EVALUATION OF AGRI-COLLE® FOR CONTROLLING *TRIALEURODES VAPORARIORUM* IN TOMATO AND CUCUMBER AND *TETRANYCHUS URTICAE* IN CUCUMBER

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Summary

The efficacy of the biorational product Agri-Colle® was assessed against the common greenhouse tomato pests *Trialeurodes vaporariorum* and *Tetranychus urticae*. Greenhouse trials showed that control of juveniles and pupae of *T. vaporariorum* in tomatoes and cucumbers was achieved with rates of 1.5 ml/L and 3.0 ml/L of Agri-Colle® with significant reductions when compared to a water control. Numbers of *T. urticae* were significantly reduced with an application rate of 3.0 ml/L of Agri-Colle® when compared to a water control on cucumbers.

Keywords: Biorational, Reduced-risk, Management, Pesticide, Integrated Pest Management, Greenhouse whitefly, Twospotted mite

INTRODUCTION

One of the world's most important pests in greenhouse vegetable and ornamental crops, the greenhouse whitefly (*Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae)), has been shown to develop pesticide resistance in a number of different insecticide classes (Zanic *et al*. 2008). A problem in protected crops, and an increasing burden to outdoor crops, the pest causes economic damage through its feeding, contamination of produce, virus transmission, and high levels of honey-dew production which creates the environment for the proliferation of problems such as sooty mould (Bi *et al*. 2002). Although biological control has been extremely successful against this pest (van Lenteren *et al*. 1996), chemical pesticides remain a valuable tool if they are compatible with an integrated pest management (IPM) regime (Wang *et al*. 2003).

The mite, *Tetranychus urticae* (Koch) (Acari: Tetranichidae) or twospotted spider mite, is a pest of similar importance around the world (Gorman *et al*. 2001). Like *T. vaporariorum*, this mite pest has been managed well with biological controls for some time (Port and Scopes 1981; Gough 1991) however there is a paucity of chemical control available, and very few chemicals that are compatible with an IPM system.

The use of biorational pesticides has been identified as an effective means of minimising resistance build-up and presents a sustainable approach to pesticide use in managing pests such as *T. vaporariorum* and *T. urticae* (Liburd *et al*. 2007). The use of multiple pesticides as well as other management approaches is vitally important in the successful management of any plant pest, but in particular *T. vaporariorum* and *T. urticae* whose lifecycles and physiology lend themselves to increased resistance build-up. Effective IPM programs require new pesticides with novel modes of action that can be used in an existing resistance management spray program to mitigate any further resistance development (Koul *et al*. 2009).

Numerous studies have indicated that a pesticide spray regime that rotates mode and action of pesticides will reduce the incidence of pesticide resistance and that local variations in the use of pesticides can influence the pattern of this resistance build-up (Hollomon 2012). Pest managers in greenhouses must be particularly mindful of the development of pesticide build-up due to the low incidence of susceptible populations reinfesting a protected crop (Gorman *et al*. 2001). Increasing the number of products available to managers who choose to use pesticides, and in particular those products that are compatible with an IPM system, is extremely important.

Agri-Colle® is a reduced-risk pesticide that has a physical mode of action that suffocates the arthropod pests and is based on natural plant extracts (Cal-Agri Products 2008). There has been a recent drive for the use of pesticides in an IPM program that are based on natural resources, particularly in the food production industry (Koul *et al*. 2009).

This study aims to evaluate the reduced-risk pesticide Agri-Colle® for use in the control of *T. vaporariorum* in tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) and *T. urticae* in cucumber.

MATERIALS AND METHODS

Three greenhouse experiments were conducted at the NSW Department of Primary Industries research station in Narara, NSW, Australia (151°19'E, 33°23'S).

Experiment 1 – *T. urticae* in cucumber

Cucumber plants were grown from 70 Khassib RZ F1 hybrid cucumber seeds (lot #100308044/3, Rijk Zwaan) which were seeded into trays and then 13 days later transferred to five litre pots, one plant per pot. *T. urticae* were cultured continuously on 24-30cm French bean plants maintained in a greenhouse kept at 25˚C ±3˚C and refreshed twice a week. Leaves were taken from this culture and adult *T. urticae* mites transferred to the experimental plants using a fine-haired paintbrush. The treatments comprised two rates of Agri-Colle® (1.5 ml/L and 3.0 ml/L), a single rate of abamectin (1.0 ml/L, Vertimec, Syngenta, Macquarie Park, Australia) and a control treatment of water. Abamectin was chosen as the industry standard based on anecdotal evidence of frequency of use and reliance in the Sydney Basin.

The experiment was conducted in two greenhouses maintained at an average temperature of 22ºC. All plants were supplied with standard nutrients (N:P:K = 5:1:10) via drip irrigation. Treatments were applied with a hand held sprayer, to the point of incipient runoff, to individual plants once a week for three weeks.
Each greenhouse contained six replicates of the four treatments arranged in three 2x2 arrays across two benches. A spatial experimental design, which accounted for trends down each bench and across the benches within a greenhouse was generated using the DiGGer software (Coombes 2002). The experimental unit was a single pot with each pot separated by plastic sheeting suspended from a one metre frame around the benches in order to minimise movement of pests. The number of *T. urticae* on four marked leaves on each plant was recorded. The experiment was repeated two weeks later making a total of 24 replicates of each of the four treatments.

A pre-treatment count was recorded to ensure a uniform starting population. Weekly assessments of the number of gravid *T. urticae* on each experimental unit were made using a head magnifying visor over four weeks, with the first three counts performed just prior to the weekly application of the pesticide treatments and the final count performed one week after the final pesticide treatment.

Experiment 2 – *T. vaporariorum* in tomato

Prior to planting out in two greenhouses maintained at an average temperature of 22ºC, 150 Tresco F1 hybrid tomato seeds (TmC5VF2Fr, De Ruiter Seeds, Auckland New Zealand) were seeded in trays. After five weeks the seedlings were transferred to pre-wetted 5L coco peat bags, one plant per bag.

T. vaporariorum were reared in cages with 40-50cm tobacco plants maintained in a greenhouse kept at 25˚C±3˚C. A uniform release of *T. vaporariorum* adults from these cages was conducted at day 8, 12, 15, 23 and 30 from transfer of seedlings to the greenhouses. A pre-treatment count of established pests was undertaken on test plants 37 days after transfer to ensure a uniform distribution throughout the greenhouses.
Two rates of Agri-Colle® (1.5 ml/L and 3.0 ml/L), a single rate of bifenthrin (0.375 ml/L, Talstar, AMC Australasia Pty Ltd, Murarrie Queensland, Australia), and a control treatment of water were applied once a week for three weeks. Bifenthrin was chosen as a standard based on anecdotal evidence of frequency of use and reliance in the Sydney Basin.

The experiment was conducted in two greenhouses, each containing three benches with a single row of tomato plants on each bench and plastic sheeting hung between each bench to reduce movement of insects. An experimental unit comprised three tomato plants with only the middle (target) plant assessed. Within a row, buffer plants were placed between each of the experimental units to reduce the effect of any movement of insects.A spatial experimental design (Coombes 2001) was used with the two Agri-Colle® treatments replicated 8 times and the control and bifenthrin treatments replicated 7 times. The experiment was repeated two weeks later.

The treatments were applied to the plants with a knapsack sprayer to the point of incipient runoff. Assessments were made weekly for four weeks, with the first three counts performed just prior to the application of the pesticide treatments. On each target plant, two leaves were assessed: a terminal leaf from a branch one third from the top of the plant and another leaf, one third from the bottom of the plant. Key lifestages (eggs, juveniles, pupae, eclosed) were recorded.

Experiment 3 – *T. vaporariorum* in cucumber

Cucumber plants were produced in a similar manner to experiment 1 and the *T. vaporariorum* were prepared as in experiment 2. Adults were released on day 20, 27, and 34 after seeding. A pre-treatment count was undertaken on all plants on day 41 from seeding to ensure a uniform population across the greenhouse. The experimental design was similar to experiment 1.

The treatments and spray regime were identical to those used in experiment 2 and a hand held sprayer was used to apply all treatments to the point of incipient runoff. Assessments were made weekly for three weeks for run 1 and four weeks for run 2, with the first three counts performed just prior to the application of the pesticide treatments and the final count performed a week after the final pesticide treatment. On each target plant a single leaf was marked, examined and key lifestages (eggs, juveniles, pupae, eclosed) recorded.

Statistical methods

Each experiment was analysed separately and in a similar manner. Counts per leaf for each insect stage at each assessment time were analysed separately using linear mixed models in GenStat (2009). The estimation of variance components used the method of residual maximum likelihood. Variance components due to the random effects of repeating the experiments in time, and trends within a greenhouse were accounted for in the analysis.

Counts per leaf were increased by one then $\log_e$ transformed prior to analysis in order to stabilise the variance. Fixed treatment effects were assessed for significance using approximate F tests (Wald statistics) with the denominator degrees of freedom calculated according to Kenward and Roger (1997). Treatment means were compared using average least significance differences (l.s.d.) at the 5% level on the transformed scale and back transformed for presentation. The results for the final assessment only are presented.

RESULTS

In all three experiments no significant treatment effects on insect counts were detected at the pre-treatment assessment (results not presented).

Experiment 1 - *T. urticae* in cucumber

At the final assessment significantly lower counts of *T. urticae* adults were observed in cucumber plants treated with abamectin than with the other treatments (F=154.84, df=3, 72.8, P<0.001). Counts of *T. urticae* in cucumber plants treated with the 3.0 ml/L rate of Agri-Colle® were significantly lower than those of the control (Table 1).

Experiment 2 – *T. vaporariorum* in tomato

Counts of juvenile stages of *T. vaporariorum* at the week 4 assessment were significantly lowest for bifenthrin compared to all other treatments and both rates of Agri-Colle® significantly reduced numbers when compared to control (F=14.66, df=3, 46.7, P<0.001) (Table 2). Numbers of *T. vaporariorum* pupae were significantly lower for all treatments, compared to the control, with both rates of Agri-Colle® being significantly lower than the bifenthrin treatment (F=7.12, df=3, 46.5, P<0.001) (Table 2). Numbers of eclosed *T. vaporariorum* were significantly higher for the control than for all treatments (F=4.25, df=3, 45.0, P=0.010) (Table 2).

***Experiment 3 - T. vaporariorum* in cucumber**

The treatments of Agri-Colle® and bifenthrin significantly reduced numbers of juveniles per leaf compared to the control (F=8.25, df=3, 31.2, P<0.001) (Table 3). All treatments reduced the incidence of *T. vaporariorum* pupae per leaf on cucumber plants when compared to the control (F=8.82, df=3, 31.1, P<0.001) (Table 3).

Table 1: Effect of a 3 weekly pesticide spray regime on *T.urticae* adults in cucumbers (experiment 1). Back transformed predicted counts with the same letter are not significantly different at P=0.05.

	T. urticae adults		
Treatment	Predicted count ($\log_e$ scale)	back transformed count	
Agri-Colle® 1.5	2.997	19.03	ab
Agri-Colle® 3.0	2.830	15.95	b
control	3.275	25.44	a
abamectin	0.021	0.02	b
average l.s.d.	0.360		

Table 2: Effect of a 3 weekly pesticide spray regime on *T. vaporariorum* juveniles, pupae and eclosed in tomatoes (experiment 2). Back transformed predicted counts in the same column with the same letter are not significantly different at P=0.05.

	T. vaporariorum juveniles		
Treatment	Predicted count (log_e scale)	back transformed count	
Agri-Colle® 1.5	4.047	56.23	c
Agri-Colle® 3.0	3.801	43.75	bc
control	5.033	152.39	a
bifenthrin	2.506	11.26	ab
average l.s.d.	0.760		

	T. vaporariorum pupae		
Treatment	Predicted count (log_e scale)	back transformed count	
Agri-Colle® 1.5	1.564	3.78	c
Agri-Colle® 3.0	1.942	5.97	bc
control	3.092	21.02	a
bifenthrin	2.479	10.93	ab
average l.s.d.	0.700		

	T. vaporariorum eclosed		
Treatment	Predicted count (log_e scale)	back transformed count	
Agri-Colle® 1.5	3.375	28.22	b
Agri-Colle® 3.0	3.445	30.34	b
control	4.642	102.75	a
bifenthrin	3.789	43.21	b
average l.s.d.	0.789		

Table 3: Effect of 3 weekly pesticide spray regime on *T. vaporariorum* juveniles and pupae in cucumbers (experiment 3). Back transformed predicted counts in the same column with the same letter are not significantly different at P=0.05.

	T. vaporariorum juveniles			*T. vaporariorum* pupae		
Treatment	Predicted count (log_e scale)	back transformed count		Predicted count (log_e scale)	back transformed count	
Agri-Colle® 1.5	1.082	1.95	bc	2.313	9.10	b
Agri-Colle® 3.0	0.674	0.96	c	1.344	2.83	c
control	2.333	9.31	a	3.237	24.46	a
bifenthrin	1.197	2.31	b	1.986	6.29	bc
average l.s.d.	0.718			0.768		

DISCUSSION

Our results demonstrate that the reduced-risk pesticide Agri-Colle®, at the rates of 1.5 and 3.0ml/L, may be considered as an effective management tool against the pest arthropod *T. vaporariorum* and as a possible supplementary management tool for the pest *T. urticae*. At the completion of a three-weekly spray regime when compared to a water control, the compound significantly reduced the numbers of both *T. urticae* on cucumber plants and *T. vaporariorum* juveniles and pupae on both tomato and cucumber plants. On several sampling occasions the reduced-risk pesticide out-performed the conventional pesticide standard it was tested against, indicating confidence may be placed in the compound successfully managing numbers of these pests.

Any effective pesticide, and in particular reduced-risk pesticide, is highly sought after in many cropping systems. The mite pest *T. urticae* currently has five registrations or permits available to cucumber growers in Australia. Of these five registrations, one is not registered in many Australian states and three were due to have their permits or registrations expire at the end of the 2010/2011 financial year. This paucity of chemical control options (InfoPest 2011), a cornerstone for effective resistance management in crops, creates a need for any new pesticides that are developed or registered to be compatible with IPM management strategies that may be using biological control agents to manage *T. urticae* numbers. The biological control agents available to manage population of *T. urticae* in Australian horticultural crops are currently limited to predatory mites. Whilst the reduction in *T. urticae* numbers as part of this experiment are not excessive, this will present an advantage when using predatory mites to manage a pest population as mortality can be expected to be similarly low and combined with spot spraying, Agri-Colle® will provide a valuable IPM tool for growers.

Similarly, the management of *T. vaporariorum* suffers from a lack of registered pesticides in tomatoes and, in particular, cucumbers. Only seven registrations, with five due to expire at the end of the 2009/2010 financial year, exist for this pest in tomatoes with even few available for cucumber growers with four registrations and one due to expire at the end of the financial year. Although growers that are attempting to manage populations of these pests have very few pesticides available to them, each pest and crop combination has a variety of modes of action available to them so resistance management is possible with those that are available. It is expected that Agri-Colle® will present an appropriate IPM tool for managing populations of whitefly due to the larvae of *Encarsia formosa* Gahan, a common biological control agent for *T. vaporariorum*, being relatively protected within the whitefly nymphs during applications (Cal-Agri Products 2008).

Whilst the addition of another pesticide, and in particular a reduced-risk pesticide, is valuable, unpublished data regarding this product's compatibility for use with common biological agents must be made available. This selectivity for efficacy against pests rather than biological control agents will be instrumental in the success of any biorational pesticides and any claims as such will require verification. This information will allow informed judgements on this compound's compatibility with IPM programs. This product will be well placed with other IPM products that complement this low toxicity against several biocontrol products available in Australia (personal communication, manufacturer). The addition of this and other similar products to the tools available to growers in an Australian horticultural context will be extremely valuable.

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