading the crop plants early in the season (Jacobson & Morley, 2009). Any infested plant material should be removed and either burned or disposed of far from the crop plants. Leaving such material on the ground can, for example, allow mealybugs the opportunity to move back onto crop plants (CABI, 2018). Physical, manual removal of mealybugs when these are observed incidentally or during visual monitoring is also commonly employed and can help reduce infestation sizes, however this is, of course, labour-intensive, expensive and typically ineffective in broad-scale production, and can be complicated by the cryptic habit of the pest.

Reducing introductions and transfer between or within crops and glasshouses

Pseudococcus viburni, and mealybugs in general, are typically introduced into new areas or spread through a crop on introduced infested plant material, equipment (such as irrigation equipment or packing material) or by attaching to and being transferred on workers' clothing, or even birds (Jacobson & Croft, 2002; Croft & Jacobson, 2007; Koppert Biological Systems, 2017). This movement can be limited by deployment of strict hygiene and quarantine methods, and there are several approaches that can be deployed. Inspection of introduced stock and equipment for mealybug presence, and subsequent removal, can be useful in identifying and removing potential sources. Where feasible, quarantining new plants for about a month, with subsequent treatment if mealybugs are detected can also be useful in limiting introduction of infested stock (CABI, 2018), and growers are advised not to introduce 'ornamental' plants into edibles nurseries to restrict potential sources of infestation (Jacobson & Croft, 2002). Plant material and debris from infested areas can be disposed of by burning, burying, or otherwise taking them far from areas of crop growth or uninfested areas (CABI, 2018). Recommendations have also been made to limit worker visits to infested areas until the end of the day (so as to avoid transfer into uninfested areas) and for provision of overalls when working in such areas that are then removed and disposed of as appropriate (Jacobson & Croft, 2002). Careful cleaning and sterilising of equipment between areas, and worker handwashing or hand-rubbing can also help reduce the likelihood of transfer. Where strong hygiene and quarantine protocols are in place, and where these are also combined with other control measures such as chemical treatment, this can result in successful control or suppression, with eradication reported on several sites (Jacobson & Croft, 2002), though clearly this is unlikely in, for example, organic settings.

Reducing survival between crops

Survival between crops can be reduced by physical means. Careful cleaning and disinfection of equipment (such as irrigation lines) and hard surfaces can help to reduce the number of nymphs eventually emerging onto a crop by destroying

overwintering stages and egg sacs. Several effective, albeit typically expensive, approaches have been advised as a result of previous AHDB-commissioned work PC 161 and PC 240.

Following removal of all plant debris, use of a propane burner applied to the surfaces of concrete dwarf and perimeter walls, post and pipe supports, pipe rails and low-level metal work, stanchions and other such surfaces, as well as the angle between soil and concrete roadway, can be used to destroy motile stages of *P. viburni*, and mealybugs in general (Jacobson & Morley, 2009). The same technique can also cause some impact on egg sacs, though some survival must be expected. Jacobson & Morley (2009) reported that though outer eggs in a mass appeared scorched and unhealthy, when egg sacs were teased apart under a microscope, eggs in the centre of a sac appeared normal and were viable when then incubated in a laboratory. Following cleaning, painting concrete blocks and walls in affected areas with thick paint or glue can also help to reduce the number of emerging nymphs (Jacobson & Croft, 2002). Thorough cleaning and disinfection of irrigation pegs and laces by means of removal from the main line, washing, and dipping in nitric acid (pH 2 or less), with the main line carefully pressure washed (Jacobson & Morley, 2009), is also important, as egg sacs in particular can be deposited in crevices on pegs, stakes and drippers that are difficult to clean.

Reducing crop invasion

Mobile *P. viburni* crawlers have been observed to rapidly migrate to plants following emergence from egg sacs early in the season, within three weeks of a glasshouse being heated (Croft & Jacobson, 2007). Jacobson & Morley (2009) devised a protocol and strategy for physical preparation of a glasshouse and plant introduction to limit crop invasion, briefly described here. Subsequent to careful cleaning between crops to reduce survival, several recommendations were made. Good quality, black-backed polythene covering was advised for use to cover any soil in the glasshouse to prevent growth of volunteer plants and provide a barrier to any mealybug emerging from such areas, with any joins off-set to beds and overlapping by at least 20cm and glued together to a provide a good seal; in their study, they utilised Thripstick 2 (a polyisobutylene emulsion with deltamethrin) as the ‘glue’. Jacobson & Morley (2009) further advised painting of Thripstick 2 to, for example, concrete surfaces such as roadway edges, stanchions, dwarf walls, and metalwork such as posts and pipe supports, before carefully covering and sealing tightly with black-backed polythene, paying particular attention to areas with increased likelihoods of harbouring mealybug egg sacs, as well as painting a sticky barrier across the beds, to isolate the plants (though they also recorded that Thripstick 2 appeared phytotoxic where it came into contact with plants).

In the Jacobson & Morley (2009) study, particular attention was paid throughout to functionally ‘isolating’ the plants from mealybug sources, by removing potential ‘green bridges’ across which the crawlers could access the plant. For example, recommendations were made to remove any lower leaves, trim excess string to avoid trailing, and painting supports with a sticky barrier to prevent their use as bridges onto plants. They also advised that such means be used jointly with careful and thorough monitoring, with physical removal and destruction of any mealybugs encountered in the plants.

Growing systems have developed and changed in the decade since completion of the Jacobson & Morley (2009) work, with most protected edibles currently grown under different conditions, for example closed systems and with the use of rockwool. Regardless, the general principles described by Jacobson & Morley (2009) remain

useful to consider – namely, a combination of careful between-crop cleaning, provision of physical barriers and removal of access points to plants to limit the number of invading mealybug, and careful monitoring and removal of any mealybug clusters found, particularly early in the season (before egg-laying), can all help towards the objective of achieving control of mealybug before layering of crops, at which point effective control becomes challenging by any means deployed.

Natural Enemies

Parasitoids

A wide range of parasitoid wasp species have been reported to attack *P. viburni*, but few are commercially produced (Blumberg & Van Driesche, 2001). Most programmes releasing these rely primarily on mealybug-specialist encyrtid parasitoids. These are typically endoparasitoids, though they can be either solitary or gregarious, and different species tend to preferentially attack different host stages. Several encyrtid parasitoids are widely available for mealybug control in the UK.

Leptomastix epona (Walker) (Hymenoptera: Encyrtidae) is native to and frequently applied in glasshouses for control of *P. viburni* in Europe (EPPO, 2019). It will attempt oviposition in all host stages from the second instar, but preferentially attacks third instar and adult *P. viburni* (Karamaouna & Copland, 2000), and is able to discriminate between parasitised and unparasitised hosts for up to four days following oviposition therein (Karamaouna & Copland, 2009b). Although it is recorded to have an increased gross reproductive rate and intrinsic rate of increase in larger hosts, *L. epona* development can start at temperatures relatively lower than that required by *P. viburni*, and also requires less heat to complete development (Karamaouna & Copland, 2009a), which suggests that the parasitoids should be able to complete more generations, and develop faster, than the pest, thus favouring control. Another *Leptomastix* species, *L. dactylopii* (Howard), typically released to control the citrus mealybug, *P. citri*, has been thought to be specific to that species in the field (Blumberg & Van Driesche, 2001), but has been observed emerging from field-gathered *P. viburni* in Californian vineyards (Daane *et al.*, 2008). Regardless, it is not commonly released for *P. viburni* control.

Two non-native species of encrytid parasitoids frequently used overseas are the gregarious *Acerophagus maculipennis* (Mercet) and *Acerophagus flavidulus* (Brèthes) (both Hymenoptera: Encrytidae; synonyms: *Pseudaphycus maculipennis* and *Pseudaphycus flavidulus*, respectively). The species are not currently available for release in UK glasshouses, but have been used with some success as part of control programmes in outdoor crops in California, France, Australia and New Zealand. Similarly to *L. epona*, *A. flavidulus* has also been shown to begun development, and to require less heat to complete development, relative to *P. viburni* (Karamaouna & Copland, 2009a). *Acerophagus flavidulus*, originating in the neotropics, also preferentially oviposits in larger hosts, but in contrast to *L. epona* is more likely to also oviposit in second instar *P. viburni* nymphs (Karamaouna & Copland, 2000), though parasitisation success and intrinsic rates of increase are lower in smaller hosts (Karamaouna & Copland, 2009a).

Acerophagus maculipennis, originally described from the Canary Islands, also prefers slightly larger hosts, with egg load increasing with age and an offspring sex ratio strongly biased towards females (Sandanayaka *et al.*, 2009). In laboratory experiments conducted at 21°C, adult females were shown to have longevity of 14 to 21 days following a development period of 20 to 21 days, with a realised fecundity of, on average, 46 eggs per female (Sandanayaka *et al.*, 2009). On average, some three

new parasitoids were reared through per mealybug, though this was increased to an average of nine when hosts were exposed to super-parasitism (where one host is attacked more than once by a single species of parasitoid, be it the same individual parasitoids attacking repeatedly, or different individuals each attacking once), suggesting that in such cases co-development of the parasitoid larvae is common (Sandanayaka *et al.*, 2009). In a series of smaller, contained glasshouse trials, Croft & Jacobson (2007) obtained a licence from Defra to release *A. maculipennis*, achieving 80-90% parasitism from a single release in tomatoes, though a maximum rate of parasitism of 40% was reported from peppers.

Anagyrus pseudococci (Girault) (Hymenoptera: Encrytidae), determined to actually comprise of two sibling species (*A. pseudococci* (Girault) and *Anagyrus* sp. nr. *pseudococci* (Girault)) is typically released for control of the vine mealybug, *P. ficus*, but is relatively polyphagous and has been recorded to emerge from *P. viburni* (Bugila *et al.*, 2015). Although able to complete development in *P. viburni*, parasitism rates of just 4.5% and emergence rates of 14.8% (the lowest values obtained from a comparison of a range of host species) were observed (Bugila *et al.*, 2015), indicating that while some parasitism may be expected, the species could not be expected to give acceptable control of *P. viburni* on a commercial scale. Indeed, Croft & Jacobson (2007) also suggested *A. pseudococci* to be the weakest potential candidate for biological control programmes for *P. viburni* when compared with *L. epona* and *P. maculipennis*.

Although credited with some level of control, varying success has been reported for parasitoid species when used on a commercial scale, with a number of reasons suggested for differences. In AHDB-funded trials, for example, Croft & Jacobson (2007) observed some 50% parasitism of *P. viburni* by *L. epona* in glasshouse tomatoes, and no parasitism in glasshouse peppers. They also reported that *L. epona* became established in a mealybug population following four weekly releases at a rate of two parasitoids per metre squared, with a 15% parasitism level 70 days after first release. Jacobson & Morley (2009) began releasing *L. epona* some 10 weeks following infestation of their trial, and stated that their results suggested coexistence of the parasitoids with the pests, rather than control in a commercial setting. In the same series of experiments, parasitism rates by *A. maculipennis* were reported at 80-90% in tomatoes and only 40% in peppers, with the differences in parasitism rates between the two crops suggested to be at least partially attributable to differences in typical locations of *P. viburni* in the two crops (namely, on lower stems in tomatoes and beneath the calyx of the fruit in peppers), affecting host-seeking behaviours in the parasitoids (Croft & Jacobson, 2007).

In addition to their cryptic habit, mealybugs are also known to engage in behavioural defence responses towards attacking parasitoids. Behaviours such as walking away, abdominal flipping and reflex bleeding have all been reported, with a comparative study of several mealybug species reporting that *P. viburni* exhibited such behaviours at a higher rate than others, including *P. citri* and *P. ficus* (Bugila *et al.*, 2014). Another defence mechanism triggered by parasitoid attack is encapsulation, which is an immune response triggered by the presence of parasitoid eggs or larvae within a host and involves the production of a multi-layered ‘capsule’ around the invader by haemocytes. In studies of encapsulation rates of parasitoids in multiple mealybug species, *L. epona* and *L. dactylopii* were recorded to have parasitism rates of 62.9% and 53.5% respectively at 23°C when attacking *P. viburni*, but at the same temperature *P. viburni* was able to achieve effective encapsulation (i.e. encapsulation of all parasitoid eggs, leading to mealybug survival) rates of 33.4% for *L. epona* and 100% for *L. dactylopii* (Blumberg & Van Driesche, 2001). In a similar study of encapsulation of *Anagyrus* sp. nr. *pseudococci* in multiple mealybug species, Bugila

et al. (2014) reported aggregated (eggs and larvae) encapsulation rates of 86% in *P. viburni*. These high rates of encapsulation may be a constituent part of the variability observed in the success of parasitoids in controlling *P. viburni* in commercial glasshouse environments. Parasitoid performance has also been linked to climate and environmental conditions, as well as preferences exhibited by certain parasitoid species (such as *A. flavidulus* and *A. maculipennis*) in regards to the geographic strain of the target (Daane *et al.*, 2012).

Predators

Predation can play an important role in managing *P. viburni* populations, and a broad number of generalist species have been recorded as attacking them. *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), a ladybird of Australian origin, has been exported world-wide as a biocontrol agent for mealybugs, and is the most well-known and, so far, the most successful at supporting control (Daane *et al.*, 2012; Kairo *et al.*, 2013). Both larvae and adults attack and kill mealybugs by consuming them. The larvae are, to some extent, mealybug mimics, covered in wax-like filaments similar to those of their target prey; this allows them to camouflage themselves from mealybug-tending ants and to forage with limited disturbance (Daane *et al.*, 2012). Prey consumption and searching behaviour are affected both by the environmental conditions and by ladybird age, where voracity usually increases with increasing larval stage with late third and fourth instars being the most voracious life stages (Hodek & Honěk, 2009; Koppert Biological Systems, 2017). Adult and early instar *C. montrouzieri* larvae are recorded as preferring eggs and young mealybugs, while older *C. montrouzieri* larvae have been seen to feed on all mealybug life stages equally (Koppert Biological Systems, 2017). Many studies have evaluated consumption rates, feeding potential and, thus, potential efficiency of the species. Koppert Biological Systems (2017) reports that at 21°C a *C. montrouzieri* larva can consume more than 250 second and third instar mealybug nymphs over the course of their development. Mani & Thontadarya (1987) reported that *C. montrouzieri* larvae were observed to consume 881 eggs, 259 nymphs or 28 adult female grape mealybugs (*Maconellicoccus hirsutus* (Green)) over the course of their development in laboratory studies. Field studies have also shown good efficacy. In studies on efficiency when feeding on *P. citri* in Tunisian citrus orchards, *C. montrouzieri* was reported to effect decreases of at least 85% in *P. citri* egg sac density one month after release (Rahmouni & Chermiti, 2013). In an Egyptian study on outdoor ornamental shrubs, percentage reductions of *P. citri* eggs, larvae and adults were observed at 41%, 42% and 57% respectively one month after release, reaching 80%, 86% and 91% respectively two months after release.

Of particular note is that fact that *C. montrouzieri* control of *P. viburni* has been shown in glasshouse trials to not be particularly affected by plant hairiness. In comparing six potential host plants (lemon, Arabica coffee, tomato, blue passionflower, potato and primrose), Heidari (1999) reported that good control was achieved by use of *C. montrouzieri* as an inundative predator irrespective of host plant, when compared with another coccinellid predator, *Nephus reunioni* (Fürsch) (Coleoptera: Coccinellidae). This is useful, as Koppert Biological Systems (2017) note that control of *P. viburni* can be particularly challenging on tomatoes, owing to the glandular hairs thereon. In the UK, *C. montrouzieri* is often recommended for periodic release in glasshouses at a rate of 4-10 per metre squared in temperatures above 16°C (D. Macdonald, *pers.comm.*).

Lacewings have also been associated with mealybug suppression, and larvae have been recorded to be effective predators of smaller mealybugs (Miller *et al.*, 2004; Daane *et al.*, 2012). Indeed, in an extensive review of predator-prey association in

the Neuroptera order of insect predators, mealybugs in the *Pseudococcus* genus, which includes *P. viburni* have been reported to be attacked by at least 14 species of neuropterid across four predator families: Chrysopidae, Coniopterygidae, Hemerobiidae and Raphidiidae (Miller *et al*, 2004). Of these families, however, only Chrysopidae are produced and used on a commercial scale for biocontrol; specifically, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). *Chrysoperla carnea* has been reported predating fortuitously on mealybugs in outdoor ornamental nurseries in Southern Europe (Beltrà *et al.*, 2013) and Egypt (Afifi *et al.*, 2010), and is a known predator in Californian orchards and vineyards (Daane *et al.*, 2007; Daane *et al.*, 2012). Although no studies could be identified on the efficacy of lacewings as a biological control agent of *P. viburni*, periodic release of lacewings into glasshouses as part of a control programme could be suggested (D. Macdonald, *pers.comm.*).

In a study on fortuitous biocontrol of the mealybug *Phenacoccus peruvianus* Granara de Willink (Hemiptera: Pseudococcidae) in Spain, Beltrà *et al.* (2013) observed the generalist predator *Orius laevigatus* Fieber (Heteroptera: Anthocoridae) preying on eggs and nymphs of the mealybug pest. Numbers could be considered relatively high – the study found 58 *C. montrouzieri*, 22 *C. carnea* and 105 *O. laevigatus* individuals in their survey. Although no comment was made in the study on predation efficacies of the species, *O. laevigatus* is a generalist predator produced for commercial use in biocontrol programmes in the UK and could be readily screened for efficacy and impact on mealybug density initially in small cage trials, with further broad-scale trials should these show promise.

Predatory flies may also present some use in control of *P. viburni*. Cecidomyiid flies are a generalist predator complement that commonly attack mealybugs (Daane *et al.*, 2012). In the UK, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) is available commercially for control of aphid species. *Coenesia attenuata* Stein (Diptera: Muscidae) has also been reported attacking a broad range of pest species in laboratory studies conducted in Portugal, with predation behaviours and production issues being studied (Martins *et al.*, 2012).

In short, in addition to species known to have good efficacy against *P. viburni*, a range of potentially useful generalist predators are available through commercial production at time of writing. Initial small-scale screening of these to investigate whether they may represent any potential use, and to determine any impact on mealybug densities, may be a useful starting point, and any showing particular promise could be investigated at a commercial scale for viability and compatibility with existing IPM programmes, and to optimise their release and integration. Important aspects such as avoidance of cross-predation or super-parasitic competition would also need to be evaluated in order to avoid trading control of one pest complement for insufficient control of another.

Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) generate significant interest as biological control agents, particularly in the families Steinernematidae and Heterorhabditidae (and their symbiotic bacteria), and some promise has been shown in regard to potential for control of *P. viburni*, and mealybugs more generally. Much of the available literature originates from South Africa, and therefore utilises South African isolates, and focuses on a range of different mealybug pest species.

In a trial investigating the susceptibility of *P. viburni* specifically to South African isolates of EPNs, Stokwe & Malan (2016) compared the performance of two isolates of *Heterorhabditis zealandica* (isolates J34, SF41), two of *H. bacteriophora* (isolates

J172, SF378), one of *Steinernema citrae* (isolate141-C) and one of *S. yirgalemense* (157-C) in laboratory studies. In screening bioassays, they observed the highest rate of mortality of 75% in *P. viburni* treated with *H. zealandica* (J34), and the lowest mortality rate of 45% when treated with *H. bacteriophora* (J172). They also observed an effect of *P. viburni* size on EPN infectivity. In further assessments of *H.* zealandica (J34), the first instar mobile crawlers were less susceptible (22% mortality), in contrast to adults (78% mortality) and intermediate instars (76% mortality). At 25°C, *H. zealandica* (J34) LD_{50} and LD_{90} values of 54 and 336 nematodes respectively were reported after 48 hours. Both *H. zealandica* and *S. yirgalemense* were also observed to successfully reproduce in *P. viburni*.

Comparable results have been obtained in screening studies against other mealybug pest species. Le Vieux & Malan (2013b) recorded mortality rates of 96% and 65% in *P. ficus* treated with *H. zealandica* (SF41) and *S. yirgalemense* (157-C), at a concentration of 100 infective juveniles in 50µl of water per mealybug. Commercially produced isolates, however, caused much lower mortality rates, with commercially produced *H. bacteriophora* and *S. feltiae* leading to 42% and 19% mortality respectively (although the differences were not reported to be statistically discernible). The LC_{50} and LC_{90} for *P. ficus* after exposure to commercially produced *H. bacteriophora* for 24 hours were reported as 36 and 555 infective juveniles per mealybug, respectively. In a similar study on the impact of EPNs on *P. citri*, van Niekerk & Malan (2012) reported that *H. zealandica* (SF41) and *S. yirgalemense* (157-C) caused mortality rates of 91% and 97% respectively 48 hours after treatment with infective juveniles at a concentration of 200 infective juveniles per mealybug, while *H. bacteriophora* (SF 351) at the same rate caused mortality of approximately 70%.

Despite promising laboratory bioassay results, several key challenges remain. Use and application on EPNs has, traditionally, been focused on the control of soil-dwelling pests, and commercial use against above-ground pests has been characterised by problems and limited and erratic success due to the challenges presented by foliar application. The infective juveniles are highly susceptible to environmental conditions, including UV radiation, desiccation and extremes of temperature (Le Vieux & Malan, 2013a), and nematodes are known to separate out of suspension by sedimentation. Insecticidal activity of the infective juvenile nematodes has been linked to available surface moisture, with the timeframe for infection of mealybug individuals also linked to the time available before the plants dried out again after application (van Niekerk & Malan, 2012). Van Niekerk & Malan (2012) reported that the two to four hours immediately following application were the most decisive time for the establishment of successful infection of mealybug targets. Platt *et al*. (2018) tested the performance of *S. yirgalemense* under different environmental conditions, and observed mortality rates of 70%, 61% and 40% in *P. ficus* at 100%, 80% and 60% relative humidity, respectively. Temperature can also affect efficacy of EPNs, with different species and isolates of EPNs known to be active and effective in different ranges of temperatures. Platt *et al.* (2018), for example, reported *S. yirgalemense* to cause mortality rates of 72% at 25°C, 45% at 30°C, and only 9% at 15°C, in *P. ficus*. On a commercial level, different species of EPN are not only suggested for different pests, but the choice is also based on expected temperature ranges around time of application.

The use of EPNs to target cryptic pests, such as mealybugs, may confer some advantage, as the relatively hidden and protected habitats at which the EPNs would need to be targeted in application may also protect the infective juveniles from prevailing abiotic conditions of the environment in which they have been applied (Le Vieux & Malan, 2013a). Potential efficacy of foliar applications can also be improved

by, for example, carrying out applications early in the morning to minimise the effects of UV radiation and avoid desiccation, and judicious selection of application technology aspects, such as nozzle, sprayer/pump type and spray pressures (Le Vieux & Malan, 2013a). The use of adjuvants in EPN suspensions can also improve potential efficacy. Van Niekerk & Malan (2015) evaluated the use of two adjuvants, the anti-desiccant polymer Zeba® and the spreader and sticker Nu-Film-P®, for impact on infectivity and survival of EPNs. Mortality of *P. citri* was increased by 22% when *H. zealandica* was applied in an aqueous suspension with 0.3% Zeba® at 80% relative humidity and a temperature cycle of 22°C for 14 hours and 11°C for 11 hours. The same formulation resulted in mortality increases of 21% and 27% at 60% and 80% relative humidity, when tested with *S. yirgalemense*. When the formulation was then combined with Nu-Film-P®, sedimentation was also delayed, leading to an increase in the average number of nematodes deposited on leaf discs. Finally, addition of Xantham gum at a concentration of 0.2% was also highly effective at delaying sedimentation, with 72% the initial nematode number reported to remain in the suspension after one hour left undisturbed. There is also evidence to suggest that combination with wax removers can also improve efficacy (Abd El Rahman *et al.*, 2012).

Although they are likely to be characterised by limited efficacy, EPNs may be of some value for UK growers. Drawing a sound conclusion on this, however, would require testing of commercially-available nematode isolates already available within the UK, with particular care taken to also evaluate the efficacy and viability of different adjuvant mixtures and available spray application technologies for foliar use in UK protected edible industry, given the important role played by foliar application in the eventual success of any programme. Although complete control appears unlikely using available commercially-produced species (Jacobson & Morley (2009), for example, stated that control measures based on parasitic nematodes proved inadequate), they might nonetheless be optimised to play a role in knocking back populations as part of an integrated approach (although the impact of any EPN applications on other biocontrol releases should also be considered).

Basic Substances

A variety of biorational, physically acting ‘insecticides’ are available for use in the UK on a very broad range of protected edible crops, with such products typically targeted at small, soft-bodied pests such as aphids, whiteflies and mealybugs and usually reliant on physical contact with the target pest. Maltodextrin (e.g. Eradicoat®, Majestik®), fatty acid insecticidal soaps (e.g. Flipper®), dodecylphenol ethoxylate (e.g. Agri 50®), and other physically acting products (e.g. SB Plant Invigorator®) are approved for use, either on- or off-label, with many requiring repeat periodic applications. The need for direct contact to be made with the target can also limit efficacy and reliability of the products when used for mealybug control, due to the cryptic nature of the pest and its tendency to reside in hidden, protected areas of the host plant.

Maltodextrin and fatty acid insecticidal soaps were evaluated for potential use in control of *P. viburni* in glasshouse tomatoes in the AHDB (then HDC)-funded work PC 215 (Croft & Jacobson, 2007). In this series of trials, Croft & Jacobson (2007) determined that seven, weekly sprays of the maltodextrin product Eradicoat T® or the insecticidal soap Savona® provided over 90% control of *P. viburni*, with Eradicoat T® performing better than Savona®. Although Croft & Jacobson (2007) recorded that the spray programme tested was adequate to supplement an approach incorporating parasitoid release, they also stated that the number of sprays must be reduced by improving application efficacy. In later work, Jacobson & Morley (2009) stated,

however, that although both Savona® and Eradicoat T® reduced mealybug populations, the overall effects in mature crops were disappointing. Specifically, the difficulty of achieving contact of the spray with the target pests, combined with the fecundity of females and the survival level leading to rapid population resurgence, necessitated frequent repeat applications to achieve population growth suppression, a strategy deemed "only partially effective and… prohibitively expensive" (Jacobson & Morley, 2009). Variability has also been reported in the efficacy of such products from field trials in other industries and against other pests. In field trials conducted in 2008 and 2009 in Italian vineyards, for example, Baldacchino *et al.* (2010) reported 54% efficacy of potassium salts of fatty acids in the 2008 trial, whereas in the 2009 trial the same product used alone did not differ from the control and only generated a significant efficacy (of 49.6%) when used in combination with a calcium polysulphide (lime sulphur) treatment. In another study, grape bunch infestation by a second generation of *P. ficus* was reduced by approximately 40% following treatment with the insecticidal soap product, Flipper®, although this difference was not found to be statistically significantly different from an untreated control (Tacoli *et al*., 2018). More modest *P. citri* mortality of 22% was reported following use of the product 2% Safer Insecticidal Soap (49% potassium salts of fatty acids) in trials on potted gardenia plants (Hollingsworth, 2005). In other pest species, non-statistically discernible reductions where observed when Flipper® was used against the leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) in two of three Italian organic vineyards used as field sites by Tacoli *et al.* (2017), with the numbers of mealybugs recorded not differing significantly from the untreated control. When applied on snap beans against thrips and whitefly, potassium salts of fatty acids were reported to reduce population densities by up to 54% (Wayfula *et al.*, 2017). Currently, use of such products is suggested as part of an integrated programme, with application of potassium salts of fatty acid products following application of a wetter and preceding release of natural enemies such as *C. montrouzieri* and mealybug-targeting parasitoids (D. Macdonald, *pers.comm.*), with such 'soft chemical' and natural enemy approaches used in pest management strategies in a diverse range of settings (including public-facing enterprises such as the Eden Project (Treseder *et al.*, 2011)).

Horticultural oils, for example petroleum oils or vegetable oils, appear to have limited efficacy when used in isolation for control of mealybug pests, but can be a useful means of 'dewaxing' mealybugs in advance of, or in combination with, other treatment applications, improving their efficacy. Hollingsworth (2005), for example, reported 12.5% mortality of mealybugs in potted gardenia plants in a glasshouse trial following treatment with a paraffinic oil product. In terms of de-waxing, however, Jacobson & Croft (2002) observed almost complete dewaxing of *P. viburni* adult females and egg sacs within 24 hours of application of 3% Crop Oil, another paraffinic oil product, applied at a rate of 30ml/L of water. Furthermore, in assessing the impact of dewaxing agents on the infection and mortality rate of the entomopathogenic fungus *Verticillium lecanii* when used in combination, Jacobson & Croft (2002) reported 80% mortality 24 hours after application in the Crop Oil + *V. lecanii* treatment, an interval too brief for the effect to have originated from *V. lecanii* infection, thus suggesting a direct effect of the dewaxing agent on mortality. Karamaouna *et al.* (2013) recorded the LC_{50} and LC_{90} for paraffin oil against third instar *P. ficus* nymphs to be 9.1mg/ml and 22.7mg/ml respectively, and 10.9mg/ml and 25.7mg/ml respectively when applied against adult *P. ficus*.

Diatomaceous earth, a nontoxic insecticide comprised of ground diatomic fossils, functions as a desiccant, absorbing waxy layers on an insect body and abrading the surfaces of insects it comes into contact with (externally or internally, particularly the digestive or respiratory systems) resulting in direct mortality. There is evidence to suggest that when used in combination with other insecticides efficacy can be

improved. Gogi *et al*. (2014), for example, reported synergistic effects of diatomaceous earth and the insect growth regulator pyriproxifen. Mortality of adult cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), females when treated with pyriproxifen alone under laboratory conditions was 73% one day post-treatment exposure, rising to 93% when the growth regulator was combined with increasing concentrations of diatomaceous earth; the quantity of wax removed was also recorded to increase with increasing diatomaceous earth concentration. Mortality of ovisacs was recorded as 60% with use of pyriproxifen alone rising to 94% with increasing diatomaceous earth concentrations (Gogi *et al*., 2014). Regardless, limited research results appear available on the efficacy of diatomaceous earth against mealybug pests, particularly for use in isolation. As such, it is not possible to determine the extent to which diatomaceous earth could be useful in protected edible crops in the UK at time of writing, necessitating further research into potential efficacy, use, and with careful consideration given to issues of crop safety and residue levels.

Kaolin is a naturally occurring clay with lethal activity against insects, available as products such as Surround® WP. Similarly to diatomaceous earth, direct mortality is caused by ingestion of the mineral particles, and through desiccation of the cuticle via adsorption by cuticle waxes or abrasion. Indirect, nonlethal effects can also be observed, and include repellence and avoidance of treated plants. For example, laboratory trials showed strong repellence and reduced feeding by the mirid pest of cocoa pods, *Helopeltis collaris* (Stal) (Hemiptera: Miridae) (Amalin *et al.*, 2015). Evidence of potential efficacy against mealybugs (and *P. viburni* in particular) is limited. Tacoli *et al.* (2018) reported mortality of citrophilus mealybug, *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae), nymphs of approximately 50% two days post-treatment (Abbot mortality of 40%) under laboratory conditions, differing significantly from an untreated control. Statistically discernible reductions were not, however, borne out in field trials in vineyards, with no differences in the numbers of *P. calceolariae or P. longispinus* observed between kaolin-treated and untreated plants. Furthermore, in both laboratory and field experiments, no statistically significant effect of kaolin treatment on numbers of *P. ficus* was reported, although the number of ovipositing *P. ficus* per bunch of grapes was approximately 20 on kaolin-treated plants in comparison to approximately 50 on untreated plants. Ware (2003) and Joubert *et al.* (2004), observed a higher number of mealybugs on citrus and mango plants following treatment with a kaolin product, and suggested that such effects may stem from non-target effects against natural enemies. Tacoli *et al.* (2018) also reported higher infestation levels of *P. ficus* on vine leaves from one of their four vineyard field sites, and suggested that the finding that kaolin-treated plants at that same site showed lower numbers of mealybugs within the bunches suggests a deterrent effect of the kaolin to migration and movement of later generations of crawlers to the fruit, an effect reported in studies on other insects (Glenn *et al*., 1999; Puterka *et al*., 2003; Sackett *et al*., 2005). By contrast, in a studying the impact of natural products on the leafhopper *S. titanus* in organic vineyards, Tacoli *et al.* (2017) reported three applications of kaolin to have an efficacy comparable to two pyrethrin applications, although both achieved sub-optimal efficacy, with early-instar nymph Abbot mortality reported at 43.2% after feeding on kaolin-treated leaves for three days.

Overall, the literature indicates variable efficacy of basic substances and biorational, physically-acting insecticides. Regardless, although in likelihood such products cannot be considered as a stand-alone solution, they may well provide use in protected edibles as a component part of a broader programme for effective control, and may improve the efficacy of other insecticidal products. It should also be highlighted that although such products are typified by broad versatility, they are also

broad-spectrum, and their impact against released biological control agents is well-recorded. As such, careful thought must be given to timings in terms of application relative to natural enemy releases. Furthermore, many such products require repeat, frequent applications, which in itself poses risk to IPM programmes despite limited residual activity, at least during application windows requiring high frequency of treatments. Additionally, applications also carry a risk of phytotoxicity. For example, Jacobson & Morley (2007) reported phytotoxicity to tomato plants following weekly applications of soft soaps and maltodextrin against *Macrolophus* spp. (Hemiptera: Miridae) population growth, while Karamaouna *et al.* (2013) observed slight phytotoxicity (leaf surface area with symptoms not exceeding 25%) when paraffin oil was applied at 25.9mg/ml, a higher rate than the LC_{90} rate for adult *P. ficus* females. As such, further evaluation of such products may be of benefit, within the context of overall IPM programmes against *P. viburni* in protected edible crops.

Biopesticides

Botanical products

In order to defend themselves from herbivorous insect pests, plants produce natural chemicals typically characterised as complex secondary metabolic compounds. Insecticides of botanical origin can thus be produced where such bioactive substances are derived from plant materials, which, although typically broad spectrum, also degrade rapidly in the environment and are thus non-persistent (and as such often compatible with existing integrated management systems, with some care taken to optimise application timings relative to natural enemy release).

Complex volatile secondary metabolites can be derived from botanical sources as plant essential oils and terpenes. These are usually characterised by strong, typically aromatic odours, and many are known to have either repellent or insecticidal properties. A number of such actives have been tested for efficacy against a range of mealybug species, with varying impact but some promise.

In trials on *P. longispinus*, Hollingsworth (2005) reported high insecticidal activity of limonene, a major constituent of citrus fruit essential oils, on *P. longispinus* mealybugs in a series of bioassays. Aqueous solutions of 1% limonene were tested in combination with different concentrations of spray adjuvants (0.5-1.5% APSA-80™, and 0.1% Silwet L-77®), and mixtures comprising 1% limonene, 0.75% APSA-80™ and 0.1% Silwet L-77® were found to cause between 69% and 100% mortality of mealybugs in a 1-minute bean-dip bioassay. When sprayed onto potted gardenia plants in a glasshouse, the same mixtures caused 44% mortality of third and fourth instar *P. citri*, higher than control levels obtained in applications of insecticidal soap (22% mortality) or horticultural oil (12.5% mortality). Hollingsworth & Hamnett (2010) reported 3-12% mortality of *P. longispinus* in bean-dip bioassays in an aqueous solution of 1% limonene with an emulsifier (Safer Insecticidal Soap® at 0.5-1% rate), increasing to 93-98% upon addition of 0.5-1% sodium lauryl sulphate, although the mixture was not stable over time. Substitution of the emulsifier for another product (Tween® at 2%) resulted in a more stable mixture with efficacy that increased through time (29% mortality if used immediately after mixing, averaging to 72% mortality 2 days after mixing, and increasing to 89% sixteen days after mixing, in bean dip bioassays). Tacoli *et al.* (2018) reported that application of citrus essential oils resulted in increased mortality of both *P. calceolariae* and *P. ficus*. In laboratory trials in New Zealand, using the same formulation as Hollingsworth & Hamnett (2010), Tacoli *et al.* (2018) reported Abbot mortality of 52-56% 20 hours post-treatment, and approximately 65-75% 40 hours post-treatment. Laboratory trials conducted in Italy, by contrast, used a commercially available citrus essential oil product (Prev-Am

Plus®, 5.88% orange oil containing at least 90% D-limonene), and observed Abbott mortality of first and second instar *P. ficus* nymphs of 48% if nymphs were sprayed and then placed on a clean agar Petri dish, rising to 84% Abbott mortality if the surface the nymphs were placed on had also been sprayed (Tacoli *et al*., 2018). In field trials in Italian vineyards, application of the commercially available citrus essential oil product against second and third generation *P. ficus* first instar crawlers led to cumulative Henderson-Tilton efficacies of 76% in one vineyard trial site (two applications against second generation crawlers), and 74.7% (one application against second generation crawlers) and 10.3% (one application against third generation crawlers) in a second vineyard trial site (Tacoli *et al.*, 2018). In trials in Tunisian vineyards, foliar application of the same essential oil product (Prev-Am®) at an application rate of 200ml/hl was found to provide control of *P. ficus* first and second instar nymphs at a comparable rate to the synthetic active imidacloprid, while providing better efficacy against egg sacs and third instar nymphs on vine trunks (Mansour *et al.*, 2010a). The authors of the study, however, stated that their findings suggested a single application of Prev-Am® would not be sufficient to adequately limit *P. ficus* population spread, but that subsequent applications may enhance such effects (Mansour *et al.*, 2010a).

Karamaouna *et al*. (2013) investigated the insecticidal activity of a range of plant essential oils against *P. ficus*, reporting LC_{50} and LC_{90} values, and noting any phytotoxic effects. The study assessed peppermint (*Mentha piperita* L.), thyme-leaved savory (*Satureja thymbra* L.), lavender (*Lavandula angustifolia* Mill), basil (*Ocimum basilicum* L.), lemon (*Citrus limon* L.) and orange (*Citrus sinensis* L.) essential oils. Essential oils derived from citrus, peppermint and thyme-leaved savory were found to have higher toxicity to *P. ficus* when compared to a paraffin oil reference product (Triona® 81 EW), while lavender and basil essential oils were less toxic. LC_{50} values for adult *P. ficus* females were reported as 2.7mg/ml for lemon, 5.4mg/ml for orange, 6.3mg/ml for thyme-leaved savory, 8.1mg/ml for peppermint, 22.5mg/ml for lavender and 44.1mg/ml for basil essential oils (in comparison to the LC_{50} of 10.9mg/ml associated with the reference paraffin oil product). LC_{90} values for adult *P. ficus* females were reported as 14.4mg/ml for lemon, 16.2mg/ml for orange, 45.9mg/ml for thyme-leaved savory, 27.9mg/ml for peppermint, 41.4mg/ml for lavender and 65.7mg/ml for basil essential oils (in comparison to the LC_{90} of 25.7mg/ml associated with the reference paraffin oil product). The essential oils also differed in terms of phytotoxic effects triggered on grape leaves. While no phytotoxic effects were reported as a result of spraying with citrus essential oils (lemon and orange), leaves sprayed with essential oils derived from the aromatic plants developed brown spots which became necrotic. Lavender (at rates over 27mg/ml), thyme-leaved savory (rates over 13.5mg/ml) and mint (rates over 9mg/ml) essential oils caused phytotoxicity symptoms on less than 25% of the leaf surface area, whereas basil essential oil triggered high levels of phytotoxic damage in most concentrations tested, with over 50% of the leaf surface showing symptoms of damage.

Evaluations of garlic, *Alium sativum*, for efficacy against mealybugs show variable efficacy. Prishanthini & Vinobaba (2014) reported an LC_{50} for a garlic extract tested under laboratory conditions of a 0.82% solution when tested for toxicity against *P. solenopsis*. Mortality was dose-dependent, with treatment at 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.5% solution concentrations resulting in 0, 3, 10, 16, 28, 51 and 75% mortalities, respectively (Prishanthini & Vinobaba, 2014). Reasonable efficacy has also been reported against *P. longispinus*. In a study carried out in Cameroon, Okolle *et al.* (2018) prepared a garlic formulation by combining powdered dried garlic bulb, and a soybean oil, detergent soap and water solution. Under laboratory conditions, treatment of cut banana leaf segments with solution at a rate of 5g/L of garlic powder

resulted in 88.9% mortality of adult female *P. longispinus*, increasing to 100% when 15g/L of garlic powder were used. Under field conditions, banana plants artificially infested with mealybugs and treated with the garlic emulsion at a rate of 10g/L of garlic powder resulted in a decrease in mealybug population density per plant of approximately 83% (Okolle *et al.*, 2018). At a rate of 20g/ml, Piragalathan *et al*. (2014) reported 87% mortality of immature *Paracoccus marginatus* (Williams & Granara de Willink) (Hemiptera: Pseudococcidae) nymphs infesting pawpaw fruits under laboratory conditions, increasing from 30% when applied at 5g/ml to 55% at 10g/ml and 80% at 15g/ml. By contrast, garlic solutions were not as effective when used to treat *P. solenopsis* on hibiscus leaves under laboratory conditions. When used in solutions at a concentration of 1%, adult female mortality of 13% was recorded 48 hours after treatment application, rising only to 23% at a 2% concentration (though mortalities of 53-63% were recorded one week after treatment) (Sardar *et al.*, 2018). Other studies reported considerably lower mortality rates, however; Cloyd *et al.* (2009), for example, reported only approximately 5% mortality of *P. citri* following application of a commercially available garlic product (Garlic Pharm, 3.8% garlic oil) at the label rate, while Cloyd & Chiasson (2007) recorded 3% *P. citri* mortality and 18% *P. longispinus* mortality five days after application (98.2% garlic juice product from Garlic GP Ltd, at a rate of 73.9ml/946ml).

Ramzi *et al*. (2018) investigated the toxicity of wormwood, *Artemisia annua*, essential oils on third instar *P. viburni* in particular. In leaf-dip bioassays, they reported LC_{50} values of 0.69% 24 hours post-treatment, and 0.42% 48 hours post-treatment, with deterrence effects increasing with increasing essential oil concentration. In laboratory tests using cinnamaldehyde, Peschiutta *et al*. (2019) reported an LC_{50} of 0.394ml/L and an LC_{95} of 5.16ml/L when applied against *P. ficus*, with fumigant treatments at 0.3ml/L air resulting in approximately 45% mortality after 24 hours of exposure, and with no reported phytotoxicity to grape leaves.

Essential oil products derived from the Mexican tea plant, *Chenopodium abrosioides*, show varied efficacy against mealybug pests, dependent on species. Although application of a commercially available product caused 55% mortality of *P. longispinus* feeding on potted red coleus four days after treatment application (at a rate of 4ml/946ml), only 3% mortality was recorded for *P. citri* feeding on green coleus five days after treatment at the same application rate (Cloyd & Chiasson, 2007).

Cloyd *et al.* (2009) evaluated a range of plant essential oil products commercially available in the US for use against a range of invertebrate pest species, according to their labels, and their evaluations included *P. citri* on potted coleus plants under glasshouse conditions. Highest mortalities observed were 100% and 97% following treatment with a soybean and rosemary oil product (Indoor Pharm™, soybean oil 3%, rosemary oil 0.1%) and a cottonseed, cinnamon and rosemary oil product (Flower Pharm™, cottonseed oil 1.5%, cinnamon oil 0.1%, rosemary oil 0.1%) respectively, though these products caused symptoms of phytotoxicity to develop on foliage. A thyme and mint oil product (Herbal Aphid Control™, thyme oil 1.05%, mint oil 0.36%) caused 89% mortality without phytotoxic effects. By contrast, however, mortality rates of only approximately 5% were reported following treatment with a garlic oil product (Garlic Pharm, 3.8% garlic oil), and approximately 32% following application with a neem product (Green Light Rose Defense, neem 70%).

The Indian neem tree, *Azadirachta indica* A. Juss (Meliaceae), can be used to derive a range of natural neem compounds, including azadirachtin, with insecticidal activity against a range of pests. Compounds are highly complex and have multiple modes of action, acting primarily through hormonal and growth regulatory effects, but,

additionally and to a lesser extent, also as deterrents and anti-feedants, and sometimes as oviposition inhibitors. Jacobson *et al.* (1987) showed that *P. citri* were deterred by a 1% hexane extract of neem seed. In laboratory choice tests, treatment of cassava plant leaves with neem kernel water extracts made them less attractive to cassava mealybugs, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), with the majority (67%) of first instar crawlers recorded to select untreated leaves on which to settle and to feed 24 hours after release (Mourier, 1997). Of those choosing to feed on treated leaves (33%), all were adversely affected, with 94% dying in the second instar. Mourier (1997) further recorded from glasshouse experiments that three, repeated applications of neem kernel water extract provided cassava plants with good protection, with limited mealybug feeding damage symptoms observed compared with an untreated control in which near defoliation was recorded in the same trial. Indeed, only 2.5% of those mealybugs treated with the weakest concentration used in the trial (1% concentration) completed development, whereas 100% of mealybugs completed development in the untreated controls (Mourier, 1997). Such findings support suggestions that application could be particularly effective if timed to target the first instar crawler life stages through a growing season.

Laboratory testing of *A. indica* extracts at increasing concentrations resulted in 3% (at 0.2% concentration), 10% (at 0.4% concentration), 20% (at 0.6% concentration), 43% (at 0.8% concentration), 75% (at 1% concentration), 86% (at 1.2% concentration) and 100% (at 1.5% concentration) mortalities of adult female *P. solenopsis* on cut coleus plant segments 24 hours after initial exposure, with an LC_{50} of 0.82% calculated (Prishanthini & Vinobaba, 2014). By contrast, leaf dip bioassays at 1%, 2% and 3% neem extract solutions yielded mortality of, on average, 40-44% in adult female and 20-45% in third instar *P. solenopsis* (Sardar *et al.*, 2018). Mamoon-ur-Rashid *et al.* (2011) observed similar results in potted cotton trials, with neem oil applied at 1.5% and 2% concentration resulting in population reductions of 43% and 52%, respectively, in *P. solenopsis*, although laboratory bioassays yielded higher mortality rates. Piragalathan *et al.* (2014) observed similar reductions, with applications of neem leaf fermented solution (at a 1g/ml rate) and neem leaf extract (at a 20g/ml rate) causing mortalities of 49% and 52%, respectively, 24 hours after treatment, and 50% and 54%, respectively, 48 hours after treatment. In field trials on established cashew in India, field efficacy of azadirachtin and crude neem oil was tested against white-tailed mealybug, *Ferrisia virgate* (Cockerell) (Hemiptera: Pseudococcidae), with reductions relative to untreated controls of 61% and 60% respectively 10 days after an initial spray, and 80% and 78% 10 days after a second spray carried out 15 days after the first (Ambethgar, 2015).

Neem extracts have been observed to have some residual activity under controlled, optimal conditions. Sardar *et al*. (2018) for example, recorded mortalities of 16-21% in *P. solenopsis* 24 hours after exposure to neem extract in leaf dip bioassays, rising to 26-33% after 48 hours, through to 63-70% after one week. Mamoon-ur-Rashid *et al.* (2012) observed sustained detrimental effects of neem oil on *P. solenopsis* for up to three months under shaded laboratory conditions, with sub-lethal effects including adverse impacts on pupal period, longevity, and fecundity observed. It is, however, very unlikely that such sustained impacts would be possible under field and glasshouse conditions, as azadirachtin and neem extract products are susceptible to degradation by UV light, leading to short residual activity in practical use. Indeed, studies have shown that such longevity of effect is not to be expected in the field, and it is widely accepted that under field conditions residual activity can only be expected for five to seven days (Mourier, 1997). For example, Moniruzzaman *et al.* (2017) reported that neem-treated fig orchard plots had a consistently higher percentage of crop infested (based on presence or absence) where applications of neem extract

were made at 30, 45 and 60 day intervals, when compared to other treatments, although applications at 7 day intervals achieved good control and, at 15 days, the percentage of crop infested did not differ from other botanical extracts. Similarly, the findings of Ambethgar (2014) also suggested a benefit of repeat, 7-day interval applications, as did those of Mourier (1997). The short residual activity also highlights the importance of crop monitoring to maximise application impact, as preventative applications are unlikely to have significant effect. Preventative application of an azadirachtin product (as a drench, taking advantage of systemic activity) resulted in 9% mortality in *P. citri* when used at the label rate, rising only to 61% when applied at four times the label rate, on potted coleus plants (Herrick & Cloyd, 2017). Such inflated rates of application are not viable in commercial settings. Additional consideration must be given to potential phytotoxic effects, with Mourier (1997) reporting impact to varying degrees, dependent on neem product concentration.

Botanically derived insecticides, though showing variability in reported efficacies in the literature, dependent on origin and mealybug species targeted, nonetheless show some promise. Although the literature suggests many are unlikely to provide commercially acceptable levels of control independently, they are likely to be a useful tool as a constituent part of a control programme and certainly warrant further evaluation for potential use against *P. viburni* in UK protected edible production, both in terms of efficacy and crop safety. Although many compounds require considerable further research and development, an increasing number are already approved or near-market in the UK and EU, for example Prev-Am® (Oro-Agri), Requiem® (Bayer), ECOguard® (Ecospray Ltd) and 3AEY® (Eden Research). It will, however, also be essential to investigate the impacts of different adjuvants, and combinations of these, as these have been repeatedly shown to not only greatly affect efficacy, but also any potential phytotoxicity to different crops (e.g. Hollingsworth, 2005; Hollingsworth & Hamnett, 2010). Issues of crop coverage and pest contact will also need to be considered, given the cryptic nature of *P. viburni* and mealybugs more generally and the need for direct contact with the target for efficacy, as will any potential for product tainting from certain botanical compounds.

Entomopathogenic fungi

The potential of entomopathogenic fungi as control agents for use against *P. viburni*, and mealybugs more generally, has been of some interest. The literature suggests some efficacy in laboratory trials, though this may be limited on a field scale, though combination with adjuvants such as dewaxing agents has been shown to improve efficacy.

Jacobson & Croft (2002) investigated the use of *Lecanicillium lecanii* (previously known as *Verticillium lecanii*) for control of *P. viburni* in protected tomatoes. In laboratory bioassays on excised tomato leaves, they investigated the infection rates of adult *P. viburni* females following application of experimental wetters and de-waxing agents, and subsequent application of the fungal product Mycotal®. Overall mortality five days after application ranged from 60-100%, dependent on the combination of adjuvants used. Subsequent laboratory bioassays further developing initial findings reported greater variability, with mortality six days after application ranging from 0% (where 0.1% Mycotal® was applied without any adjuvants) to 100% (where 0.1% Mycotal® was applied after the dewaxing agent 2% Savona®). Similar variability was reported for second instar nymphs (10-90% dependent on adjuvant), and egg sacs and first instar crawlers (11-56% dependent on adjuvant). Highest rates of mortality were typically reported following applications of Savona® (at either 2% or 4%), with Mycotal® application in isolation or with Addit® only typically leading to mortality rates at the lower end of the range.

The important role played by adjuvants such as dewaxing agents in improving the efficacy of entomopathogenic fungi has also been reported by Curkovic *et al*. (2019). In laboratory evaluations using a Potter tower, exposure of *P. viburni* females to non-lethal concentrations of the agricultural detergent TS-2035® as a co-adjuvant in formulations of *Beauveria bassiana* (strain GHA) or *Metarhizium anisopliae* (strain Qu-M984) resulted in changes to mortality rates and LC_{50} values of the solutions. Mortality rates increased with increasing concentration of fungal pathogens. One day after application of *M. anisopliae* in isolation, mortality rates of *P. viburni* were reported to be approximately 7, 20 and 40% at *M. anisopliae* concentrations of 1, 10 and 100% w/v. Three days post-treatment, mortality rates were reported to be approximately 10, 25 and 50%, respectively, rising to approximately 30, 40 and 62% respectively six days after treatment. Combination with TS-2035® as a co-adjuvant at a non-lethal rate of 0.001% v/v resulted in increases to mortality of 10-30%. The highest mortality rate of *P. viburni* following *M. anisopliae* treatment was observed at a rate of 100% w/v of the fungal pathogen with TS-2035® at a rate of 0.001% v/v, with mortality of 75-85% one to six days after application. In the case of the *B. bassiana* formulation tested, increasing concentration of the fungal pathogen resulted in increased mortality, similar to the trend observed for *M. anisopliae*, but this was not found to be increased by addition of TS-2035®. Regardless, combination of both fungal pathogens with TS-2035® reduced the LC_{50} of the solutions. For *M. anisopliae*, the LC_{50} in colony forming units (CFU) per ml were significantly reduced from 8.6×10^7, 3.3×10^7 and 3.0×10^7 at one, three and six days after treatment respectively, to 8.8×10^6, 7.8×10^6 and 6.1×10^6 respectively. The reduction for *B. bassiana*, by contrast, was only found to be statistically discernible at three days after application, with the CFU LC_{50} reduced from 1.6×10^4 to 9.5×10^3.

Potential efficacy has also been explored in other mealybug species. Chartier FitzGerald *et al*. (2016) screened South African field-collected strains of *M. anisopliae* and *B. bassiana* against *P. citri* in a laboratory plate bioassay. A commercial *B. bassiana* product (BroadBand®) achieved 77% mortality of first instar crawlers, and approximately 42% mortality of adults. Treatment with other field-collected strains of *B. bassiana* resulted in mortality rates ranging from approximately 37-54% for adult *P. citri*, and approximately 44-68% for first instar crawlers. In the case of *M. anisopliae*, mortalities of approximately 52-68% of first instar crawlers, and approximately 38-48% of adult *P. citri* were observed. In laboratory bioassays on whole potato tubers dipped in fungal pathogen solutions, mortalities of 48, 34 and 28% were reported three days after treatment with *B. bassiana*, *Paecilomyces lilacinus* and *P. fumosoroseus*, respectively, rising to 100, 82, and 96%, respectively (Karaca *et al.*, 2016). Plate bioassays of efficacy of *Isaria farinosa* (synonym: *Paecilomyces farinosus*) against *P. citri* indicated mortalities of 89% in egg sacs, 78% in first instar crawlers, 84% in second instar nymphs and adult females at 95% relative humidity, following application at a rate of 1×10^8 conidia/ml, with mortality decreasing with relative humidity, though significant infection was nonetheless observed above 70% relative humidity (Demirci *et al*., 2011). Similar reductions in mortality with decreasing relative humidity have also been reported in evaluations of *I. farinosa* for efficacy against *P. ficus*; when applied at a rate of 1×10^8 conidia/ml, adult *P. ficus* female mortality was reported to 78% at 95% relative humidity, decreasing to around 50% at 70 and 80% relative humidities (Muştu *et al.*, 2015). Increasing mortality with increasing conidial concentration, as observed in the Curkovic *et al*. (2019) *P. viburni* study is also a recurring theme. For example, Mohamed (2016) reported the same trend in a study on *P. ficus*. In dip bioassays of adult female *P. ficus*, mortalities of 74, 52 and 32% resulted from submersion in fungal solutions of *B. bassiana*, *M. anisopliae*, and *L. lecanii*, respectively, at 5×10^5

conidia/ml. By contrast, mortality rates of 98, 86 and 74%, respectively, were reported following the same treatment at a rate of 5×10^7 conidia/ml.

Banu *et al*. (2010) evaluated the susceptibility of *P. marginatus* to various entomopathogenic fungal species under laboratory conditions. Maximum mortalities of 80% seven days after treatment with *L. lecanii*, and 75% and 70% mortalities nine days after treatment with *M. anisopliae* and *B. bassiana*, respectively, were reported in adult *P. marginatus*, with maximum mortalities of 100% of nymphs by all three fungal species nine days after treatment (Banu *et al.*, 2010).

Although some potential is suggested by laboratory assays, further investigation and research at a commercial field scale would be required to fully validate the efficacy of entomopathogenic fungi for industry, as this is likely to be constrained, or subject to high levels of variability, due to a number of potential limitations. Infection relies primarily on direct contact, which could be challenging given the cryptic nature of *P. viburni*. Temperature and humidity are known to affect fungal spore germination, with efficacy repeatedly shown to be affected by relative humidity levels. Furthermore, the role of strain within fungal species has also repeatedly been shown to affect efficacy (e.g. Panyasiri *et al.*, 2007; Chartier FitzGerald *et al.*, 2016), Regardless, efficacy has been shown to improve with adjuvant and co-adjuvant use (e.g. Jacobson & Croft, 2002; Curkovic *et al*., 2019). A range of entomopathogenic fungi are also commercially available, and as such screening, with consideration given to the use of such adjuvants in a protected setting and to impact and timing within an integrated approach, may be of benefit.

Viral pathogens

Literature searches have not suggested any entomopathogenic viral diseases in mealybugs that may have control potential against the pest. As such, at time of writing, this is not a viable avenue of investigation in terms of management strategies for *P. viburni*.

Semiochemicals

Semiochemicals, such as sex pheromones, can be used as pest control tactics, beyond or in addition to their use in monitoring strategies. They can be used to alter pest behaviour, and thus can facilitate potential management through either disruption of behaviour, such as those associated with mating, or through the development of ‘lure and kill’ strategies. Although the identification of the *P. viburni* sex pheromone has opened such possibilities, no work appears to have been undertaken to develop such approaches on a practical scale for this species. Limited work has, however, been undertaken on other mealybug species, with variable results.

Franco *et al.* (2004) describe a two-year study, conducted in small plots in Portuguese and Israeli citrus orchards, in which mass trapping of *P. citri* males was carried out by deploying traps baited with 200µg of sex pheromone at a rate of one per tree. While the researchers trapped and significantly reduced numbers of male *P. citri*, these reductions were not found to significantly reduce infestation levels on the citrus fruits. Cocco *et al*. (2014) investigated the potential of pheromone-baited traps as mating disruptors of *P. ficus* in two Sardinian vineyards. They observed 86-95% reductions of male numbers captured in disrupted plots, as well as significantly lowered mealybug densities and modified population age structure (with a decreased percentage of ovipositing females, and higher proportion of preovipositing females) at

an active ingredient rate of 93.8g/ha (150mg per dispenser), though these impacts were not found to have a significant impact on damage levels.

Although such studies suggest the potential for pheromone-baited traps to be deployed as control options in integrated management strategies, significant development of approaches remains necessary. The design of such trapping systems, as well as the commercialisation of the *P. viburni* sex pheromone in particular, will be essential before mass trapping and 'lure and kill' approaches can become viable options for UK growers.

Conventional Insecticides

Chemical control of mealybugs has, historically, been the most common management strategy deployed. Although many actives used were often quite effective (for example, Frick (1952) reported the organophosphate ethyl parathion provided adequate control of *P. maritimus* at a rate of 48g a.i./ha), issues of resistance against a broad range of actives have emerged (Franco *et al.*, 2004). Furthermore, many of the actives used, past and present, are characterised by broad-spectrum activity and are known disruptors of IPM programmes, and thus are, generally, of limited compatibility within UK glasshouse crop IPM strategies. Remedial chemical control is also difficult to achieve on a commercial scale. The cryptic behaviours of mealybug pests, whereby they settle into protected areas of crops, results in limited contact with treatment applications. This protects the targets from actives reliant on direct contact for efficacy. Some newer, systemic products might be expected to overcome such challenges, although there is growing evidence to suggest this may not be the case in glasshouse systems, as a result of mealybug feeding behaviours (for example, the type of tissue fed on, and the location on the host plant at which feeding takes place) conferring some limitations on active ingredient quantities ingested (Herrick & Cloyd, 2017; Herrick *et al.*, 2019). Mealybugs are further protected by their typically hydrophobic waxy secretions, which prevent penetration of water-based insecticide solutions (Franco *et al.*, 2004), and their high reproductive rates allow rapid recovery of populations. Timing is often of critical importance, with early instars often more susceptible to treatments, and with first instar crawlers more exposed as they more around on hosts (Daane *et al.*, 2012).

Synthetic product use can also be challenged by the need to consider harvest intervals in an application programme, particularly in continuously harvested crops such as tomatoes and peppers. Regardless, effective synthetics may be of interest where these can be used to achieve maximum possible control ahead of fruit set, or to knock back large populations that threaten unacceptable levels of economic damage.

Identified literature pertaining to control of *P. viburni* or, where this is not available, other mealybug pest species is summarised herein, as well as any observations on active ingredient approval as at November 2019 (LIAISON database, Fera Science Ltd, 2019; EU Pesticides Database, EU Commission, 2019; BCPC, 2019; databases accessed November 2019). For any such chemical insecticides, potential for use should be considered with harvest intervals in mind, and the impacts on existing IPM management strategies for both mealybug targets and other invertebrate pests.

Pyrethroids

In laboratory bioassays, pyrethroid insecticides have been shown to be effective against mealybug targets, and this has carried through on a field scale for some

actives, though variability across crops and studies should be noted. Saeed *et al*. (2007) screened a number of commercially available pyrethroids for efficacy against *P. solenopsis* (under the synonym *Phenacoccus gossypiphilous*) under laboratory and field conditions. In laboratory hibiscus leaf-dip bioassays at a field application rate (825ml/ha) equivalent, mortality rates were observed as follows:

- Bifenthrin (Talstar® 10EC) – 50% after 24 hours, 35% after 48 hours, and 40% after 72 hours;
- Cypermethrin (Arrivo® 10EC) – 22.5% after 24 hours, 75% after 48 hours, and 90% after 72 hours;
- Deltamethrin (Decis® Super 10.5EC) – 52.5% after 24 hours, 75% after 48 hours, and 90% after 72 hours;
- Esfenvalarate (Sumi Alpha®) – 17.5% after 24 hours, 35% after 48 hours, and 47.5% after 72 hours; and
- β-cyfluthrin (Bulldock® 2.5EC) – 25% after 24 hours, 50% after 48 hours, and 57.5% after 72 hours.

Under field conditions, where nursery hibiscus plants were treated by knapsack sprayer at the same application rate (825ml/ha), mortality rates of mealybugs present on leaves were as follow:

- Bifenthrin – 66.3% after 24 hours, 80.8% after 48 hours, and 83.9% after 72 hours;
- Cypermethrin – 73.3% after 24 hours, 79.4% after 48 hours, and 82.0% after 72 hours;
- Deltamethrin – 82.1% after 24 hours, 82.8% after 48 hours, and 87.8% after 72 hours;
- Esfenvalarate – 41.3% after 24 hours, 80% after 48 hours, and 80.6% after 72 hours; and
- β-cyfluthrin – 60.5% after 24 hours, 78.7% after 48 hours, and 78.8% after 72 hours.

It should be noted that reduced efficacy was reported in terms of mortality on apical branch segments (e.g. at 72 hours after application mortality rates of 63.1% for bifenthrin, 53.4% for cypermethrin, 47.7% for deltamethrin, 66.6% for esfenvalarate and 66.5% for β-cyfluthrin were reported). The results obtained by Saeed *et al.* (2007) suggest reasonable efficacy of several of the pyrethroid actives, with particular importance in the field screening trials, as often promising laboratory results fail to translate to effective control under field conditions for mealybug pest species. In particular, deltamethrin, under field conditions, appeared to provide a rapid effect on mealybug populations, although other actives increased in efficacy through time. In testing LC_{50} values on known susceptible laboratory populations against field-gathered populations of *P. solenopsis* in Pakistan, Saddiq *et al*. (2017) reported the LC_{50} for a commercial deltamethrin formulation (Decis® Super 10EC) of, on average, 8.59μg/ml in laboratory populations, and 45.32μg/ml for field populations.

Other field evaluations have also suggested good efficacy. Karar *et al*. (2010) screened a range of commercial actives for efficacy against mango mealybug, *Drosicha mangiferae* Green (Hemiptera: Pseudococcidae), in mango orchards in Pakistan. Twenty-four hours after application, treatment with λ-cyhalothrin (Karate® 2.5EC at 50ml/100L) resulted in mortality rates of 74.85% in first instar crawlers, 63.43% of second and third instar nymphs, and 31.89% of adult females. This rose to 86.32%, 74.88% and 52.4%, respectively, after seven days post-treatment. In the case of deltamethrin (Decis® 2.5EC at 50ml/100L), mortality rates of 77.93% in first instar crawlers, 70.72% of second and third instar nymphs, and 45.44% of adult females were observed 24 hours after treatment, rising to 85.2%, 77.22% and 61.58%, respectively, seven days post-treatment. Treatment with cypermethrin

(Ripcord® 10EC at 100ml/100L) resulted in mortalities of 4.8% in first instar crawlers, 37.61% of second and third instar nymphs, and 21.87% of adult females after 24 hours, rising to 64.92%, 45.83% and 32.96%, respectively, seven days post-treatment. Reductions of 91-92.5% have been reported in field evaluations of λ-cyhalothrin in cashew orchards, when targeted against *F. virgata* (Ambethgar, 2015).

By contrast, limited efficacies have also been reported on both a laboratory and field scale. For example, treatment with cypermethrin resulted in mortalities in *P. citri* of only approximately 30% twenty-four hours after treatment in laboratory toxicity bioassays carried out by Arshad *et al.* (2015), and Agnello *et al*. (1992) reported that treatment with esfenvalarate was no more effective at controlling the Comstock mealybug, *Pseudococcus comstocki* (Kuwana) (Hemiptera: Pseudococcidae), in New York pear orchards than applications of distilled water.

Overall, literature suggests reasonable efficacy of pyrethroids. Deltamethrin, in particular, is approved for use in UK and EU production, with valid approval for use against mealybug pests in many protected crops. Cypermethrin, λ-cyhalothrin, β-cyfluthrin and esfenvalarate are also approved for use in the UK and in the EU; bifenthrin is not approved for use in either the UK or the EU. Regardless, these products are often considered incompatible with existing IPM programmes, and therefore their use should likely be limited to situations were unacceptable economic damage is threatened, and where other IPM-compatible measures have been exhausted. Consideration of potential resistance issues should also be considered, as should application methods and potential use of adjuvants to improve efficacy. Pyrethroids remain a potentially useful tool, and as such a programme to evaluate effective application rates and treatment scenarios may be of benefit to UK growers, though this would seem less a priority than evaluation of potential resistance, or evaluations of newly available actives to market.

Pyrethrins

Pyrethrins are a fast-acting contact insecticide extracted from *Chrysanthemum* pyrethrum. They often have shorter harvest intervals, and are considered more compatible with IPM approaches, when compared to synthetic pyrethroids. The literature suggests varied efficacy. In evaluating a range of commercially available products for the domestic market, Cloyd *et al*. (2009) reported *P. citri* mortality rates of approximately 50% for one formulation (Pyola™; 89.5% canola oil, 0.5% pyrethrins), and approximately 75% for another (Garden Safe Houseplant & Garden Insect Spray™; 1.0% canola oil, 0.01% pyrethrins) on potted coleus plants, while Hogendorp & Cloyd (2013) reported comparable mortality of 69% following application of another such product (Schultz Expert Gardener®), also *P. citri*. These mortality rates, however, contract with those observed by Taskin *et al.* (2014), for example, who reported less than 10% mortality of *P. ficus* in laboratory bioassays. Furthermore, Jacobson & Morley (2009) stated that control programmes targeting *P. viburni* and incorporating applications of pyrethrins were not considered to provide adequate levels of control in a commercial setting.

A number of pyrethrin products are approved for use in the UK (e.g. Pyrethrum 5EC®, Spruzit®), including for use against mealybugs, in some protected edible crops. It may be that efficacy could be improved with use of adjuvants, and it may be useful to evaluate this on a commercial scale. Regardless, literature would seem to suggest limited efficacy, although value may nonetheless be derived from greater compatibility within existing IPM programmes.

Organophosphates

Organophosphates as a class are one of the most widely used chemical insecticides for mealybug control in orchards and vineyards worldwide, with chlorpyrifos use particularly widespread (Franco *et al.*, 2004; Daane *et al.*, 2012; Le Vieux & Malan, 2013a), though issues of resistance have been recorded. Mortality of *P. viburni* treated with a label rate (1.2ml/L, or 576 ppm) of a commercially available chlorpyrifos product (Lorsban® 4E, 48% a.i.) in a laboratory Potter tower bioassay were approximately 70% 24 hours after treatment, rising to approximately 78% after 72 hours; when combined with an adjuvant (TS-2035® at 0.001% v/v), mortality was increased to above 90% at both observation times post-treatment (Curkovic *et al.*, 2019).

In the Saeed *et al*. (2007) screening trial, commercial formulations of chlorpyrifos (Lorsban® 40EC), profenofos (Curacron® 50EC) and triazophos (Hostathion® 50EC) were evaluated at field application rates (2500ml/ha) under laboratory and field conditions for efficacy against *P. solenopsis*. Laboratory hibiscus leaf-dip bioassays resulted in the following mortalities:

- Chlorpyrifos – 82.5% after 24 hours, rising to 100% after 48 hours;
- Profenofos – 92.5% after 24 hours, rising to 100% after 48 hours; and
- Triazophos – 47.5% after 24 hours, 72.5% after 48 hours, and 82.5% after 72 hours.

Under field conditions, nursery hibiscus plants were treated at the same application rate (2500ml/ha) with a knapsack sprayer, and again good mortality rates were observed for most organophosphate actives tested among mealybugs on foliar surfaces:

- Chlorpyrifos – 68% after 24 hours, 69% after 48 hours, rising to 85.2% after 72 hours;
- Profenofos – 68.2% after 24 hours, rising to 93.9% after 48 hours and 94.3% after 72 hours; and
- Triazophos – 65.7% after 24 hours, rising to 75.2% after 48 hours and 74.8% after 72 hours.

As was reported for pyrethroid class actives, reduced mortality rates were recorded on apical branch segments (e.g. at 72 hours after application mortality rates of 76.9% for chlorpyrifos, 80.1% for profenofos, and 63.5% for triazophos).

In mango orchards in Pakistan, similar results have been reported following application of organophosphates against *D. mangiferae* by Karar *et al.* (2010). Treatment with chlorpyrifos (Lorsban® 40EC at 50ml/100L) resulted in mortality rates of 66.7% in first instar crawlers, 61.94% of second and third instar nymphs, and 35.19% of adult females 24 hours after treatment. Seven days post-treatment, mortality had risen to 77.68% in first instar crawlers, 70.11% of second and third instar nymphs, and 52.99% of adult females. Treatment with profenofos (Curacron® 500EC at 30/100L) resulted in mortalities of 74.42% in first instar crawlers, 70.02% of second and third instar nymphs, and 50.11% of adult females after 24 hours, rising to 83.68%, 78.84% and 64.44%, respectively, seven days post-treatment. In the case of triazophos (Hostathion® 20EC at 100ml/100L), mortality rates of 52.94% in first instar crawlers, 47.34% of second and third instar nymphs, and 30.12% of adult females were observed 24 hours after treatment, rising to 68%, 62.03% and 35.55%, respectively, seven days post-treatment. Efficacy of chlorpyrifos has also been shown in pear orchard field trials, with application at a rate of 170g a.i./379L achieving *P. comstocki* mortality rates of 81.3% (with the remaining recorded to be moribund) (Agnello *et al.*, 1992); the same study reported 56.8% mortality following application of methyl parathion, although phosmet, azinphos-methyl and mevinphos all returned lower mortality rates, at 21.3, 29.8, 36% respectively (Agnello *et al.*,

1992). In testing LC_{50} values on known susceptible lab populations against field-gathered populations of *P. solenopsis* in Pakistan, Saddiq *et al*. (2017) reported the LC_{50} for a commercial chlorpyrifos formulation (Lorsban® 40EC) of, on average, 13.19μg/ml in laboratory populations, and 32.75μg/ml for field populations.

Both profenofos and triazophos resulted in mortalities of *F. virgata* of over 90% in cashew crops (Ambethgar, 2015), with profenofos mortality rates of over 96% 24 hours post-treatment also reported in laboratory bioassays against *P. marginatus* (Piragalathan *et al*., 2014). Laboratory bioassays against *P. marginatus* indicated mortality rates of 66.7% following treatment with acephate, rising only to 69.4% after 48 hours (Piragalathan *et al.*, 2014). Application of acephate (Commando® 97DF) under field conditions in a cotton crop resulted in *P. solenopsis* population reductions of 70.35% after 24 hours, and 86.3% after 72 hours (Mamoon-ur-Rashid *et al*., 2011), while a study on ferns found three applications at 7-12 day intervals of the active at a rate of 780mg/L reduced *P. longispinus* mealybug populations, though with phytotoxic effects (Martin & Workman, 1999).

Despite the efficacy shown by organophosphates, the majority of the actives listed in this section are not approved for use in the UK or the EU, with chlorpyrifos a notable exception (although this is only approved for use in stored grain) and phosmet approved for use in some other EU countries. Products in this class are being phased out across Europe, are not compatible with IPM approaches, and therefore should not be considered to represent a viable or useful option for protected edible growers in the UK at this time.

Neonicotinoids

The neonicotinoid class of insecticides is characterised by systemic and long-lasting residual activity, especially when applied as a seed treatment or in the growing medium. As systemic substances, there has been much interest in their use as a management tool for mealybug pests, as by being transported around the plant, the active should be more likely to be taken up by the target mealybugs, despite the cryptic behaviours that protect them from many other active ingredient classes.

Imidacloprid is the most commonly used (and investigated) neonicotinoid in terms of mealybug control (Mansour *et al.*, 2018), with the efficacy typically related to the mode of application. In evaluations in Tunisian vineyards, it was shown that application of imidacloprid through furrow irrigated systems did not result in acceptable levels of control (Daane *et al*., 2006; Mansour *et al*., 2010a), suggested to result from an effectively diluted application rate due to root structure and distribution differences when compared with those observed in drip-irrigated systems (Daane *et al.*, 2006). In testing a commercial formulation of imidacloprid (Confidor® at a rate of 3ml/l/vine) Mansour *et al.* (2010a) reported overall Abbot mortality rates of 23.45%, 39.32%, 27.27% and 54.4% of *P. ficus* 3, 7, 14 and 21 days following treatment application in a furrow-irrigated Tunisian vineyard system. By contrast, when applied through a drip-irrigated system, imidacloprid (Spector®) applied at rates of 1-2ml/vine was reported to reduce mealybug egg populations by 86% 20 days after treatment, reaching 100% mortality 40 days after treatment, and achieving good long-term control in Tunisian vineyards from the lower rate (Mansour *et al.*, 2010b). Daane *et al.* (2006) also reported reduced grape cluster damage when imidacloprid was applied through drip-irrigation, in comparison to furrow irrigation. Similar results have also been observed in New Zealand vineyards, where Lo & Walker (2011) reported reductions in mealybug abundance of up to 99% following application of imidacloprid soil drenches at 0.525g a.i./vine when compared to untreated vines. In cashew crops,

reductions of *F. virgata* of over 90% were reported following treatment with imidacloprid (Ambethgar, 2015).

Karar *et al.* (2010) evaluated the efficacy of imidacloprid and acetamiprid against *D. mangiferae* in mango orchards in Pakistan. Treatment with imidacloprid (Confidor® 200SI at 100g/100L) resulted in mortality rates of 70.47% in first instar crawlers, 57.89% of second and third instar nymphs, and 40.28% of adult females 24 hours after treatment. Seven days post-treatment, mortality had risen to 77.5% in first instar crawlers, 67.76% of second and third instar nymphs, and 48.61% of adult females. In the case of acetamiprid (Mospilan® 20EC at 100g/100L), mortality rates of 79.72% in first instar crawlers, 65.25% of second and third instar nymphs, and 58.13% of adult females were observed 24 hours after treatment, rising to 90.57%, 81.42% and 70.57%, respectively, seven days post-treatment.

Variability is, nonetheless, possible, with Castle & Prabhaker (2011) commenting that, over the course of a two-year trial, they observed greater efficacy of applications of imidacloprid and thiamethoxam, in certain mulberry trees, leading the study authors to suggest that aspects such as population growth trajectories relative to starting population densities, timing of application and variables associated with uptake and distribution of systemic actives within each tree could play a role in eventual product efficacy. Regardless, by the end of the trial, they observed 45-50% of treated mulberry trees to be free from *M. hirsutus* infestation, with substantial reductions in the numbers of the mealybugs on treated trees in comparison to increasing densities on untreated controls (Castle & Prabhaker, 2011).

Curative applications of imidacloprid (Discus® at a rate of 1 tablet per pot, 2.5g) achieved mortality of only 34.3% in *P. citri* on potted coleus 14 days after application in glasshouse conditions, while two other neonicotinoid actives, dinotefuran (Safari® at a rate of 1.7g/946ml) and thiamethoxam (Flagship® at a rate of 0.6g/946ml), achieved lower rates of 23.2% and 26.4% under the same conditions (Herrick & Cloyd, 2017). Mortality was also limited following preventative applications of imidacloprid in tablet (Discus® at a rate of 1 tablet per pot, 2.5g) and liquid (Marathon II® at a rate of 0.48ml/946/ml) forms at the label rate, reaching 14.5% and 12.8%, respectively, 31 days after application (Herrick & Cloyd, 2017). By contrast, trials on *P. citri* on potted coleus treated with acetamiprid resulted in 68% mortality of five days after application (TriStar® at a rate of 0.093g/946ml) in one study (Cloyd & Chiasson, 2007), and mortality of 84% seven days after application (TriStar® at a rate of 0.05g/L) in another (Hogendorp & Cloyd, 2013). In a trial investigating the impact of light, insecticide concentration and time on the efficacy of imidacloprid applied against *P. citri* in potted poinsettia, mortality values were not found to exceed 50% combinations tested (Cloyd *et al*., 2012). In evaluating dinotefuran, the same trial did not observe mortality rates above 50% under any combination (Cloyd *et al*., 2012). In potted ferns, by contrast, mealybug infestations were reduced to 1-4% of fronds infested with *P. longispinus* following application of acetamiprid, compared to 83% of fronds infested in untreated control pots (Martin & Workman, 1999).

In laboratory leaf-dip bioassays, imidacloprid resulted in approximately 18% mortality of *P. citri* third instar nymphs 6 days after treatment, rising to approximately 58% after 26 days (Arshad *et al*., 2015). Mamoon-ur-Rashid *et al.* (2011) evaluated the efficacy of imidacloprid and thiamethoxam under laboratory and field conditions. In leaf dip bioassays, imidacloprid (Confidor® 20SL at a rate of 250ml/acre) caused mortality rates in second instar *P. solenopsis* nymphs of 59.3% after 24 hours, rising to 66.05% and 81.57% 48 and 72 hours after treatment, respectively. In the case of thiamethoxam (Actara® 25WG at a rate of 24g/acre), mortality rates of 82.98, 91.49 and 95.24% were observed after 24, 48 and 72 hours, respectively. Under field

conditions, pooled mortality on a cotton crop of 73.29% for imidacloprid and 74.36% for thiamethoxam were reported (Mamoon-ur-Rashid *et al.*, 2011).

The literature suggests variable efficacy of neonicotinoid actives when used against mealybugs, though good to excellent mortality rates have been achieved in several studies. As a class of systemic actives, the reduced emphasis on complete spray coverages, often required with other products, could be useful, particularly in mature crops where this can be difficult to achieve, and against mealybug pests such as *P. viburni* in particular. With that in mind, however, it could be expected that efficacy should be improved, as direct, external contact with the target surface should not be required, however there is evidence to suggest that, despite being sap-sucking pests, mealybugs may not be as susceptible as might be expected. Differences in feeding behaviours and locations of mealybugs, relative to other Hemipteran pests such as whiteflies and aphids have been suggested as explanations for this (Cloyd *et al.*, 2012; Herrick & Cloyd, 2017). For example, differences in the type of plant tissue fed on, whether the pest feeds on terminal growth and foliage (such as whiteflies and aphids) or plant stems (as do many mealybugs), and differences in the number or time-span of intercellular punctures, ingestion, or stylet motility during phloem searching could all alter the exposure rates of different pest targets to systemic actives. Were neonicotinoids to be evaluated for potential usefulness to UK protected growers, great care would need to be taken to minimising impact on IPM and pollination programmes.

Avermectins

Limited literature is available on the efficacy of avermectins, naturally derived actives, on mealybug pests in general, though variable and limited efficacy is suggested. In field trials on cotton in Egypt, treatment with a commercial formulation of emamectin benzoate (Proclaim® 5% SG) led to *P. solenopsis* population decreases of 24% (El-Zahi *et al.*, 2016), while in Sri Lankan laboratory bioassays on sprayed papaya fruit, treatment with abamectin (Mitsu®) led to 66% mortality in *P. marginatus* after 48 hours (Piragalathan *et al.*, 2014). In testing LC_{50} values on known susceptible laboratory populations against field-gathered populations of *P. solenopsis* in Pakistan, LC_{50} values for a commercial emamectin benzoate formulation (Proclaim® 1.9EC) were reported to be, on average, 11.16µg/ml and 137.76µg/ml for laboratory and field populations respectively (Saddiq *et al.*, 2017). The risk for the potential development of resistant populations has also been identified (Afzal & Shad *et al.*, 2015), and as such any application schedules should take into consideration insecticide resistance management. Further evaluations on the efficacy of avermectin formulations as a control measure against *P. viburni* would be required at a commercial, field scale before conclusions could be drawn about their potential use by UK protected edible growers. Abamectin-based pesticides are, however, available on- and off-label for glasshouse pests, and, with relatively short harvest intervals, benefit may be derived from such products.

Carbamates

The literature available, though limited, suggests good efficacy of carbamate products against mealybug pests. In screening tests, Saeed *et al*. (2007) evaluated commercially available methomyl (Lannate® 40WP) and thiodicarb (Larvin® 80DP) formulations at field application rates (1250g/ha and 1125g/ha, respectively) for efficacy against *P. solenopsis*. Laboratory leaf-dip bioassays resulted in mortality rates of:

- Methomyl – 62.5% after 24 hours, 82.5% after 48 hours, and 90% after 72 hours; and

- Thiodicarb – 35% after 24 hours, 65% after 48 hours, and 75% after 72 hours.

Under field conditions, where nursery hibiscus plants were treated by knapsack sprayer at the same application rates, mortality rates of mealybugs present on leaves were as follows:

- Methomyl – 89.7% after 24 hours, 91.5% after 48 hours, and 91.8% after 72 hours; and
- Thiodicarb – 77% after 24 hours, 78% after 48 hours, and 79.4% after 72 hours.

Although reduced mortality rates were recorded on apical branch segments (e.g. at 72 hours after application mortality rates of 82.9% for methomyl and 70.2% for thiodicarb), methomyl nonetheless caused the greatest mortality on these sections of plants when compared to other commercial pyrethroid, organophosphate and organochlorine formulations. In similar evaluations of efficacy against *P. solenopsis* on cotton, application of a methomyl product (Lannate® 40% SP at a rate of 250g/acre) led to 73.5% mortality of second instar nymphs three days after application in a laboratory leaf-dip bioassay, while population reductions of 89.63% and 92.46% were reported three days and seven days after treatment applications under field conditions, though pooled mortality over a ten day period averaged at 70.93% (Mamoon-ur-Rashid *et al.*, 2011). In field trials on cotton in Egypt, application of a methomyl product (Neomyl® 90% SP at the rate recommended by the Egyptian Ministry of Agriculture) led to reductions in population size of *P. solenopsis* of 92.3%. In New York pear orchards, mortality rates of 72 and 67.6%, respectively, were reported following field rate application of methomyl (Lannate® 1.8L at a rate of 102g a.i./379L) and carbaryl (Sevin® 50% WP at a rate of 454g a.i./379L) against *P. comstocki*, with two applications of methomyl timed to target each of two generations of first instar crawler reported to provide acceptable levels of control (Agnello *et al.*, 1992). As such, the literature suggests that carbamates may have some use as a potential active for use against *P. viburni* as part of a management strategy, though efficacy against this particular pest and crop safety should be confirmed by evaluations. Pirimicarb, for example, is available for off-label use against aphid pests in protected peppers and several other protected crops.

Ryanoids

Overall, the literature does not suggest that ryanoid class actives would have strong impact against *P. viburni* as a potential control option, though screening targeted at this particular mealybug species has not been conducted. Although laboratory bioassays may suggest high efficacy, with mortality rates of 92.2% reported in *P. marginatus* 24 hours after treatment with chlorantraniliprole (Coragen® at 0.18ml/L) in a leaf-dip bioassay (Piragalathan *et al.*, 2014), other available studies suggest significantly lower efficacy. For example, a leaf-dip bioassay of chlorantraniliprole (Coragen® at 50g a.i./ha) led to *P. solenopsis* mortality of only 15% after 48 hours, rising to 34% after 96 hours (Nagrare *et al.*, 2016), while preventative application of cyantraniliprole (Mainspring® at 0.9ml/946ml) in potted coleus was found to be ineffective, causing mortality of introduced *P. citri* of 2.2% and 12.8%, respectively, 24 and 31 days after treatment application.

Flonicamid

Flonicamid is a feeding inhibitor, relatively selectively used against hemipteran and thysanopteran pests. Investigations on potential efficacy on mealybug pests have, however, not suggested strong efficacy against mealybug species. Leaf dip laboratory bioassays of a commercially available product (Ulala® at a rate of 200gm/ha) resulted in 16.76% mortality of *P. solenopsis*, rising to 25.05% and

55.18% after 3 and 4 days, respectively (Nagrare *et al.*, 2016). Field trials of another commercially available product (Teppeki®) on cotton applied at a recommended field rate reduced *P. solenopsis* populations by 30.8% (El-Zahi *et al.*, 2016), while in potted coleus mortality rates of *P. citri* never exceeded 20% when flonicamid (Aria®) was applied at the label rate (0.3g/946ml) as a systemic drench (Herrick *et al.*, 2019). Although evaluation screenings for efficacy against *P. viburni* have not been reported in the literature, as with the ryanoid class of actives, it seems unlikely that considerable efficacy against this particular species would be observed, although assessment of impact following foliar spray applications may be of interest to provide information on the potential of this application method.

Flupyradifurone

Flupyradifurone is a nicotinic acetylcholine receptor agonist, with known action against mealybugs (Nauer *et al.*, 2014; Daane *et al.*, 2018), though results appear to suggest variable levels of efficacy. In potted coleus trials conducted under glasshouse conditions, treatment with flupyradifurone (Altus® at a rate of 14 fl.oz/100 gallons) led to reductions in the mean number of madeira mealybug egg sacs of 61.23%, 36.9% of immature stages and 68.64% of adults 14 days after treatment relative to an untreated control (Vafaie, 2019a), while 49 days after treatment reduction of 63.4% for adults and 70% for immature stages were reported relative to an untreated control (Vafaie, 2019b). In evaluating the efficacy of flupyradifurone against *M. hirsutus* in Californian dates, Ganjisaffar *et al.* (2019) reported good control under laboratory bioassay conditions, using sprayed leaf discs on an agar arena. Although adult females were not significantly reduced in number following sprayed applications of a commercially available product (Sivanto Prime®) at the maximum label field rate (1023ml/ha), the percentage of ovipositing females was reduced by 60%. Spraying of egg sacs resulted in a reduced number of eggs hatching, with only on average 21.4% eggs hatching from each egg sac following treatment application, and of the first instar crawlers that emerged only 0.3% were found to have survived on average. Furthermore, mortality rates of *M. hirsutus* nymphs were reported as 60% 24 hours after treatment, rising to 73.3% two days after treatment up to 97.8% six days after treatment. It was also recorded that all nymphs stopped feeding for six days post-treatment. Although no studies appear to screen flupyradifurone against *P. viburni*, the results obtained in the limited number of studies published suggest that the active could provide, potentially, useful levels of control for UK protected edible growers, although this and crop safety would need to be confirmed against this target pest under commercially relevant conditions before sound conclusions could be drawn, with consideration given to implications and impacts on existing IPM programmes. Flupyradifurone is currently approved in several EU member states, but not the UK at time of writing.

Indoxacarb

Indoxacarb is an oxadiazine active compound targeted, primarily, at lepidopteran pests and would not be expected to have good efficacy against mealybugs. Saddiq *et al.* (2017) determined LC_{50} values of known susceptible laboratory populations and field-collected populations of *P. solenopsis* in Pakistan cotton crops, as 21.04μg/ml and 129.37μg/ml for laboratory and field populations respectively. Literature on field efficacy against mealybug pests could not be identified at time of writing, though the presence of literature on the mechanisms of mealybug insecticidal resistance to this particular active suggest its use has been considered or that it has been deployed against *P. solenopsis* (Afzal *et al.*, 2015; Afzal & Shad, 2016).

Pymetrozine

There is limited literature available on the efficacy of pymetrozine against mealybug pests specifically. Barbosa *et al.* (2018) reported low mortality of little more than approximately 20% in third instar *Ferrisia dasylirii* (Cockerell) (Hemiptera: Pseudococcidae) mealybugs. Regardless, at time of writing approval for pymetrozine has been withdrawn in the EU and UK, and, as such, products containing this active represent no potential utility for UK protected edible growers for the control of mealybug.

Spinosad

Spinosad is an active compound based on chemical compounds found in *Saccharopolyspora spinosa*, a bacterial species, with little literature available to support efficacy against mealybug pests (though much research has been conducted on impacts on the natural enemies of mealybug pests). Saddiq *et al.* (2017) observed LC_{50} values of, on average, 18.88µg/ml and 97.7µg/ml for known susceptible laboratory and field populations of *P. solenopsis* treated with a commercially available spinosad product (Tracer® 240SC at a recommended rate). Further testing would be required in order to determine potential efficacy against *P. viburni* within management programmes, however given that spinosad-based products are approved for use in the UK, on- and off-label, including in protected crops (with three-day harvest intervals), and for application via irrigation systems, sound evaluation in a glasshouse system may be of benefit to UK growers.

Sulfoxaflor

Ganjisaffar *et al*. (2019) evaluated the efficacy of sulfoxaflor against *M. hirsutus* mealybugs in a laboratory sprayed cotton leaf bioassay. Treatment of egg sacs with a commercially available formulation (Closer® SC) at the label rate (300ml/ha) resulted in hatch rates of only 2.8% in each egg sac, with no first instar crawlers recorded to survive after emergence. Mortality of second instar nymphs was reported to increase through time after treatment application, with mortality of 8.9% reported one day after treatment, rising to 22.8% after two days through to 79.4% six days after treatment. Although adult females were found to be negligibly affected by sulfoxaflor treatment (1.7% mortality six days after treatment application), sub-lethal effects were nonetheless observed, with a 50% reduction in the number of successfully ovipositing females reported. Good control of *P. solenopsis* in semi-field and field trials 3-10 days after treatment application has also been reported, though the active was seen to have slower initial impact than an organophosphate reference product (Lysandrou *et al*., 2012). Further testing of efficacy against *P. viburni* would be needed before sound evaluations could be made in terms of usefulness to UK growers, however the limited literature available suggests potential use where sulfoxaflor is targeted at, in particular, early stages of the mealybug lifecycle. Assessment of such targeted applications in a commercially relevant setting, as part of a management programme rather than as a stand-alone application, may also provide greater relevance for industry.

Lipid synthesis-disrupting actives

Lipid-synthesis disruptors, and particularly spirotetramat, appear able to achieve reasonable control of mealybug pests, though highest efficacy is focused on immature life stages, with variable efficacies reported. Brück *et al.* (2009) reported that, in eleven European vineyard field trials, application of a commercially available spirotetramat product (Movento®) at rates of 72-88g a.i./ha resulted in mean efficacy of 92%, while trials undertaken in four vineyard field trials in the U.S.A. using the

same product and application rates led to a mean efficacy of 99%. Mansour *et al.* (2010a) observed absence of *P. ficus* eggs and adult females from Tunisian vineyards three weeks after application of spirotetramat (Movento® 150OD at a rate of 120ml/hl), and further reported over 90% efficacy in prevention of further spread of first and second instar nymphs on grapevine leaves. Mortality of 100% has been reported for *P. citri* on sour orange plants in Turkey (Satar *et al.*, 2013), while spirotetramat has also reportedly been used to successfully control *P. citri* in Tunisian citrus orchards (Mansour *et al.*, 2017).

Ganjisaffar *et al.* (2019) reported that spray treatment of *M. hirsutus* egg sacs with a commercial formulation of spirotetramat (Movento® 240SC at a rate of 731ml a.i./ha) resulted in a reduction of eggs successfully hatching, with only 28.5% hatching in each egg sac compared with 85.7% hatching from untreated control egg sacs. The number of first instar crawlers surviving after emergence was also reduced, with 53.1% surviving; which was less than from untreated and water treated controls (at 93.2 and 82.9% survival respectively). The egg hatch rate and first instar crawler survival rates, however, were the highest of the tested treatments (compared with bifenthrin, flupyradifurone, fenpropathrin, buprofezen, sulfoxaflor and acetamiprid). Although negligible mortality in adult females was reported (0.6% six days after treatment), 50% of females were observed to not complete oviposition successfully, suggesting sub-lethal impact. The reported mortality of second instar nymphs was also modest, with a rate of 42.8% recorded six days after treatment. In two systemic insecticide screening trials on potted coleus under glasshouse conditions, spirotetramat applied as a systemic drench was never reported to cause mortality that exceeded 22% when applied as either a curative or preventative treatment (Herrick & Cloyd, 2017; Herrick *et al*., 2019).

The literature available suggests the potential for good efficacy of spirotetramat. Spirotetramat, and other lipid synthesis-disruptors such as spirodiclofen, are approved for use in the UK (off-label in a number of protected crops), and spirotetramat is currently considered to have a reasonable IPM profile (Mansour *et al.*, 2018). Testing to confirm efficacy against *P. viburni* in protected edible glasshouse systems would be necessary before a sound evaluation on potential use for UK growers could be drawn, but the relatively strong IPM profile, short harvest intervals (three days) and potential for efficacy support consideration.

Summary of potential products and actives

A summary of some of the actives discussed as part of this review, with examples of products, is provided in Table 1 below. Where not approved for use in UK or, at least, Europe (LIAISON database, Fera Science Ltd, 2019; EU Pesticides Database, EU Commission, 2019; BCPC, 2019; databases accessed November 2019), classes/products have been excluded.

Table 1. Potential actives that could warrant further investigation/development for the control of *Pseudococcus viburni* in UK glasshouses. Examples of products, approved crops and targets are not exhaustive and are provided for context. Targets are on-label unless specified. Key crops have been identified, where possible, as being of most relevance to protected edible crops in which *P. viburni* pose the greatest threat. Information accessed via LIAISON database (Fera Science Ltd., 2019), with non-UK EU approvals confirmed via EU Pesticides Database (EU Commission, 2019), in November 2019.

Insecticide type	Active	Example products	Approved for use in…	Target	Notes
Biopesticides					
Entomopathogenic nematodes	e.g. *Heterorhabditis*, *Steinernema* spp.				Untested as foliar applications in protected crops. Efficacy dependent on strain.
Entomopathogenic fungi	*Beauveria bassiana*	Naturalis-L®	Protected edibles	Thrips, whitefly	Variable, limited to moderate efficacy suggested, dependent on dewaxing.
	Metarhizium anisopliae	Met52 OD®	Protected aubergine, cucumber, pepper, tomato	Pests (none specified)	Variable, limited to moderate efficacy suggested, dependent on dewaxing.
Neem extracts	Azadirachtin	Azatin®	Protected ornamentals	Thrips	Variable, though potential for good efficacy suggested.
Plant extracts	Garlic	ECOguard®, NEMguard DE®	Protected carrots and parsnips, and some outdoor crops	Nematodes	Variable, though potential for good efficacy suggested.
	Orange oil	Prev-Am®	EU - wide range of crops	Range of pests	Potential for good efficacy suggested.
Terpenoids		3AEY®, Requiem EC®	EU (in progress for UK)	Aphids, whitefly, mites, thrips	Variable, though potential for good efficacy suggested.

Insecticide type	Active	Example products	Approved for use in…	Target	Notes
Conventional insecticides					
Pyrethroids	Deltamethrin	Decis®	Wide range including protected cucumber, pepper, tomato	Aphids, caterpillars, mealybugs, scale insects, whitefly	Moderate to good efficacy suggested.
	Cypermethrin	Cythrin 500 EC®	Wide range, including protected brassicas and edible podded peas	Wide range including aphids	Moderate to good efficacy suggested.
	Beta-cyfluthrin	Gandalf®	Outdoor arable crops and brassicas	Aphids, beetles, midges and moths	Moderate efficacy suggested.
Pyrethrins		Pyrethrum 5 EC®, Spruzit®	Protected cucumber, pepper, tomato	Pests (none specified)	Limited efficacy to moderate efficacy suggested.
Neonicotinoids	Acetamiprid	Gazelle SG®	Protected pepper, tomato	Aphids, whitefly	Variable, moderate efficacy suggested.
Avermectins	Abamectin	Dynamec®	Protected pepper, tomato	Mites, pests (none specified)	Limited evidence available, limited efficacy suggested.
	Pirimicarb	Aphox®	Protected peppers, courgette	Aphids	Potential for moderate control suggested.
Ryanoids	Chlorantraniliprole	Coragen®	Protected tomato (off-label)	*Tuta absoluta*	Variable, limited efficacy suggested.
	Cyantraniliprole	Verimark 20 SC®	Protected brassicas, strawberries	Pests (none specified)	Limited evidence, likely limited efficacy suggested.
Other	Flonicamid	Mainman®	Protected cucumber, tomato (off-label)	Aphids, whitefly, mealybugs	Limited efficacy suggested.
	Flupyradifurone	Sivanto® prime	EU – wide range of crops	Range of primarily sucking pests (e.g. aphids, whitefly)	Moderate to good potential efficacy suggested.

Insecticide type	Active	Example products	Approved for use in…	Target	Notes
	Indoxacarb	Rumo®, Steward®	Protected cucumber, pepper and tomato	Pests (none specified)	No evidence, likely to have poor efficacy.
	Spinosad	Spindle®	Protected cucumber, pepper, tomato	Pests (none specified)	Limited evidence available.
	Sulfoxaflor	Sequoia®	Protected cucumber, pepper, tomato	Pests (none specified)	Potential for good efficacy over time, where targeted at immature stages.
Lipid biosynthesis disruptors	Sirotetramat	Batavia®, Movento ®	Protected salads, range of outdoor crops	Aphids, whitefly	Potential for good efficacy over time, where targeted at immature stage.
	Spirodiclofen	Envidor®	Protected cucumber, pepper, tomato	Spider mite	Limited evidence, but potential for good efficacy over time, where targeted at immature stages.
Basic substances					
	Maltodextrin	Eradicoat®, Majestik®	Protected edibles	Aphids, whitefly, spider mite	Variable, modest to good efficacy suggested. Crop safety concerns at required application frequency.
	Fatty acid soaps	Flipper®	Protected tomatoes, cucumber, peppers	Aphids, whitefly, spider mite	Variable, modest to good efficacy suggested. Crop safety concerns at required application frequency.
	Dodecylphenol ethoxylate	Agri 50 E®	Edible crops	Mealybug, aphids, leafhoppers, whitefly, spider mite	Variable, possibly limited efficacy suggested.
	Potassium salts of fatty acids	Jaboland®, Jabolim®, Nakar®	Protected tomatoes, peppers	Pests (none specified)	Variable, modest to good efficacy suggested.

Insecticide type	Active	Example products	Approved for use in…	Target	Notes
	Diatomaceous earth	SilicoSec®	EU – stored grains	Stored product pests	Limited efficacy in isolation suggested, improved in mixture with other products.
	Kaolin (aluminium silicate)	Surround WP®	EU - orchards	Leafhopper, psyllid, moth	Limited efficacy in isolation suggested, improved in mixture with other products.

Current Overseas Control Practices and Opportunities for Application in the UK

Current management practice overseas appears to rely on a combination of careful monitoring, cultural control and manual removal (particularly, use of thorough hygiene protocols to limit spread), and, most typically, on the application of broad-spectrum conventional chemical insecticides. Of these, pyrethroids, neonicotinoids and, primarily in outdoor crops, organophosphates have been the most utilised and effective, historically. Biological control using, primarily, the mealybug predator *C. montrouzieri* and parasitoid wasps is also widely deployed, with inundative and augmentative releases common.

In terms of remedial, or indeed preventative, control, the current literature review has not identified strong opportunities for application in UK protected edible industry of overseas control practice that is not already utilised. Conventional insecticide applications are the norm in U.S. outdoor orchards and vineyards (Waterworth *et al.*, 2011a). With many organophosphate actives being phased out across Europe, and their incompatibility with IPM approaches used in current glasshouse crops, these actives are not a useful avenue despite potential for good efficacy and common use in South African viticulture. Pyrethroids have been used with relative success, and are already available for use in the UK, though given their relative incompatibility with IPM programmes they should probably be used as a 'last resort', where unacceptable economic damage becomes likely. In Italian vineyards, two pyrethrin applications are considered standard practice (Tacoli *et al.*, 2017). In cotton crops in Pakistan, *P. solenopsis* is managed, primarily, through conventional chemical spray programmes, though issues of resistance are leading to increasing interest in alternative novel chemistry and products. In Californian vineyards, applications of spirotetramat are part of insecticide spray programmes against *P. ficus* and vine mealybugs from late spring to early summer, and post-harvest (Daane *et al.*, 2012).

Related Research from Other Industries in the UK and Overseas

Much recent research has been conducted for outdoor crops, particularly in viticulture, orchards, and cotton, reflecting the relative persistence of mealybugs as a pest in often high-value crops, in areas of relatively high population load potential, or in areas where they are a developing or emerging pest problem of particular importance (e.g. cotton in Pakistan).

Overall, there is growing interest overseas, with the literature suggesting particularly in viticulture, on the role of plant-derived products and other biopesticides (including entomopathogenic nematodes), particularly where these are organically approved, for control of mealybug pests, as well as novel chemistries, with growing bodies of literature investigating their efficacy in a seemingly more structured manner than older chemistries and products. Although such products have not yet become normal practice for remedial control, and further evaluations for efficacy in glasshouse systems against *P. viburni* would be required, such products are likely to represent a stronger opportunity for application in the UK protected edible industry, given their relative IPM compatibility. Trials in these industries also suggest potential efficacy in otherwise challenging crops, which could translate into glasshouse production, with a number of botanically derived products and relatively novel chemistries showing the possibility for good levels of control. There is also considerable interest in the roles played by different adjuvants and co-adjuvants in improving efficacy of insecticidal products, with research showing that their impact as affected by crop and target mealybug species.

Of note is the identification and synthesis of the *P. viburni* sex pheromone. Investigation and development of pheromone-baited trap regimes for monitoring and to inform spray programmes in Chile, South Africa and New Zealand show that deployment of such programmes could improve control strategies against *P. viburni*, and that findings are robust. Production of the lures, however, is not yet undertaken on a commercial scale, and as such would currently not be economically viable in the UK industry. Should commercial development and availability be realised in the future, however, this would likely represent a useful opportunity for application in the UK.

Conclusions

Pseudococcus viburni, and mealybugs more generally, remain a challenging and chronic pest for the UK protected edible sector, and can cause significant economic pressure on production. Remedial control is particularly challenging to achieve due to the cryptic, concealed nature of the pest and the protection afforded by the waxy coating that develops on individuals as they mature. Potential shown by products under laboratory conditions does not often translate into good efficacy at a commercial scale, and many products that would be expected to exert good control fail to achieve acceptable levels of control (typically considered to be above 80% efficacy) when deployed in commercial conditions. Although several on- and off-label plant protection products are available these are often limited in efficacy, challenging to use as part of IPM strategies, or typically incompatible with organic systems. Physical control and monitoring methods can be effective, but such options are often labour intensive and expensive. Several relatively novel chemistries have shown some promise, as have a range of biopesticidal options. Although some may not achieve complete control in isolation, others may have considerable promise, and judicious use of adjuvants may further support efficacy. Near-market gains may be possible through evaluations and validation of some such products, supporting management progress in IPM and organic systems.

Monitoring

The identification and synthesis of the *P. viburni* sex pheromone could provide a useful tool in the monitoring of populations, and in informing management strategies and spray programmes. This has not yet been commercially developed and as such is not cost effective for the UK protected edible industry at this time. An opportunity for application in UK glasshouses is evident, however, should lures become commercially available in the future.

Cultural control

Cultural management remains time intensive and expensive. Efforts are primarily focused around limited spread of existing populations into new areas. The use of thorough hygiene and quarantine protocols can be effective in this regard, and careful, thorough cleaning down between crops to reduce the number of *P. viburni* surviving between crops and invading new crops is also important.

Natural enemies

Several natural enemies are already commonly deployed against mealybug pests. Of these, *C. montrouzieri* remains one of the most widely known and effective, and release is often recommended in combination with a mealybug parasitoid mix, with *L. epona* used for control of *P. viburni*. Lacewing larvae have also been observed to

have some impact on mealybug pests. Several other generalist predators may have some efficacy but are unlikely to provide better control than existing options. Entomopathogenic nematodes may also provide some benefit, but this would be dependent on overcoming environmental and application limitations imposed by foliar sprays.

Basic substances

Basic substance products are highly versatile and typically possess strong safety profiles. Mixed results have been obtained in terms of efficacy, however, and their broad spectrum combined with the need for frequent, repeated applications for meaningful impact are likely to cause incompatibility with biological control programmes, as well as have impact on crop safety. Careful evaluation of these aspects should be undertaken were further trials to be conducted on the efficacy of such products in glasshouse systems.

Biopesticides

A number of biopesticidal products, particularly of botanical origin, hold particular promised for control of *P. viburni*, and the considered use of adjuvants, dependent on crop and mealybug target, have been shown to further improve the efficacy of such products. The increasing availability of such products making their way into the UK market suggests the potential for relatively near-market gains to be made in this area in particular, though care must be taken to ensure any evaluations are made under commercially relevant conditions and application strategies.

Conventional insecticides

Several relatively novel chemistries and actives show evidence of potential use in targeting *P. viburni*, some already approved on- or off-label for use in protected edible crops, with short harvest intervals, or which are relatively near-market. Insecticides applied through irrigation with systemic activity do not necessarily exert as much of an effect as might be expected, and studies have hypothesised that this is due to mealybug feeding behaviours, though they may show foliar contact activity regardless. Efficacy is often most strongly elicited against immature life stages of the pest, particularly for some actives, so this, and potential sub-lethal effects, should be considered in evaluating the potential of products as part of an integrated strategy, further highlighting the importance of careful monitoring of crops as part of management programmes for *P. viburni* in glasshouse systems.

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Agrii, Alpha Biocontrol Ltd, Andermatt, Arysta Lifescience, BASF, Bayer, Belchim, Bionema Limited, Certis Europe, Dow, DuPont, Eden Research, Fargro Limited, FMC, Gowan, Interfarm, Lallemand Plant Care, Novozymes, Oro Agri, Russell IPM, Sumitomo Chemicals, Syngenta, UPL.

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