MONITORING CODLING MOTH *CYDIA POMONELLA* L. (LEPIDOPTERA: TORTRICIDAE) IN VICTORIAN POME FRUIT ORCHARDS WITH PEAR ESTER

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Summary

Sex pheromone mediated mating disruption (MD) is widely used to control codling moth *Cydia pomonella* L. in Australian pome fruit orchards. The high concentrations of pheromone in MD treated orchards interferes with sex pheromone traps used for monitoring the pest. Ethyl *(E, Z)*-2, 4-decadienoate (pear ester), a pear kairomone was investigated as an alternative to sex pheromone lures in sticky traps for monitoring of codling moth in apple (Granny Smith and Cripps Pink) and pear (European pear and nashi) orchards. In MD treated orchards, pear ester lures caught significantly more codling moths (both sexes combined) than did 10 mg sex pheromone lures. Pear ester lures caught on average three times more codling moth males than females in any of the four tested varieties at the same height under the same MD treatment (i.e. ±MD). Both pear ester and 10 mg sex pheromone lures were more effective when placed higher in the tree canopy. Pear ester used for attraction and monitoring of codling moth males and especially females in pome fruit orchards has great potential to improve pest control. Use of pear ester lures in traps placed high in the tree canopy for monitoring of codling moth female activity in pome fruit orchards under MD is advocated.

Keywords: female monitoring, pear kairomone, sex pheromone, mating disruption

INTRODUCTION

Codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) is the most serious pest of pome fruit worldwide and the most damaging pest of commercial apple, pear, quince and nashi orchards in Australia. Codling moth is widely distributed in all Australian States except Western Australia, which is free of codling moth after a successful eradication program (Poole 2004). The pest is also not present in the Northern Territory.

Broad-spectrum organophosphate insecticides were widely used for codling moth control, but in the early 1990s resistance to azinphos-methyl was confirmed in Australia (Thwaite *et al.* 1993). Application of sex pheromone mediated mating disruption (MD) can be an effective alternative to the use of organophosphate insecticides for codling moth control. MD could significantly reduce the number of insecticide sprays and substantially support Integrated Pest Management (IPM) as an environmentally friendly tool for effective pest control in orchards (Vickers and Rothschild 1991, Brown and Il'ichev 2000). Many fruit growers in Australia use MD to control codling moth damage. However, MD works best when the level of the codling moth population is low and often requires supplementary insecticide sprays if the codling moth population is high (Vickers *et al.* 1998). Currently, MD is the key-stone of IPM in the fruit growing areas of Victoria (Williams and Il'ichev 2003).

Synthetic sex pheromones have been used for codling moth monitoring in orchards for more than 35 years (Thwaite and Madsen 1983). Unfortunately, monitoring traps containing 1 mg codling moth sex pheromone lures have been unreliable in orchards treated with pheromone mediated MD probably because of strong competition from the higher concentration of sex pheromone used in the MD dispensers. To overcome this, sex pheromone traps baited with 10 mg sex pheromone lures (Vickers *et al.* 1998) became widely used for monitoring. However, such lures were also sometimes inhibited by the higher sex pheromone concentration used for MD, with decreased catches of codling moth males leading to incorrect estimates of pest populations (Gut and Brunner 1998, Vickers *et al.* 1998). Pome fruit growers who used codling moth MD were urged to check trees for evidence of fruit infestation and not rely totally on codling moth captures in monitoring traps containing 10 mg sex pheromone lures (Penrose *et al.* 2000).

Black Light Traps (Biocontrol Ltd. Mt. Crosby, Queensland, Australia) have been used successfully to monitor codling moth populations in orchards treated with MD, but because they attract many non-target insects to the light source, including natural enemies of codling moth and other beneficial insects, they are inconvenient and difficult to use (Rivkina *et al.* 2000). There is a need for an effective, easy to use, specific tool for codling moth monitoring in orchards, particularly those under MD.

Codling moth adults are attracted to the volatiles from apples and in particular to *(E, E)*-ά-farnesene (Wearing and Hutchins 1973, Sutherland *et al.* 1974). Unfortunately, instability and rapid breakdown of *(E, E)*-ά-farnesene substantially restricted the development of lures based on this material for monitoring codling moth in orchards (Cavill and Coggiola 1971).

Investigation of improved *(E, E)*-ά-farnesene stability and the use of other host plant volatiles from apples for codling moth male and female attraction continues (Bengtsson *et al.* 2001, Yan *et al.* 2003, Coracini *et al.* 2004, Hern and Dorn 2004). Various blends of different host plant volatiles, mostly derived from apples, synergise response of codling moth males to sex pheromone (Witzgall *et al*. 2001, Yang *et al.* 2004, Ansebo *et al.* 2004) and affect oviposition of females (Witzgall *et al.* 2005).

Light *et al.* (2001) discovered that the pear-derived kairomone, ethyl *(E, Z)*-2, 4-decadienoate (later named pear ester or "PE"), was a species-specific attractant for males and, most importantly, for codling moth females. The effectiveness of PE in codling moth male and female attraction and potential usefulness for monitoring in orchards has been tested in western North America (Light *et al.* 2001, Knight and Light 2005a, b, Knight *et al.* 2005), northern Italy (Ioriatti *et al.* 2003), eastern Ontario in Canada (Trimble and El-Sayed 2005) and in northern Victoria (Il'ichev *et al.* 2002, Il'ichev 2004) and central New South Wales (NSW) (Thwaite *et al.* 2004) in Australia.

Field trials established in 2001 in northern Victoria (Il'ichev *et al.* 2002) demonstrated that pure PE and PE mixed with the codling moth sex pheromone component *(E, E)*- 8, 10-dodecadienol, also known as Codlemone, were highly specific and attractive for codling moth males and females in orchards. However, the performance of PE lures was not significantly better than various sex pheromone lures particularly in orchards without MD treatment (Il'ichev 2004). In a Granny Smith apple orchard under MD treatment in central NSW, PE lures as a monitoring tool were superior compared to various sex pheromone lures (Thwaite *et al.* 2004).

The variability in the results from these independent trials warranted further investigation. This paper reports such an investigation with standardised methodology to assess the effectiveness of PE lures as a codling moth monitoring tool in a range of pome fruit varieties in Victoria, Australia.

MATERIALS AND METHODS

Experimental design, locations and treatments

Field sites were located in commercial pome fruit orchards at Ardmona (William Bon Chrétien (WBC) pears, Cripps Pink and Granny Smith apples) and Bunbartha (Nijiseiki nashi) in northern Victoria. When available, codling moth monitoring information from the previous season was used to identify blocks likely to have sufficient trap captures to allow statistical analysis of the data. Eight experimental blocks, two of each of four varieties (WBC pears, Cripps Pink apple, Granny Smith apple and Nashi) were selected for the trial. All eight experimental pome fruit blocks were square shaped, similar in size (1.4 ha), and located at the same elevation of about 115 m. The two blocks for each variety (always on the same orchard) were randomly assigned the two levels of MD treatment (+MD or -MD).

MD dispensers (Isomate CTT®) for codling moth control were placed in all trees within the +MD treated blocks at the registered rate (500 dispenser ha^{-1}) in mid-September. Isomate CTT® is a double-tubing controlled release dispenser that contains the codling moth sex pheromone components *(E, E)*-8, 10-dodecadienol (215 mg $dispenser^{-1}$), dodecanol (120 mg $dispenser^{-1}$) and tetradecanol (27.5 mg $dispenser^{-1}$). If MD treatment was not applied (-MD) for codling moth control, fruit blocks were treated with insecticides (parathion-methyl and/or azinphos-methyl) applied about 7-14 times during the season, according to normal practice of the orchardists.

Within each block four replicates of each combination of two lure types at each of two heights (high or low) were assigned, via a 4 × 4 Latin square design with plots not less than 24 m × 24 m. A separate randomisation of treatments (Lure Type × Height) was used for each block. PE lures (Code DA2313, Trécé 8693, Batch 40052183) made from grey halo butyl rubber septa dispensers loaded with 1 mg of PE were used at all sites and "Bubble" lures (PheroTech Codling Moth Super Lures (Code RD-0405/000, Batch CM SL 032003) loaded with 10 mg of Codlemone) were used in +MD blocks. "BioLures" consisting of controlled release membranes loaded with 1mg of Codlemone (Suterra BioLure®, Batch 39011902) were used as sex pheromone lures in –MD blocks. Bubble lures were suspended from the apex of the trap and BioLures were attached by a sticky strip to the inside of the trap. There was only one lure in each trap. Bubble lures and BioLures will subsequently be referred to as sex pheromone lures.

Easiset® delta traps with sticky inserts were mounted on the middle tree in each plot in each experimental site. All traps were separated from each other by 24 m to avoid interference. "Low" traps were placed on a tree limb at shoulder height (about 1.5 m) in the lower part of the tree canopy. "High" traps were attached to a bamboo rod for easy servicing and placed within the top 0.5 m of the tree canopy (about 2.5 m) but no closer than 50 cm to a MD dispenser. Traps were suspended with the sticky base placed horizontally.

Monitoring of codling moth catches

Traps were placed in the orchard at the beginning of apple flowering (in early October in Victoria) and checked weekly for the presence of codling moth, which were counted and removed from the sticky inserts. The trial started with the first codling moth catches in the traps and ran for 24 weeks. PE and Bubble lures were changed after 12 weeks, but BioLures were changed after eight weeks according to label recommendations. All sticky inserts were changed regularly at four weeks or earlier if the surface lost stickiness or caught many insects.

Identification of codling moth sex

During weekly monitoring of all field trials, the sex of each codling moth on the sticky inserts of the traps with PE lures was identified by examining external genitalia, using magnification if necessary. Codling moths from traps with sex pheromone lures were not examined for sex identification because these lures were not expected to attract females irrespective of the treatment.

Statistical analysis

Trap catches over the entire 24-week monitoring period were analysed using a linear mixed model (LMM) fitted using ASREML (Gilmour *et al.* 2006). This software incorporates the modified F-test of Kenward and Rogers (1997) for testing fixed effects. Tests of significance for random effects that were not part of the design strata were based on the likelihood ratio test. Design strata random effects, eg. Blocks, were retained in the model irrespective of their level of significance. The response variable was taken as Y=log (Total+1) to better meet the assumptions required to apply LMM in ASREML. On the log scale, the differences between average values for treatment A and treatment B would be D=log (A) - log (B)= log (A/B). The 'ratio' of A to B on the original scale is then given as A/B=antilog (D). This ratio of A to B is referred to as 'relativity' since it reflects the average value in treatment A relative to the average value in treatment B.

The first analysis was restricted to PE lure types only and here Total corresponds to the sum of each sex caught in a trap over the 24 week monitoring period. The second analysis was unrestricted with respect to lure type but with Total taken as the sum of all moths (irrespective of sex) caught in a trap over the 24 week monitoring period.

For the analysis restricted to PE lure types only, the fixed effects included in the full model were effects for MD (+MD or -MD), Variety (European Pears, Cripps Pink apple, Granny Smith apple or Nashi), Height (high or low), Sex (male or female) and interactions between these main effects but excluding terms involving interactions between MD and Variety. The interaction between MD and Variety was confounded with Block and was fitted as a random effect. Also included as random effects in the model were interactions between Blocks and the effects for Height, Type and Height × Type interaction, effects for Rows within Blocks, Columns within Blocks and a random error term. The random effects for Blocks, Rows within Blocks and Columns within Blocks were not tested for significance as these were design strata associated with the experiment.

For the second analysis of the total moth catches (irrespective of sex) across both lure types the full model initially fitted was similar to the analysis of the PE lure type analysis outlined above, but the effect for Sex was replaced by Type (PE lure vs. Sex pheromone lure).

RESULTS

Significant effects on total moth catches of each sex with PE lures

The total numbers of codling moths caught over the 24 week period in each block for each combination of Height and Lure type, separated on each sex for PE lures, are presented in Table 1. Summarised results of the statistical analyses below are given as relativities of the numbers under pairs of treatments, or as relativities of females to males.

Analysis indicated that the random effects for Block × Height and Block × Height × Sex were not significant ($P > 0.05$) but there was a significant ($P < 0.05$) Block × Sex random effect. Hence the relative numbers of the two sexes differ significantly across the blocks. Of the fixed effects all main effects (Variety, MD, Sex and Height) and the interactions Variety × Height and MD × Height were significant ($P < 0.05$). The remaining fixed effects in the initial model fitted were not significant. The interaction effects for Variety × Height and MD × Height, after adjusting for the other significant fixed effects, were significant at <0.001 and 0.020 respectively.

PE lure traps, averaged over blocks of the same variety, MD treatment, and trap height placement caught approximately 3.01 (s.e. 0.72) times more males than females. The magnitude of the effect of trap height on catch of each sex differed across varieties and MD treatments but low traps always caught significantly fewer moths in each case (Table 2).

PE lure traps in –MD blocks also trapped more moths of each sex than the corresponding traps in +MD blocks having the same variety on the same orchard, but with the relativities different across traps placed high and traps placed low. The model estimated there were 3.16 (s.e. 0.86) times more moths of a particular sex caught in high traps in blocks not treated with MD

Table 1. Total number of codling moths males and females caught over the 24 weeks in the Pear Ester and Sex pheromone lures, at each height (low or high within the tree canopy) in each block.

MD Variety	PE Lure Female	PE Lure Male	Sex. Ph Male	Total
-MD Granny Smith - high	211	841	1814	2866
-MD Granny Smith - low	94	343	805	1242
Total	**305**	**1184**	**2619**	**4108**
-MD Nashi - high	40	45	231	316
-MD Nashi - low	28	22	180	230
Total	**68**	**67**	**411**	**546**
-MD Cripps Pink - high	124	627	792	1543
-MD Cripps Pink - low	92	352	610	1054
Total	**216**	**979**	**1402**	**2597**
-MD Pear - high	108	388	699	1195
-MD Pear - low	28	144	500	672
Total	**136**	**532**	**1199**	**1867**
+MD Granny Smith - high	122	144	91	357
+MD Granny Smith - low	64	52	29	145
Total	**186**	**196**	**120**	**502**
+MD Nashi - high	26	72	67	165
+MD Nashi - low	6	32	22	60
Total	**32**	**104**	**89**	**225**
+MD Cripps Pink - high	107	118	80	305
+MD Cripps Pink - low	49	52	21	122
Total	**156**	**170**	**101**	**427**
+MD Pear - high	76	81	69	226
+MD Pear - low	10	14	9	33
Total	**86**	**95**	**78**	**259**

compared to those caught in high traps in +MD blocks. For low traps, the model estimated that those in –MD blocks caught 4.31 (s.e. 1.17) times as many moths as those in +MD blocks.

Effect of lure type on total moth catches (irrespective of sex)

The random effects for Block × Height, Block × Type and Block × Height × Type were not significant ($P > 0.05$). Of the fixed effects Height × Variety, Height × Type, Height × Type × MD were not significant ($P > 0.05$) leaving as significant fixed effects for Variety, Height, Type, Height × MD, MD × Type and Variety × Type. The level of significance of the three significant interaction effects, after adjusting for the other significant fixed effects, were < 0.001, < 0.001 and 0.013 respectively.

Based on the fitted model, low traps of either type (PE or sex pheromone lures) within the same block were estimated on average to catch approximately 0.41 (s.e. 0.05) times as many moths as high traps for +MD blocks and 0.73 (s.e. 0.08) as many moths as high traps for -MD blocks, independent of variety. The relativities for total catches (i.e. combining males and females) for PE lures to sex pheromone lures within the same block

Table 2. The estimated relativities (Low total : High total) and associated standard errors for male or female catches using Pear Ester lures within the same block either with (+) MD or without (–) mating disruption (MD).

MD Variety	Relativity (Low : High)	Std. Error
-MD Granny Smith	0.63	0.09
-MD Nashi	0.71	0.11
-MD Cripps Pink	0.75	0.11
-MD Pear	0.35	0.05
+MD Granny Smith	0.46	0.07
+MD Nashi	0.52	0.08
+MD Cripps Pink	0.55	0.08
+MD Pear	0.26	0.04

Table 3. The estimated relativities (PE total: Sex pheromone total) and associated standard errors for total catches at either height (low or high within the tree canopy) within the same block either with (+) MD or without (–) mating disruption (MD).

MD Variety	Relativity (PE : Sex. Ph)	Std. Error
-MD Granny Smith	0.84	0.15
-MD Nashi	0.45	0.08
-MD Cripps Pink	0.90	0.16
-MD Pear	0.68	0.12
+MD Granny Smith	4.46	0.81
+MD Nashi	2.39	0.44
+MD Cripps Pink	4.76	0.87
+MD Pear	3.59	0.66

Table 4. The estimated relativities with (+) mating disruption (MD) total : without (-) MD total and associated standard errors for catches within an orchard (same variety) for each trap type (Pear Ester (PE) or Sex pheromone (Sex. Ph) at either height (low or high within the tree canopy).

Type	Height	Relativity (+MD : -MD)	Std. Error
PE. Lure	High	0.79	0.33
PE. Lure	Low	0.45	0.19
Sex. Ph	High	0.15	0.06
Sex. Ph	Low	0.08	0.04

depends on Variety and MD, but not on height. These estimated relativities and associated standard errors are given in Table 3.

The relative number of total moths caught within an orchard for a +MD block relative to a –MD block depends on the lure Type (PE or sex pheromone lure) and Height (high or low) but are not significantly different across the four varieties. These estimated relativities and associated standard errors are given in Table 4.

DISCUSSION

Based on 24-week totals (Table 1), traps placed high in the tree canopy caught more codling moths than traps placed lower irrespective of lure type, treatment and fruit variety. This is not unexpected, since Weissling and Knight (1995) demonstrated that codling moth males and females in apple orchards were distributed mostly high in the canopy, where they mate. Knight and Light (2005b) demonstrated that significantly more male but not female moths were caught in PE lure traps placed high versus low in the tree canopy. Yang *et al.* (2005) showed in wind tunnel studies that pre-exposure to sex pheromone increased the behavioural responses to PE of codling moth males, but not females. Our results demonstrated that PE lure traps caught about three times more males than females in any of the four tested varieties at the same height (i.e. high or low) under the same MD treatment (i.e. +MD or – MD). Also in our study PE lure traps placed higher caught significantly more female moths than PE lure traps placed low. Field trials in Europe demonstrated that 10 mg PE lures caught significantly more moths (both male and female) in pheromone-treated orchards under MD than in orchards treated with insecticide (Yang *et al*. 2005). The opposite results were found in our study which used only 1 mg PE lures and where direct comparisons were possible (Table 4). For all four cultivars irrespective of the trap height, significantly more moths (both male and female) were trapped in insecticide-treated (-MD) orchards than in MD-treated (+MD) orchards. Our study in MD treated orchards, where direct comparisons were possible (Table 3), demonstrated that 1 mg PE lures caught significantly more codling moths (both sexes combined) than did 10 mg sex pheromone lures. Comparing the efficacy of these two lure types was one of the key aims of our study and the results clearly support the use of PE lures as a tool for monitoring the pest in orchards under MD.

By contrast, our results support continued use of 1 mg sex pheromone lures to monitor insecticide-treated orchards since the codling moth catches in traps with 1 mg sex pheromone lures were either higher than (Granny Smith apple, nashi and pear) or not significantly different (Cripps Pink apples) to PE lures (Table 3). There could be a case for using a trap baited with a combination of PE and sex pheromone lures. Il'ichev (2004) demonstrated significant increase in codling moth catches for dual lures compared to 1 mg sex pheromone lure, particularly in pome fruit orchards without MD treatment.

Our results also demonstrated that PE lures placed in fruit blocks treated with MD had an advantage in attracting large numbers of codling moth females. The use of PE lures for attraction and monitoring of codling moth females has great potential to improve monitoring techniques, particularly in pome fruit orchards treated with MD, because it may allow better prediction of egg hatch than is currently achieved with a male-based phenology model. Recent research conducted in the United States of America demonstrated the potential of using PE lures for early-season prediction of egg hatch time (Knight and Light 2005c) and development of an action threshold for codling moth control in MD treated apple orchards (Knight and Light 2005d). Although most codling moth females caught with PE lures were already mated, Knight and Light (2005c) did not use mating status in establishing biofix dates because it required further investigation in different pome fruit varieties and climatic zones.

Based on our findings, we advocate the use of 1 mg PE lures in pome fruit orchards under MD. Irrespective of the lure type used, traps should continue to be placed higher in the tree canopy. Our results also confirmed that 1 mg sex pheromone baited traps placed higher in the tree canopy of insecticide treated orchards are more efficacious than at the presently recommended shoulder height (about 1.5 m). But since treatment thresholds have been developed for the lower placement there is no justification for changing recommended placement of the traps (Thwaite and Madsen 1983). Further work is planned to develop female based biofix dates and to revise the predictive model (Williams 1993) currently used in Victoria to forecast egg hatch.

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