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Terpene based biopesticides as potential alternatives to synthetic insecticides for control of aphid pests on protected ornamentals

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ABSTRACT

Biopesticides based on plant extracts offer a promising alternative to the use of conventional synthetic pesticides. However, biopesticide products must provide acceptable levels of control. To date, few studies have investigated the efficacy of biopesticide products under conditions that reflect commercial practice. Here we report results from three experiments, one completed under glasshouse conditions in 2014 and two completed under polytunnel conditions, in 2015 and 2016, respectively. These experiments tested the efficacy of three terpene based biopesticides used to control two aphid species, peach-potato aphid (*Myzus persicae*) and melon and cotton aphid (*Aphis gossypii*), on ornamental crops. The three biopesticide products tested were orange oil (60 g active ingredient per litre, formulated as a soluble liquid), the essential oil from *Chenopodium ambrosioides* variety nr. *ambrosioides* (16.75% active ingredient, formulated as an oil dispersion) and neem oil (1% active ingredient, formulated as emulsifiable concentrate). The biopesticides tested were applied as foliar sprays using a water volume of 600 l/Ha and all experiments were done at Harper Adams University, Shropshire, UK.

The biopesticide products tested gave statistically similar levels of control of *M. persicae* populations on pansy plants as the conventional synthetic insecticide flonicamid (500 g/kg active ingredient, formulated as a wettable granule) and spirotetramat (150 g/l active ingredient, formulated as an oil dispersion). All products reduced numbers of aphids by at least 85% during the experimental period. Orange oil also gave a similar speed of kill to flonicamid and was faster acting than spirotetramat, two conventional synthetic insecticides that are widely used to control aphid pests of ornamental crops. Against *A. gossypii* on *Hebe*, orange oil gave similar levels of control (90% reduction in aphid numbers) as flonicamid (98% reduction in aphid numbers), when applied with a spray interval of three days (as per label recommendation). The essential oil from *Chenopodium ambrosioides* variety nr. *ambrosioides* was not as effective as flonicamid but did significantly reduced (80% reduction in aphid numbers) numbers of *A. gossypii* on *Hebe* compared to a water control when applied with a spray interval of five days. Neem oil was not effective against *A. gossypii*. Importantly, there was little evidence of any phytotoxicity caused by any of the biopesticide products tested. The potential to use these products as part of an Insecticide Resistance Management (IRM) programme are discussed.

1. Introduction

Continued reliance on synthetic pesticides to provide effective control of crop pests, weeds and diseases is under increasing pressure. The number of active ingredients permitted for use in the European Union (EU) has declined from *c.* 1000 actives in 1993 to around 250 in 2011 (Chapman, 2014). This decline is largely the consequence of regulatory changes, e.g. implementation of EU Regulation 1107/2009 and an associated shift from risk to hazard based assessment of pesticide safety in terms of human health and the environment. There are also financial and time constraints on the development of active ingredients. The cost of bringing a new active ingredient to market has increased from $152 million in 1995 to $256 million in 2005 (Chapman, 2014) while the time taken to develop and register a new conventional pesticide is now around 10 years (Glare et al., 2012). As a result, while there were around 70 new active ingredients in the development pipeline in 2000, this number had dropped to 28 in 2012 (Chapman, 2014). These challenges facing the industry are compounded by increasing numbers of cases of pesticide resistance, with over 580 arthropod species being recorded as having developed resistance globally (Sparks and Nauen, 2015).

Biopesticides offer a promising alternative to the use of

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conventional synthetic pesticides due to reduced risk of resistance developing in pest populations, lower development costs, higher target specificity, lower environmental persistence, and generally improved compatibility with biological controls (Copping and Menn, 2000; Chandler et al., 2011; Hubbard et al., 2014; Seiber et al., 2014). Plant essential oils are synthesised through secondary metabolic pathways and have long been recognised to possess insecticidal and/or repellent properties (Regnault-Roger et al., 2012; Isman, 2016). Although plant essential oils are typically complex mixtures, they are often dominated by two or three chemical compounds that can be usually classified into two chemical groups, terpenes or phenylpropanoids (Edris, 2007; Bakkali et al., 2008; Regnault-Roger et al., 2012).

There are many examples of biopesticides based on terpenes, including orange or citrus oils, essential oil derived from *Chenopodium ambrosioides* variety nr. *ambrosioides* (Chenopodiaceae) and neem extracts. Orange or citrus oils include *d*-limonene as the major component (Hink and Fee, 1986) and are known to be toxic to a wide range of insect pest species (Sheppard, 1984; Hink and Fee, 1986; Karr and Coats, 1988; Hollingsworth, 2005; Raina et al., 2007). Products based on orange or citrus oils work through contact as well as, in some cases, fumigation action. The essential oil derived from *Chenopodium ambrosioides* variety nr. *ambrosioides* L., consists of a mixture of 14 monoterpenes and is known to be toxic to a range of insect and mite pest species (Chiasson et al., 2004a, 2004b; Cloyd and Chiasson, 2007). Finally, oil extracted from *Azadirachta indica* A. Juss. (neem) seeds includes, as the major compound, azadirachtin A (a triterpene), which has been shown to have antifeedant and repellent properties as well as inducing sterility in insects, such as the peach-potato aphid (*Myzus persicae* (Sulzer)) (Mordue (Luntz) et al., 1996; Chaudhary et al., 2017). Although biopesticides based on plant essential oils have previously been shown to have insecticidal effects, their modes of action are poorly understood. The few studies so far completed suggest that plant essential oils are cytotoxic and/or neurotoxic (Pavela and Benelli, 2016), but further work is required in this area. It is also possible that any insecticidal effects observed are the consequence of synergy between different plant essential oils that have differing modes of action. A review of the biological effects of plant essential oils is provided by Bakkali et al. (2008).

Around 100 species of aphid are considered to be agricultural pests of a wide range of crops (van Emden and Harrington, 2007). Two of the most important aphid pest species are *M. persicae* and the melon and cotton aphid (*Aphis gossypii* Glover) due to the wide range of crops that they may infest and their ability to develop resistance to conventional synthetic insecticides, such as carbamates (e.g. Furk and Hines, 1993; Foster and Blackshaw, 2012), pyrethroids (e.g. Marshall et al., 2012; Foster and Blackshaw, 2012) and neonicotinoids (Bass et al., 2011; Herron and Wilson, 2011).

Both *M. persicae* and *A. gossypii* are important pests of ornamental crops (Alford, 2003), where they present a major challenge to growers due to a lack of tolerance to damage and difficulty in achieving effective control using available conventional synthetic insecticides.

Despite increasing interest in, and availability of, biopesticides few studies have investigated the efficacy of biopesticide products relative to currently used conventional synthetic insecticides under conditions that reflect commercial practice. This study investigates the potential of three terpene based biopesticides for the control of *M. persicae* and *A. gossypii* on protected ornamental crops.

2. Materials and methods

2.1. Insects

A population of a single clone of *M. persicae*, known to be resistant to pyrethroid and carbamate insecticides, was maintained on pak choi (*Brassica rapa* L.) seedlings prior to use in experiments. *Aphis gossypii* were collected from a commercial ornamentals nursery and maintained on cotton seedlings (*Gossypium hirsutum* L.) prior to use in experiments. Both aphid populations were maintained in fine mesh insects cages of size 47.5 × 47.5 × 47.5 cm (BugDorm, MegaView Science, Taichung City, Taiwan). Plants were changed each week and fresh plants infested with aphids taken from the discarded plants. Insect cages were placed in controlled environment rooms (Weiss Technik UK Ltd, Ebbw Vale, UK) set to 20 °C, 60% relative humidity and 16:8 (light:dark) hours.

2.2. Plants

Pansy (*Viola* x *wittrockiana* var. Lubega F1 Mix) and *Hebe* 'Purple Pixie' plug plants were planted into 9 cm diameter pots in May in each year of the project. Pansy plants were potted into a peat based growing medium (M3 Pot/Bedding Compost, Levington, Frimley, UK) while *Hebe* plants were potted into a soil based growing medium (John Innes No. 2, J Arthur Bower's, Lincoln, UK). Pots were placed into a ventilated polytunnel and stood on capillary matting to allow for watering. Plants were grown on until they had begun to flower (July/August) before being transferred to a ventilated glasshouse compartment (pansy plants) or ventilated polytunnel (*Hebe* plants).

2.3. Infesting plants with aphids

A small number of pansy plants were placed into the insect cages in which *M. persicae* were reared and a similar number of *Hebe* plants were placed into the insect cages in which *A. gossypii* were reared. Plants were placed close together so that aphids were able to colonise the pansy and *Hebe* plants. Once aphid populations had become established on pansy and *Hebe* plants, completing at least two generations, these plants were then used to infest the remaining pansy and *Hebe* plants. The remaining plants were infested by carefully placing aphid infested leaves of the same plant species onto a previously uninfested plant. In this way each plant was infested with approximately 10 mixed age aphids. Aphid populations were allowed to establish for two weeks before the start of the experiment.

2.4. Treatments

Two conventional insecticides were used in this study, flonicamid (Mainman) and spirotetramat (Movento) (see Table 1). Flonicamid works primarily through ingestion and inhibits aphid feeding. Spirotetramat, also works mainly through ingestion, and is an acetyl-CoA carboxylase inhibitor. Both insecticides are widely used for control of aphids on ornamental crops. The three biopesticide products are based on plant essential oils (see Table 1). Azadirachtin A is obtained from neem seeds (*Azadirachta indica*) and is formulated as an emulsifiable concentrate containing 1% azadirachtin A. *Chenopodium ambrosioides* variety nr. *ambrosioides*, variously known as wormseed, Jesuit's tea or Mexican-tea is formulated as an oil dispersion containing 16.75% of the essential oil, which is composed of a mixture of 14 monoterpenes. Orange oil is formulated as soluble liquid containing 60 g/l of the active ingredient of which *d*-limonene is the major component.

2.5. Treatment applications

Treatments and a water control were applied using an Oxford Precision Sprayer (MDM Engineering Ltd, Bristol, UK) fitted with a hollow cone nozzle (HC/1.74/3, Hypro EU Ltd, Cambridge, UK) delivering a droplet size of 200–300 μm. Spray applications were made using three 3 bar pressure. The concentration of each product used was recommended by the manufacturer (see Table 1) and water volumes were standardised at 600 l/Ha.

2.6. Experimental design

Individual plots were created on benching within a glasshouse