FIRST AUSTRALIAN TRIALS OF ETHYL (2E, 4Z)-2,4-DECADIENOATE FOR MONITORING OF FEMALE AND MALE CODLING MOTH *CYDIA POMONELLA* L. (LEPIDOPTERA: TORTRICIDAE) IN POME FRUIT ORCHARDS

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Summary

Codling moth (CM) is the most important pest of commercial pome fruit orchards in Australia. Sex pheromone mediated mating disruption is widely used in Victoria to control CM but monitoring of CM populations with sex pheromone traps in orchards treated with mating disruption is difficult. Ethyl (2E, 4Z)-2,4-decadienoate (DA) has been reported to be a species-specific, highly attractive pear-derived kairomone for male and female CM. The first field trials in Australia of different formulations of DA were conducted in the 2001-02 season in apple, pear and nashi pear orchards and continued in the 2002-03 season on different varieties of apples. Pure DA and it's mixture with CM sex pheromone (E, E)-8-,10-dodecadien-1-ol (codlemone) were highly specific and attractive for CM males and females in orchards. Further work is warranted to determine optimum combinations of DA and codlemone.

Keywords: codling moth, pear-derived kairomone, monitoring of females, pome fruit.

INTRODUCTION

Codling moth (CM) *Cydia pomonella* L. (Lepidoptera: Tortricidae) is the most damaging and widely distributed pest of commercial apple, pear and nashi (Asian pear) orchards in Australia. Uncontrolled CM larvae can destroy significant amounts of the crop (Geier 1981). CM has developed resistance to commonly used broad-spectrum organophosphate insecticides such as azinphos-methyl (Thwaite *et al.* 1993). Sex pheromone mediated mating disruption was demonstrated to be an environmentally friendly, effective alternative to the use of broad-spectrum organophosphate insecticides for CM control (Vickers *et al*. 1998, Brown and Il'ichev 2000). Many fruit growers in Victoria use sex pheromone mediated mating disruption to control CM damage. Currently, mating disruption is the cornerstone of ecologically balanced integrated pest management in the fruit growing areas of northern Victoria (Williams and Il'ichev 2003).

Synthetic sex pheromones have been used for pest monitoring in orchards for more than 30 years (Thwaite and Madsen 1983). These very effective and species specific monitoring tools also permit observations of CM mating behaviour (Witzgall *et al*. 1999, 2001). Unfortunately, monitoring traps with sex pheromone dispensers are not very effective in orchards treated with pheromone mediated mating disruption. For example, the dispensers with 1 mg of the CM sex pheromone, that are usually used to monitor CM in conventional orchards, do not attract male CM under mating disruption treatment. This is probably because of strong competition from the higher concentration of sex pheromone created by the mating disruption dispensers. Sometimes dispensers with 10 mg of CM sex pheromone could also be inhibited by the higher CM sex pheromone concentration used for mating disruption and hence the number of CM males attracted to monitoring traps would be noticeably decreased (Gut and Brunner 1998, Vickers *et al*. 1998).

Black Light Traps (BLT) (Biocontrol Ltd. Australia) have been used successfully to monitor CM populations in orchards treated with mating disruption, but attract many non-target insects to the light source. This makes monitoring difficult (Rivkina *et al.* 2000). Therefore, the demand for an effective and specific tool for CM monitoring in orchards, particularly those under mating disruption, is very high. It was known that CM adults were attracted to the volatiles from apples and in particular to (E, E)-α-farnesene (Sutherland *et al*. 1974). Unfortunately, instability and rapid chemical breakdown substantially restricted the development of (E, E)-α-farnesene for CM monitoring in orchards (Cavill and Coggiola 1971). Investigation of different host plant volatiles from apples for CM male and most importantly for female attraction in the orchards continues (Yan *et al*. 1999, Coracini *et al*. 2004).

Discovery of the pear-derived kairomone ethyl (2E, 4Z)-2,4-decadienoate, which is able to attract males and females of CM, was very important. This kairomone, which later became known as DA was reported to be a species-specific and highly potent attractant for males and, most importantly, for CM females (Light *et al*. 2001).

The first field trials in Australia were conducted during the 2001-02 season to test the effectiveness and the species-specific attraction of different formulations of DA for CM males and females in apple, pear and nashi pear orchards treated with, and without mating disruption. Preliminary results were presented by Il'ichev *et al*. (2002) but here the final results of trials conducted from 2001 to 2003 are reported. These trials aimed to evaluate the effectiveness of pure DA and its mixture with codlemone for monitoring CM males and females in different pome fruit orchards treated with, and without mating disruption.

MATERIALS AND METHODS

Experimental sites

Commercial orchards located in the Greater Shepparton fruit production area of northern Victoria, Australia were used as field sites. Different formulations of DA were tested in the 2001-02 season in apple (variety Pink Lady), pear (variety Packham) and nashi pear (variety Nijiseiki) orchards and continued in the 2002-03 season on different varieties of apples (Pink Lady and Granny Smith).

During the 2001-02 season field trials were set up in five different orchards. Orchards 1 and 2 contained nine pear and three nashi pear blocks respectively and were treated with mating disruption (MD). Orchards 3, 4 and 5 contained four pear, three apple and four nashi pear blocks respectively and were not treated with MD (no MD), but were treated with insecticides. A set of four traps containing different dispensers for trials was placed in each orchard block. Each orchard therefore became an experimental site with each block within each orchard being considered a replicate in a randomised block design.

Dispensers of Isomate C (Shin-Etsu Chemical Co. Ltd., Japan for Biocontrol Ltd. Australia) were applied at the registered rate of 1000 dispensers ha^{-1} in MD pome fruit blocks during the middle of September of each season. Isomate C is a controlled release formulation of single tubing dispenser for mating disruption with CM sex pheromone that contains codlemone (99 mg $dispenser^{-1}$), dodecanol (53.5 mg $dispenser^{-1}$) and tetradecanol (12.5 mg $dispenser^{-1}$). If MD treatment was not applied (no MD) for codling moth control, orchards were treated with insecticides (parathion-methyl and/or azinphos-methyl) applied during the season about 7-14 times. This is common practice in many commercial pome fruit blocks in the Shepparton district of northern Victoria.

During the 2002-03 season, field trials were set up in commercial orchards as a 4 x 4 Latin square design in Granny Smith apples under MD treatment and in Pink Lady apples without MD treatment. Pherocon Delta VI traps (Trece Inc., Salinas, CA, USA) with CM sex pheromone, placed in the orchards early in the season, were used to identify the initial level of CM population and orchard suitability for comparative trials. Monitoring information from the previous season about the CM infestation was also used to ensure sufficient trap captures to allow statistical analysis of the data.

Dispensers and traps for field tests

During the 2001-02 season dispensers (Trece Inc., Salinas, CA, USA) loaded with either 1 mg of pure DA kairomone (DA2313, Trece 8693), or a mixture of 0.5 mg DA with 0.5 mg codlemone (CM/DA, Trece 9183) were tested in comparison with standard 10 mg CM sex pheromone dispensers (10X CM Mega Lure, Trece Inc.) and commercial 'Long Life' codlemone dispensers (CM-L2, Trece Inc. USA). Tested dispensers were randomly allocated to traps that were equally spaced along a tree row within each block.

During the 2002-03 season on Pink Lady apples without MD treatment, the attractiveness of DA was compared with the attractiveness of BioLure (Concep Inc. Oregon, USA) (1 mg of CM sex pheromone dispensers), with 1 mg (CM-L2) and 10 mg (10X) codlemone dispensers. On Granny Smith apples under MD treatment, dispensers of DA, CM/DA and 10X were compared with the catches of CM in BLT.

In both seasons, grey halo butyl rubber septa dispensers for trials were placed individually in the middle of the sticky base of Pherocon Delta VI traps. Delta traps were hung in the tree canopy at a height of about 1.5-2.0 m from the ground. Dispensers were changed every 8 weeks and the sticky bases in the traps were cleaned weekly during the monitoring period and replaced when they lost stickiness or caught many insects. All traps were placed a minimum of 36 m (approximately five pear or apple trees in between traps) apart within and across replications.

Monitoring of codling moth catches

Traps were monitored weekly. The number of moths caught was recorded and all moths were collected for sex identification. The monitoring of the experiments during both seasons started in early September, before the start of the first CM flight in spring, and stopped in the middle of March, when the last CM

flight had finished. The total CM catch per trap over the whole monitoring period was used for statistical analyses.

Identification of codling moth sex and female mating status

During weekly monitoring of all field trials, the sticky bases of the DA and CM/DA traps with CM catches were collected and delivered to the laboratory for identification of the sex and female mating status. The sex of the moths caught was identified by examining genitalia under the dissecting microscope. For identification of the female mating status, the abdomen of CM females was dissected and bursa copulatrix inspected for presence of spermatophores. Traps with 1 mg (CM-L2 and BioLure) and 10 mg (10X) sex pheromone dispensers were expected to catch only male CM in all orchards irrespective of the treatment so sticky bases from these traps were not collected for sex identification, assuming that all moths caught would be males. CM samples from BLT were sorted by sex and the mating status of females was determined.

Statistical analysis

The total trap catch of codling moths over the monitoring period was used for statistical analyses. Total number of CM caught was analysed using Analysis of Variance (ANOVA), with individual means being compared using Fisher's unprotected least significant difference (LSD) test (p=0.05). Statistical analysis of the results from the field trials in 2001-02 was based upon a randomised block design under both MD treatment and no MD treatment. Analysis of the results from the field trials on apples in 2002-03 was based upon a 4 x 4 Latin square design in Granny Smith apples under MD treatment and in Pink Lady apples without MD treatment.

RESULTS

Field trials during the 2001-02 season

The results of the 2001-02 trials in pear and nashi pear orchards are presented in Tables 1 and 2. There were significant differences in total number of CM caught by the four dispenser types ($p<0.01$) in nashi pear orchards without MD treatment. 10X and CM/DA dispensers caught significantly more CM than DA or CM-L2 dispensers and 10X caught significantly more CM than CM-L2 dispensers (Table 1). In the nashi pear orchards treated with MD the catches were low and did not differ significantly among 10X, DA and CM/DA dispensers, but CM-L2 did not attract CM at all (Table 1).

During the trials on pears without MD treatment the total number of moths caught in 10X and DA was not significantly higher than that in CM/DA dispensers. Also there were no significant differences between CM-L2, 10X and CM/DA. However, CM-L2 dispensers caught significantly more moths than did DA dispensers (Table 1).

CM was detected in pears under MD treatment in only three of the nine replications and only catches from these three replications were used in calculations. CM/DA demonstrated continuous catches with an average of 1.5 CM per trap per week (CM t^{-1} w^{-1}), whereas 10X caught an average of 0.61 CM t^{-1} w^{-1}. In general, CM catches on pears under MD were low and did not differ significantly among the three types of dispensers (Tables 1 and 2.). There were no CM catches at all recorded in these four different types of dispensers in Pink Lady apples treated with, and without MD during the 2001-02 season. Dispensers with 1 mg of codlemone (CM-L2) were not attractive for CM males in the orchards treated with MD irrespective of the fruit variety

Table 1. Mean total codling moth (CM) trap catch per replication for the four different dispensers in pear and nashi pear orchards with and without mating disruption (MD) treatment in the 2001-02 season.

TRIAL	DISPENSER TYPE				ANOVA	
	10X	DA	CM/DA	CM-L2	p-value	LSD(5%)
Nashi-no MD	257.0	108.0	220.0	176.0	0.007	73.9
Nashi-MD	9.7	2.0	4.0	0	0.486	16.8
Pears-MD	5.7	5.3	14.3	0	0.392	18.3
Pears-no MD	17.0	3.7	16.7	32.7	0.072	20.6

Table 2. Total trap catch of male and female codling moth (CM) for the four different dispensers in pear and nashi pear orchards with and without mating disruption (MD) treatment in the 2001-02 season.

TRIAL		DISPENSER TYPE			
	Sex	**DA**	**CM/DA**	**CM-L2**	**10X**
Nashi-no MD	males	412	844	705	1027
	females	19	38	0	0
Nashi-MD	males	6	11	0	29
	females	0	1	0	0
Pears-no MD	males	11	48	98	51
	females	0	2	0	0
Pears-MD	males	16	42	0	17
	females	0	1	0	0
Total	CM	464	989	803	1124
Sex ratio	M:F	21.68:1	22.21:1	100:0	100:0

Table 3. Mean total codling moth (CM) trap catch per replication for the four different dispensers in Pink Lady and Granny Smith apples treated with and without mating disruption (MD) in the 2002-03 season.

TRIAL	DISPENSER TYPE						ANOVA	
	DA	**CM/DA**	**10X**	**CM-L2**	**BioLure**	**BLT**	**p-value**	**LSD(5%)**
Pink lady-no MD	25.0	NT [1]	120.0	108.2	132.8	NT[1]	0.004	43.9
Granny Smith-MD	96.0	130.0	99.0	NT	NT	408.0	0.008	269.6

[1] not tested

Table 4. Total trap catch of male and female codling moth (CM) in different traps and dispensers in Granny Smith and Pink Lady apples treated with and without mating disruption (MD) respectively in the 2002-03 season.

TRIAL		DISPENSER TYPE					
	Sex	**DA**	**CM/DA**	**CM-L2**	**BioLure**	**10X**	**BLT**
Granny Smith-MD	males	361	430	NT[1]	NT	397	1428
	females	24	92	NT	NT	0	203
Pink Lady-no MD	males	80	NT [1]	433	531	480	NT
	females	20	NT	0	0	0	NT
Total	CM	485	522	433	531	877	1631
Sex ratio	M:F	10.02:1	4.67:1	100:0	100:0	100:0	7.03:1

[1] not tested

probably because of the inhibition by much higher sex pheromone concentration of mating disruption dispensers.

Field trials during the 2002-03 season

The results of the 2002-03 trials in apple orchards are presented in Tables 3 and 4. There were no significant differences in catches between BioLure, CM-L2 and 10X dispensers, but all three types of dispensers caught significantly more ($p<0.01$) CM than DA dispensers (Table 3). During the trials on Granny Smith apples under MD treatment, BLT caught significantly higher ($p<0.01$) numbers of CM than the other traps. There were no significant differences between catches in DA, 10X or CM/DA (Table 3).

The results of field trials during two consecutive seasons indicated that CM catches with DA and CM/DA were not significantly different ($p>0.05$) from catches with 10X sex pheromone in orchards under MD treatment. DA and CM/DA dispensers were attractive for CM irrespective of MD treatment and very species-specific for CM males and most importantly for CM females.

Sex ratio and mating status of codling moth caught in kairomone traps

The results of the total trap catches for males and females of CM are given in Tables 2 and 4. During field trials in both 2001-02 and 2002-03 seasons all females caught on DA and CM/DA dispensers had mated. Sex ratio between males and females in DA and CM/DA dispensers in 2001-02 was about 22:1 (Table 2).

BLT caught 203 mated females in Granny Smith apples treated with MD but was not used at all in Pink Lady apples without MD because sex pheromone traps are more convenient and easy to use in the orchards not treated with mating disruption. Sex ratio between males and females in DA dispensers was 10:1, in CM/DA dispensers was about 5:1 and in BLT was 7:1 (Table 4).

DISCUSSION

Currently only sex pheromone traps are used as the standard tool for monitoring pest populations and information is based only on male moth catches. The relationship between the time when the first male moth appears in sex pheromone traps and the time when eggs laid by the female moth begin to hatch is used in integrated pest management programs for pest control (Williams and McDonald 1991).

The results of the trials reported here demonstrate that dispensers with 1 mg of the CM sex pheromone (CM-L2) that are attractive for CM in conventional pome fruit orchards (no MD), do not perform well in orchards under mating disruption (Tables 1 and 2). Although there were no significant differences between 10X, DA and CM/DA dispensers in orchards under mating disruption, there was a trend towards higher catches by the CM/DA dispensers, even in pear orchards. The low catches of CM with DA in pear orchards might be based on its origin from ripe Bartlett pears. The low attractiveness of DA in pears could be explained by the olfactory inhibition or competitive effects of the same or similar esters and other natural semiochemicals released from pears (Light *et al.* 2001). The apparent trend towards higher CM catches with CM-DA warrants further investigation to determine optimum combination of codlemone and DA.

It is very important for effective control to target as precisely as possible the pest in the most vulnerable stage of its development. To predict the time for first CM egg hatch, day-degree models are used. These models use the date in spring when the first CM male is caught in the sex pheromone trap as an indication of the beginning of the first flight (Williams and McDonald 1991). Usually male CM fly first followed a few days later by the females. Most of the day-degree models do not take into account the sex of the target pest. The current day-degree model developed for CM has a potential error in estimating egg hatch based on the number of males trapped.

The use of DA as a species-specific and attractive pear-derived kairomone for males and most importantly for females of CM could be as effective as 10X sex pheromone for monitoring in pome fruit orchards treated with mating disruption. This monitoring tool could also be used to make the calculations of day-degree models more accurate by providing a better estimate of the first CM egg hatch (assumed to coincide with the capture of the first mated female). The use of this pear-derived kairomone for attraction and monitoring of mated CM females, particularly in the orchards treated with mating disruption has great potential and should be investigated further.

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REFERENCES

Brown, D.J. and Il'ichev, A.L. (2000). The potential for the removal of organophosphate insecticides from stone-fruit production in the Goulburn Valley, Australia. *Acta Horticulturae* **525:** 85-91.

Cavill, G.W.K. and Coggiola, I.M. (1971). Photosensitized oxygenation of α-farnesene. *Australian Journal of Chemistry* **24**: 135-142.

Coracini, M., Bengtsson, M., Liblikas, I. and Witzgall, P. (2004). Attraction of codling moth males to apple volatiles. *Entomologia Experimentalis et Applicata* **110:** 1-10.

Geier, P.W. (1981). The codling moth, *Cydia pomonella* (L.): profile of a key pest. In: Kitching, R.L. and Jones, R.E. (eds.) *The ecology of pests: some Australian case histories.* CSIRO Melbourne. pp.109-129.

Gut, L.J. and Brunner, J.F. (1998). Pheromone-based management of codling moth (Lepidoptera: Tortricidae) in Washington apple orchards. *Journal of Agricultural Entomology* **15**: 387-406.

Il'ichev, A.L, Rivkina, O.G. and Light, D.M. (2002). First trials of the "pear ester" for codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) monitoring in Australia. In: *Abstracts of the 33rd Annual General Meeting and Scientific Conference of the Australian Entomological Society, Perth, 22-27 September 2002*: 45.

Light, D.M., Knight, A.L., Henrick, C.A., Rajapaska, D., Lingren, B., Dickens, J.C., Reynolds, K.M., Buttery, R.G., Merrill, G., Roitman, J. and Campbell, B.C. (2001). A pear-derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L.). *Naturwissenschaften* **88**: 333-338.

Rivkina, O.G., Sexton, S.B., and Il'ichev, A.L. (2000). The black light trap for monitoring of codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) in pome fruit orchard under mating disruption. In: *Abstracts of the XXIth International Congress of Entomology, Foz do Iguassu, Brazil, 20-26 August, 2000, book 1:* 284.

Sutherland, O.R.W., Hutchins, R.F.N. and Wearing, C.H. (1974). The role of the hydrocarbon α-farnesene in the behaviour of codling moth larvae and adults. In: Browne L. B. (ed.) *Experimental analysis of insect behaviour*. Springer: Berlin, Germany, pp. 249-263.

Thwaite, W.G. and Madsen, H.F. (1983). The influence of trap density, trap height, outside traps and trap design on *Cydia pomonella* (L.) captures with sex pheromone traps in New South Wales apple orchards. *Journal of the Australian Entomological Society* **22**: 97-99.

Thwaite, W.G., Williams, D.G. and Hately, A.M. (1993). Extent and significance of azinphos-methyl resistance in codling moth in Australia. In: Corey, S., Dall, D., and Milne, W. (eds.) *Pest Control and Sustainable Agriculture*. CSIRO Publishing, pp. 166-168.

Vickers, R.A., Thwaite, W.G., Williams, D.G. and Nicholas, A.H. (1998). Control of codling moth in small plots by mating disruption: alone and with limited insecticide. *Entomologia Experimentalis et Applicata* **86**: 229-239.

Williams, D.G. and Il'ichev, A.L. (2003). Integrated Pest Management in Australia. Book Chapter 28, In: Maredia, K. M., Dakouo, D. and Mota-Sanchez, D. (eds.). *Integrated Pest Management in the Global Arena.* CAB International, London, UK. pp. 371-384.

Williams, D.G. and McDonald G. (1991). Computer models and pest management in Victorian agriculture. *Plant Protection Quarterly* **6**: 45-47.

Witzgall, P., Backman, A., Svensson, M., Koch, U., Rama, F., El-Sayed, A., Brauchli, J., Arn, H., Bengtsson, M. and Lofqvist. J. (1999). Behavioral observations of codling moth, *Cydia pomonella*, in orchards permeated with synthetic pheromone. *BioControl* **44**: 211-237.

Witzgall, P., Bengtsson, M., Rauscher, S., Liblikas, I., Backman, A., Coracini, M., Anderson, P., and Lofqvist. J. (2001). Identification of further sex pheromone synergists in the codling moth, *Cydia pomonella*. *Entomologia Experimentalis et Applicata* **101**: 131-141.

Yan, F., Bengtsson, M. and Witzgall, P. (1999). Behavioral response of female codling moth, *Cydia pomonella*, to apple volatiles. *Journal of Chemical Ecology* **25**: 1343-1351.