

IMPROVING THE PHEROMONE LURE FOR DIAMONDBACK MOTH

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ABSTRACT

Pheromone traps have potential to assist with integrated pest management of diamondback moth (*Plutella xylostella* (Linnaeus)). Trapping experiments were set up in brassica crops in Canterbury and Auckland to test the potential for improving lures for pheromone traps. Catch was improved with higher purity of synthetic lures containing (Z)-11-hexadecenyl acetate and (Z)-11-hexadecenal and (Z)-11-hexadecen-1-ol (30:60:10). The initial lures tested caught 7.5% of the number of moths caught in traps baited with virgin females. This increased to 20-47% using better lures. Coupled gas chromatography and electrophysiology confirmed the presence of three active peaks from the chemicals listed above, plus a fourth unidentified compound. Traps with improved lures were used in a pilot IPM programme in Pukekohe and will assist brassica growers to minimise insecticide usage.

Keywords: Brassica, diamondback moth, *Plutella xylostella*, pheromone.

INTRODUCTION

Pheromones and other behaviour-modifying compounds have been useful for managing insects in a wide variety of ways, including decision support (Suckling & Karg 2000). In New Zealand, there are already a number of examples of pheromone applications in pest management (Suckling 2001). The first step in the development of pest management tactics based around a pheromone is the development of a successful attractant blend. For diamondback moth (*Plutella xylostella* (Linnaeus)), a wide range of pheromone blends have been reported to be attractive to male moths (Tamaki et al. 1977; Ando et al. 1979; Chisholm et al. 1983; Koshihara et al. 1978; Maa et al. 1984; Macaulay et al. 1986; Zilahi-Balogh et al. 1995). The basis of geographical variation in diamondback moth male response to different pheromone blends is unclear. The origin of diamondback moth in New Zealand is also unclear, although there is evidence of importation of strains from Australia (Voice & Chapman 2000).

Earlier tests in New Zealand indicated that only very poor catches were achieved using lures based around (Z)-11-hexadecenyl acetate (Z11-16:Ac) and (Z)-11-hexadecenal (Z11-16:Ald), averaging under 0.1 moth per trap per day (D.M. Suckling & P. Brookbanks, unpubl. data). This led us to investigate improved lures for decision support in pest management of *P. xylostella*.

MATERIALS AND METHODS

Chemicals and Insects

Synthetic lures used in Experiment 1 were made up from commercial components (Blend 1, Sigma, typically 95% purity), or purchased as lures from Pherobank (Wageningen, The Netherlands) (Blend 2) or Agrisense (UK) (Blend 3). The higher purity compounds used in Experiments 2-4 were purchased from Pherobank, and the

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components loaded onto rubber septa. Component purity was checked by gas chromatography.

Plutella xylostella females were taken from colonies maintained at Lincoln and Auckland, depending on the location of the trapping experiment. The original moths for these colonies came from Christchurch and Pukekohe respectively.

Experiment 1

Pheromone sticky traps (Suckling & Shaw 1990) were baited with one of three synthetic pheromone lures or a gauze cage containing three virgin female moths. Synthetic lures contained either 100 µg of a 60:40 ratio of (Z)-11-hexadecenyl acetate (Z11-16:Ac) and (Z)-11-hexadecenal (Z11-16:Ald) at two isomeric purity levels (97% and >99% purity), or a ternary blend ratio of 30/60/10 of Z11-16:Ac, Z11-16:Ald and (Z)-11-hexadecen-1-ol (Z11-16:OH) (Table 1). Three replicates of each treatment were placed in a randomised complete block design at 0.4 m height in a brassica crop at each of two Canterbury locations (Ladbrooks and the Biological Husbandry Unit, Lincoln University). There was at least 5 m between treatments and 40 m between blocks. Traps were checked weekly from 5 February - 6 March 1998.

Experiment 2

To determine the reproducibility of the results from Experiment 1, in a separate trial later in the season pheromone sticky traps were baited with either the best synthetic pheromone blend from Experiment 1 (>99% purity of 100 µg at the ratio 60/40 Z11-16:Ac / Z11-16:Ald) or a gauze cage containing three virgin female moths. Three replicates of each treatment were operated on an organic vegetable farm at Marshlands, Canterbury from 2-16 May 1998.

Experiment 3

Pheromone sticky traps were baited with one of four blends at two different loadings, three virgin female moths or remained unbaited. The synthetic treatments included two, three and four component blends taken from the literature (Table 2). The additional component of Z9-tetradecenyl acetate (Z9-14:Ac) was added in low quantities because of reports that it improved catches (Chisholm et al. 1983). Traps were placed 10 m apart in a randomised complete block design with one replicate in each of five vegetable brassica crops at Pukekohe, and assessed weekly from 15 December 1999 – 9 February 2000.

Experiment 4

Pheromone sticky traps were baited with one of five synthetic blends, three virgin female moths, or left unbaited. The synthetic treatments included the best three component blends from earlier experiments, as well as a four component blend. Two treatments were loaded at lower rates than in Experiment 3, in order to check for effects of overloading. Five replicates were operated at Pukekohe, from 8 April to 5 May 2000.

Gland extract and coupled gas chromatograph-electroantennogram detection

Virgin female diamondback moths 24-48 h old were dissected between 2100 - 2200 h, which was during active calling behaviour, and the pheromone glands were extracted into hexane. Two extracts were prepared (20 and 27 females per extract), and 4 µl was injected into a Varian 3800 gas chromatograph, operating in splitless mode. The carrier gas was nitrogen at 1 ml per min, and the temperature programme was maintained at 80°C for 1 min and ramped up 20°C/min to 240°C. The eluent from a 30 m x 0.25 mm ZB wax column (Phenomenex, Torrance, CA) was split between the flame ionisation detector (FID) and an electroantennogram recorder (Syntech, Hilversum, The Netherlands). A male diamondback moth antenna was excised and connected between glass capillaries containing BE Ringer's solution with 10% polyvinylpyrrolidone (Molecular Weight 360 000). A moist charcoal filtered airstream was maintained over the antennae at 500 ml/min.

Statistics

Trap catches were compared between treatments after log transformation to stabilise the variance, using analysis of variance or *t*-tests. Blank traps and virgin

female-baited traps were included in all tests, and trap positions were rotated between readings.

RESULTS AND DISCUSSION

Experiment 1

A total of 1489 moths were caught (Table 1), with highly significant differences between treatments due to the presence of virgin females as lures ($P < 0.0001$). The best synthetic blend achieved 36.4% of the catch of virgin females, although the same binary blend of compounds at lower purity (Blend 1) was less attractive. Blend 1, found to be unsatisfactory in 1997 (D.M. Suckling & P. Brookbanks, unpubl. data), was confirmed here as relatively ineffective. The other two blends differed in ratio and one component, but no significant difference in catch was present. Both of the acetate and aldehyde ratios (60:40 and 40:60) have been reported in the literature as effective lures (Tamaki et al. 1977; Koshihara et al. 1978).

TABLE 1: Catch of male diamondback moths in traps baited with a range of pheromone compounds loaded on rubber septa compared to traps baited with three virgin female moths, tested in Canterbury (n=6 reps). Letters indicate significant differences from Fisher's least significant differences (LSD).

Pheromone compounds	Blend 1 ¹ (µg)	Blend 2 ² (µg)	Blend 3 ³ (µg)	Virgin females
Z11-16:Ac	60	60	30	
Z11-16:Ald	40	40	60	
Z11-16:OH			10	
Mean no. male moths	11.3	54.5	39.7	149.7
SEM	4.9	20.4	7.2	30.1
Fishers LSD	a	a	a	b
% of virgin females	7.5%	36.4%	26.5%	100%

¹Purity 97%.

²Purity > 99%.

³Ratio confirmed by gas chromatography.

Experiment 2

The catch of male diamondback moths in Canterbury in traps baited with a binary pheromone blend of high purity compounds (Blend 2 in Experiment 1) was 20.0 males per trap (SEM=5.5), compared to a catch to virgin females of 142.0 males per trap (SEM= 40.0) ($t = -4.69$; $P < 0.05$; $df = 3$). A total of 486 moths were caught to both lures. In this repetition of the previously best treatment, the catch of diamondback moths was only 14% of the catch to virgin females. This result led to the need for further tests with a wider range of blends, in an effort to improve catches relative to virgin females.

Experiment 3

A total of 5965 moths were caught in the 10 treatments (Table 2). High loadings of synthetic lures (1 µg) were less attractive than the mid-range loading rates (100 µg) ($P < 0.0001$). In addition, three or four component lures were more attractive than the two component lures ($P < 0.0001$). However, in this test, the best synthetic lures (Blend 2) at the lower dose caught only 28% of the catch to virgin females. There appeared to be a dose-related inhibitory effect from the addition of Z9-14:Ac, as has been reported by Chisholm et al. (1983), although this was not significant.

TABLE 2: Catch of male diamondback moths in traps baited with a range of pheromone compounds (μg per rubber septum) compared with traps baited with three virgin females, tested at Pukekohe. Letters indicate significant differences from Fisher's least significant differences (LSD).

Blend	1	2	3	4	5	6	7	8	Females	Blank
Z11-16:Ac	40	30	30	30	400	300	300	300		
Z11-16:Ald	60	60	60	60	600	600	600	600		
Z11-16:OH		10	9	9		100	90	90		
Z9-14:Ac			1	0.1			10	1		
Mean no./trap	37.8	170	76	126	6.4	65.6	33	59	616	2.8
SEM	9.5	15.6	10.6	31.8	1.7	6.7	2.4	5.7	91.7	1.4
Fisher's LSD	c	b	bc	bc	d	bc	c	b	a	d
% catch to females	6.1	27.7	12.3	20.5	1.0	10.7	5.4	9.6	100	0.5

Experiment 4

A total of 138 moths was caught in this experiment at Pukekohe (Table 3). Trap catches to the mid-range (100 μg) and low (10 μg) pheromone trap loadings were not significantly different. The ternary blend without Z11-16:Ac (Blend 3) was significantly less attractive than the other blends, and similar to unbaited traps. In this test, Blend 1 achieved 47% of the catch to virgin females.

TABLE 3: Catch of male diamondback moths in traps baited with a range of pheromone compounds (μg per rubber septum) compared with traps baited with three virgin female moths, tested at Pukekohe. Letters indicate significant differences from Fisher's least significant differences (LSD).

Blend	1	2	3	4	5	Virgin females	Blank
Z11-16:Ac	30	30		3	3		
Z11-16:Ald	60	60	75	6	6		
Z11-16:OH	10	0.01	10	1	1		
Z9-14:Ac			15		0.001		
Mean no./trap	5.0	4.6	0.2	2.8	4.4	10.6	0
SEM	1.0	0.8	0.2	0.6	0.7	2.5	0
Fisher's LSD	ab	b	c	b	b	a	c
% catch to females	47.2	43.3	1.8	26.4	41.5		

Coupled gas chromatograph-electroantennogram detection

Three electrophysiologically-active peaks were identified by comparing their retention times with those of standards, and were found to be present in the ratio of 60:10:30 for Z11-16:Ac, Z11-16:Ald and Z11-16:OH (Fig. 1). No Z9-14:Ac was found, but another active peak was detected, which has not yet been identified. This additional electrophysiologically-active compound in the gland extract could account for the inferior catch of all synthetic blends tested, compared with virgin female moths. Further work will be required to identify this compound. Gland analysis can potentially give erroneous ratios compared to calling virgin females because either female effluvia may change over time, there is individual variation between moths or some compounds may be released differentially from gland contents. Hence the difference between the ratios of the three components found during gland analysis and trapping studies reported here

may not be enough to significantly affect catches. An analysis of effluvia from calling moths did not give satisfactory results.

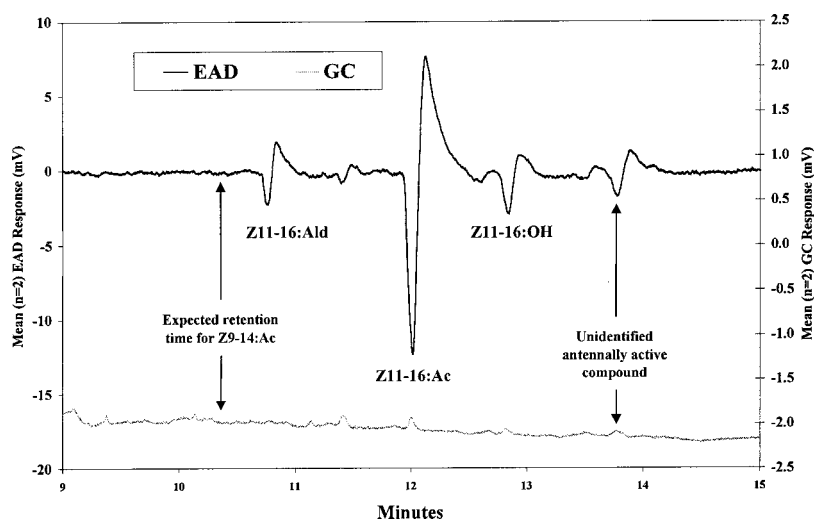


FIGURE 1: Coupled gas chromatograph (lower) and electroantennogram detection (upper) of female diamondback moth pheromone gland extracts. Traces are averages of two runs on separate extracts.

These experiments have demonstrated a considerable improvement over previously available blends for pheromone traps for diamondback moth in New Zealand. The most attractive blend here was a ternary mix of 30:60:10 of Z11-16:Ac, Z11-16:Ald and Z11-16:OH loaded at 100 µg. Although a range of synthetic blends and ratios was highly attractive, none were as attractive as three calling females. This suggests that isolation of a more attractive synthetic blend may be possible.

The extension of these results into an integrated pest management programme against diamondback moth has successfully commenced (Rogers et al. 2000; Walker et al. 2001), with the recommendation of both pheromone trapping and scouting for pest monitoring. The best blend reported here has also been used in a study of diamondback moth dispersal in the context of resistance management (Cameron et al. 2002).

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