Available online at www.sciencedirect.com
ScienceDirect
Crop Protection 27 (2008) 248–255
Crop Protection
www.elsevier.com/locate/cropro

Development of PVC-resin-controlled release formulation for pheromones and use in mating disruption of yellow rice stem borer, *Scirpophaga incertulas*

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Received 24 November 2006; received in revised form 24 May 2007; accepted 24 May 2007

Abstract

Half lives of a range of Lepidopteran sex pheromone components incorporated into plasticized polyvinylchloride (PVC)-resin formulations were determined under a range of conditions in a laboratory wind tunnel and in the field in Egypt, Pakistan and India. Increasing aliphatic chain length by two methylene groups increased half lives by a factor of approximately 7.5 at 27 °C. Half lives of saturated aliphatic acetates decreased with increase in temperature by 79.2%, 85.5% and 95% for dodecyl acetate, tetradecyl acetate and hexadecyl acetate, respectively, between 22 and 34 °C. Addition of the polymer-soluble dye, Waxoline Black, improved the stability of labile aldehydes and polyunsaturated compounds incorporated into plasticized PVC under field conditions, although the antioxidant BHT had no effect. Studies confirmed that the formulation was ideally suited for the release of 14-carbon acetates and 16-carbon aldehydes with typical field lives of 70–100 days. A PVC-resin formulation of sex pheromone was tested for mating disruption of yellow rice stem borer, *Scirpophaga incertulas*, in replicated 1 ha plots in India over two seasons. Even at an application rate of 10 g a.i./ha/season there was a significant reduction in damage relative to that in plots treated with insecticides. However, application rates of 30–40 g a.i./ha/season are recommended for general use by farmers.
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Keywords: Mating disruption; Pheromones; PVC-resin formulation; Controlled release; *Scirpophaga incertulas*

1. Introduction

Lepidopteran female sex pheromones utilize straight-chained aliphatic compounds of between 10 and 23 carbon atoms, derived from fatty acids. A typical Lepidopteran sex pheromone consists of two or three structurally related compounds in a specific ratio. The compounds often contain an oxygenated functional group; these can be terminal groups such as acetates, alcohols or aldehydes or functional groups positioned at specific locations on the carbon chain, such as epoxides and ketones. Unsaturation is common in Lepidopteran sex pheromones usually comprising between one and three double bonds with specific positional and geometric stereochemistry (Witzgall et al., 2004). The specificity and high level of bio-activity of Lepidopteran sex pheromones have attracted considerable interest in crop pest control (Howse et al., 1998; Wyatt, 1997) using a range of techniques, including disruption of male moth orientation leading to mating disruption (Sanders, 1997) and attraction to a lethal source, as in lure-and-kill and mass trapping (Cork et al., 2003; El-Sayed et al., 2006) or, more recently, a non-lethal source as in auto-confusion (Howse, 2004). Control by techniques that involve male attraction invariably require less pheromone than mating disruption and so are potentially more cost-effective, although this advantage can be mitigated by the need purchase and maintain trap systems and

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doi:10.1016/j.cropro.2007.05.011

re-application (Yamanaka, 2007). Similarly, most controlled release formulations have first-order release kinetics whereby release is proportional to the amount of pheromone present. Thus, optimal pheromone use is best achieved by multiple low-dose applications over a season rather than a single high-dose application (McDonough et al., 1992). However, the choice between single or multiple applications per season may ultimately be dependent on the relative costs of labour and pheromone (Fitzpatrick et al., 2004) rather than achieving the lowest seasonal application rate.

Cork et al. (1989) described a polyvinylchloride (PVC)-resin formulation (Fitzgerald et al., 1973; Hendricks et al., 1987; Sanders, 1981) that was modified to optimize release rate characteristics of several pheromone components and achieve a high level of protection of chemically labile compounds. The present study was undertaken to determine the release rate characteristics of a wide range of pheromone components, with different protectants under different conditions in order to assess the scope of the PVC-resin formulation for use in mating disruption. An optimized formulation was then utilized to determine the pheromone application rates required to achieve control of a model pest species, the yellow rice stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera, Pyralidae), in replicated trials on 1 ha plots.

2. Methods and materials

2.1. Pheromone dispensers

PVC-resin dispensers for release rate studies were prepared by mixing 40% by weight vinyl chloride/vinyl acetate emulsion copolymer (Vinnol E5/65C, Wacker Chemicals Ltd., UK) with a 1:1 mixture of the plasticizers Cereclor (S45, ICI Ltd., UK) and di-(2-ethylhexyl)-phthalate (DEHP, Sigma Aldrich Ltd., UK). Pheromone components, antioxidant and UV screener Waxoline Black (WB, ICI Ltd., UK) were then added to the prepolymer as required, typically 1% each by weight. The prepolymer was thoroughly mixed and degassed on a rotary evaporator for 1 h under vacuum, poured into a mould composed of two glass plates with suitable spacers (0.1 cm) and cured by heating to 150 °C for 15 min. The resulting PVC sheets were removed from the moulds and cut into 1 cm squares. Compounds tested were either prepared at NRI or procured from Sigma-Aldrich Ltd. (Gillingham, Dorset, UK) and were at least 98% chemically and isomerically pure by gas chromatography (GC).

PVC-resin dispensers utilized for mating disruption trials with *S. incertulas* were provided by Agrisense-BCS (UK) in the form of extruded lengths of 3 mm diameter. The exact composition of the formulations is proprietary information but was basically similar to those prepared at NRI. Each 'string' dispenser contained a 1:3 mixture of (*Z*)-9-hexadecenal (Z9–16:Ald) and (*Z*)-11-hexadecenal (Z11-16:Ald) (5% w/w).

2.2. Release rate studies

PVC-resin dispensers ($1 \times 1 \times 0.1$ cm) were weighed and hung from wires suspended in a Perspex wind tunnel providing a constant 8 km/h wind speed. The temperature was maintained at 22, 27 or 34 °C throughout the duration of each experiment. Related studies were conducted in crop fields in El Fayoum, Egypt (29°26′N, 30°38′E), Medchal, India (17°38′N, 78°30′E) and Multan, Pakistan (30°13′N, 71°27′E) by suspending 30 PVC-resin dispensers from a line in direct sunlight with typical maximum and minimum temperatures of 34 ± 2 and 28 ± 2 °C, respectively. Two PVC-resin strips were removed at typically 0, 1, 2, 3, 5, 10, 15, 21, 27, 33 and 40 days of exposure, re-weighed and stored at −20 °C in heat-sealed, tri-laminated metalized sachets, until the end of the trial when they were analysed for residual pheromone by GC at NRI.

2.3. GC analysis of residual pheromone

PVC-resin dispensers exposed in a wind tunnel at NRI were extracted individually with hexane (5 ml) containing an internal standard, typically tridecyl acetate, at RT for 8 h (Hendricks et al., 1987). Samples (ca. 1 cm lengths) of Agrisense 'string' PVC-resin dispensers exposed in the field were cut, weighed and then extracted with hexane as for the NRI samples. Hexane extracts were analysed on a Carlo Erba 5300 Mega series gas chromatograph fitted with flame ionization detector, Carlo Erba A200s autosampler and packed glass columns (1.5% Carbowax 20 M on Chromosorb G AW DCMS) temperature programmed from 100 °C at 4 °C/min to 200 °C with nitrogen carrier gas (25 ml/min). Isomeric ratios were determined on a fused silica capillary CP Wax 52CB column ($30\,\text{m} \times 0.32\,\text{mm}$ ID, Chrompack, UK) using splitless injection at 220 °C and an oven temperature of 70 °C held for 2 min; the temperature was programmed at 20 °C/min to 120 °C and then at 4 °C/min to 220 °C using helium carrier gas (0.4 kg/cm^2). The quantity of residual compound was corrected for the initial weight of PVC-resin sample and expressed as a percentage of the initial loading. Results are the mean of two samples.

Depletion of pheromone components from the PVC-resin formulations was characterized by the exponential equation $y = ae^{-bt}$, where y is the percent remaining, t is the time (days), e is the base of natural logarithms and a and b are constants. The latter constants and regression coefficient (r) were calculated using SigmaPlot 2001 (Systat Software Inc., USA). Half lives ($t_{1/2}$) for compounds were determined from the exponential equation substituting calculated values of a and b and setting y to 50.

2.4. Field trial location and application

Mating disruption trials were conducted in 1995 and 1996 in dry season rice crops (December–April) in Medchal, India. The predominant rice variety grown was Tellahamsa, an indigenous medium-duration variety (125 days) known to be susceptible to *S. incertulas* (N.H.P. Rao, pers. comm.). Each trial involved four treatments with two checks (farmers' practice), each replicated three times. Treatment and check plots were approximately 1 ha in area, so the total trial area was 18 ha. Typical farmers' holdings of 0.3–0.7 ha required the co-operation of some 40 farmers to undertake each season's trials. Trial plots were laid out with 100 m spacings between pheromone-treated plots and farmers' practice check plots (local check). Additional farmers' practice plots (distant check) were laid out in farmers' fields approximately 2 km from the main trial site. Farmers with plots in the pheromone-treated areas refrained from applying insect pest control measures, while farmers with check plots were requested to continue their normal insect pest control routines. Farmers used a single application of insecticide per season, typically a granular systemic insecticide, Phorate (ranging from 2.5 to 12.5 kg/ha), between 20 and 35 days after transplanting (DAT).

Trials were each laid out in 1 day, working with approximately 20 labourers on 4 February 1995 and 12 February 1996, 18 and 25 DAT, respectively. Dispensers were each attached to 1 m split bamboo canes and placed, by hand, in a 4 m × 4 m grid in the 1 ha trial plots (625 dispensers/ha) in 1995 and in a 5 m × 4 m grid in 1996 (500 dispensers/ha). PVC-resin dispensers were cut to different lengths to generate different application rates/ha. At harvest all canes and dispensers were collected, the dispensers were removed and the canes stored for future re-use.

2.5. Stem borer larval damage assessments

Stem borer damage in check and pheromone-treated plots was assessed approximately every 10 days, generating at least three assessments of dead heart (DH) and two assessments of white heads (WH). Each assessment was made by examining all tillers in a total of 50 hills at approximately 4 m intervals along two randomly selected transects of each trial plot. DH and WH damage was expressed as mean percentage for each trial plot and subjected to analysis of variance (ANOVA). Where significant differences in treatment means were indicated at the 5% level or lower, the means were compared by Newman–Keuls test (Statgraphics, Version 2.00).

2.6. Yield assessments

At least three sample areas (5 × 5 m) from each trial plot were harvested and threshed by hand to obtain wet weight grain yield estimates. Dry weight estimates were calculated by reducing the grain weight by 10% and converting to kg/ha. Most crop-cuts were taken at the same time as the farmers' harvest and as a result the data were collected over several days. Averaged yields for each trial plot were subjected to ANOVA, and where significant differences in treatment means were indicated at the 5% level or lower the means were compared by Newman–Keuls test (Statgraphics, Version 2.00).

3. Results

3.1. Physicochemical properties of PVC-resin formulations

Release rates of saturated, straight-chained acetates from the standard PVC-resin formulations aged in the wind tunnel varied in a non-linear manner, with increases in carbon chain length and temperature (Table 1). Increasing aliphatic chain length by two methylene groups resulted in half lives increasing by a factor of approximately 8.5, 7.6 and 5.2 at 22, 27 and 34 °C, respectively. Half lives of saturated aliphatic acetates decreased with increase in temperature by approximately 86% over the range tested (range 79.2%, 85.5% and 95% for dodecyl acetate (12:Ac), tetradecyl acetate (14:Ac) and hexadecyl acetate (16:Ac), respectively, between 22 and 34 °C). Half lives of decyl acetate (10:Ac) were too short and octadecyl acetate (18:Ac) too long to be measured accurately over all the time and temperature ranges tested.

Addition of the plasticizer Cereclor to DEHP to produce a 50:50 blend had no apparent effect on release rates of saturated acetates compared with DEHP alone (Table 1). However, pheromone loading in the standard PVC-resin dispensers was found to influence release rates of 12:Ac,

Table 1
Effect of temperature and plasticizer on release of saturated aliphatic acetates from PVC-resin dispensers (1 × 1 × 0.1 cm sheets) exposed in a wind tunnel (8 km/h)

Temperature (plasticizer)[a]	Compound	Half life (days)	First-order release parameters[b]		
			a	b	r^2
22 °C (DEHP + C)	10:Ac	0.9	98.27	0.735	0.991
	12:Ac	7.7	91.56	0.078	0.985
	14:Ac	65.3	97.31	0.001	0.974
	16:Ac	858.0	97.64	0.001	0.191
	18:Ac	> 1500	96.87	0	0.018
27 °C (DEHP + C)	10:Ac	0.7	99.52	0.978	0.998
	12:Ac	5.4	97.50	0.123	0.996
	14:Ac	39.1	100.50	0.018	0.929
	16:Ac	306.8	98.99	0.002	0.715
	18:Ac	> 1000	100	0	0
34 °C (DEHP + C)	10:Ac	0.8	10.0	2.098	0.999
	12:Ac	1.6	98.75	0.438	0.988
	14:Ac	9.5	94.51	0.067	0.989
	16:Ac	43.0	93.28	0.015	0.966
	18:Ac	113.8	96.77	0.006	0.732
27 °C (DEHP)	10:Ac	0.8	99.83	0.85	0.999
	12:Ac	5.7	97.50	0.12	0.880
	14:Ac	45.2	98.44	0.015	0.972
	16:Ac	563.2	99.57	0.001	0.199
	18:Ac	1521.1	99.50	0.001	0.049

[a]DEHP = di-(2-ethylhexyl)-phthalate, C = cereclor.
[b]Percentage remaining = ae^{-bt}; r^2 = regression coefficient.

Table 2
Effect of loading of saturated aliphatic acetates on release from standard PVC-resin dispensers ($1 \times 1 \times 0.1$ cm sheets) exposed in a wind tunnel (27 °C, 8 km/h)

Percent loading	Compound	Half life (days)	First-order release parameters[a]		
			a	b	r^2
1	12:Ac	5.5	94.20	0.12	0.985
	14:Ac	39.2	93.63	0.16	0.855
	16:Ac	115.2	88.53	0.005	0.333
2	12:Ac	6.5	95.63	0.100	0.966
	14:Ac	48.3	97.92	0.014	0.652
	16:Ac	199.6	97.65	0.003	0.303
5	12:Ac	7.7	101.40	0.092	0.976
	14:Ac	56.0	100.70	0.013	0.934
	16:Ac	212.4	96.07	0.003	0.501
10	12:Ac	5.9	100	0.116	0.988
	14:Ac	46.9	99.85	0.015	0.967
	16:Ac	196.6	91.28	0.003	0.366
20	12:Ac	5.4	98.54	0.125	0.993
	14:Ac	44.2	95.64	0.015	0.887
	16:Ac	148.3	88.70	0.004	0.250

[a]Percentage remaining = ae^{-bt}; r^2 = regression coefficient.

14:Ac and 16:Ac (Table 2), with half lives increasing with 1–5% loadings but decreasing with 10% and 20% loadings.

When exposed in the field in Egypt, 14:Ac and hexadecanal (16:Ald) had similar half lives in formulations containing WB and BHT (Table 3). However, half lives of the corresponding monounsaturated and diunsaturated compounds were reduced, suggesting that the compounds were degraded in situ rather than released. Removing BHT from these formulations had no effect on half lives. Half lives for all aldehydes and unsaturated acetates for formulations containing no protectants or BHT alone were significantly reduced compared with that of 14:Ac, which was essentially unchanged (ca. 20 days). This further suggested that differences in half lives between 14:Ac, 16:Ald and the unsaturated compounds resulted from degradation in situ rather than release. The lack of protection offered by BHT was unexpected but presumably reflected the high release rate of the compound from the PVC-resin formulation ($t_{1/2}$ of less than 1 day). Later formulations developed contained a polymerized form of BHT that increased field longevity of compounds subject to oxidation (unpublished).

In a related experiment, 14-carbon, mono-unsaturated compounds with different terminal functional groups were aged in the field in Egypt (Table 4). (*Z*)-9-Tetradecenal (Z9-14:Ald) had a half life (4.9 days) that was similar to that of 12:Ac in the wind tunnel at 27 °C (5.4 days) but half that of (*Z*)-9-tetradecen-1-ol (Z9-14:OH) even though their molecular weights were comparable. Half lives of (*Z*)-9-tetradecenyl acetate (Z9-14:Ac) and Z11-16:Ald were found to be similar, as observed in earlier studies but twice as long as in previous trials (Table 3), suggesting that losses from degradation had been significantly reduced.

Table 3
Effect of different protectants on release of compounds[a] from PVC-resin dispensers ($1 \times 1 \times 0.1$ cm sheets) exposed in Egypt

Protectant[b]	Compound	Half life (days)	First-order release parameters[c]		
			a	b	r^2
None	14:Ac	20.6	99.83	0.034	0.984
	Z9-14:Ac	3.1	100.80	0.228	0.987
	ZE9, 11-14:Ac	0.5	99.78	1.282	0.997
	16:Ald	1.3	98.47	0.508	0.988
	Z11-16:Ald	0.5	99.07	1.357	0.962
	EE10, 12-16Ald	0.2	100	3.423	0.999
BHT	14:Ac	20.4	101.60	0.038	0.996
	Z9-14:Ac	4.0	106.20	0.187	0.991
	ZE9, 11-14:Ac	0.6	98.90	1.088	0.966
	16:Ald	4.8	103.80	0.153	0.980
	Z11-16:Ald	3.2	89.35	0.179	0.921
	EE10, 12-16Ald	1.4	100.40	0.483	0.993
WB	14:Ac	16.7	99.50	0.041	0.991
	Z9-14:Ac	10.1	101.20	0.069	0.997
	ZE9, 11-14:Ac	4.1	104.40	0.182	0.994
	16:Ald	16.3	100.20	0.043	0.957
	Z11-16:Ald	10.3	95.920	0.063	0.983
	EE10, 12-16Ald	4.9	105.60	0.154	0.987
WB + BHT	14:Ac	17.0	100.40	0.041	0
	Z9-14:Ac	10.9	103.70	0.067	0.997
	ZE9, 11-14:Ac	4.7	105.20	0.16	0.986
	16:Ald	16.6	101.60	0.043	0.957
	Z11-16:Ald	10.7	102.40	0.067	0.993
	EE10, 12-16Ald	5.3	106.40	0.142	0.979

[a]Compounds present in equal amounts and totalled 1% w/w of the PVC-resin formulation.
[b]BHT = butylated hydroxytoluene, WB = Waxoline Black.
[c]Percentage remaining = ae^{-bt}; r^2 = regression coefficient.

Table 4
Influence of terminal functional group on release from standard PVC-resin dispensers exposed to direct sunlight in Egypt

Country	Compound	Half life (days)	First-order release parameters[a]		
			a	b	r^2
Egypt[b]	Z9-14:Ald	4.9	99.02	0.138	0.966
	Z9-14:OH	9.9	98.60	0.069	0.992
	Z9-14:Ac	25.2	95.67	0.026	0.957
	Z11-16:Ald	29.7	96.42	0.022	0.958
Pakistan[c]	Z11-16:Ald	22.2	104.50	0.033	0.984
	EE10,12-16:Ald	22.1	108.60	0.035	0.969
	7, 11-16:Ac[d]	44.8	106.90	0.017	0.927

[a]Percentage remaining = ae^{-bt}; r^2 = regression coefficient.
[b]PVC-resin sheet dispensers ($1 \times 1 \times 0.1$ cm) containing a 1:1:1:1 blend of compounds.
[c]Extruded PVC string dispensers (3 mm dia.) 1:1:1 blend of compounds 5% w/w a.i.
[d]50:50 blend of Z,Z- and Z,E-isomers.

Extruded PVC-resin dispensers were exposed under field conditions in Pakistan with a 5% loading of the sex pheromone of *Pectinophora gossypiella*, a 50:50 blend of (*Z*,*E*)- and (*Z*,*Z*)-7,11-hexadecadienyl acetate (ZE7, 11-16:Ac and ZZ7,11-16:Ac) and components of the pheromone of *Earias insulana*, Z11-16:Ald and (*E*,*E*)-10,12-hexadecadienal (EE10,12-16:Ald). The mono- and di-unsaturated aldehydes had similar half lives (22 days) (Table 4), suggesting that little, if any, degradation of the diene aldehyde occurred. Isomers of 7,11-16:Ac had half lives of almost 45 days, which were comparable to 16:Ac in a wind tunnel at 34 °C, also suggesting little, if any, loss by degradation.

3.2. Mating disruption trials

Residual pheromone was analysed in aged PVC-resin dispensers containing the sex pheromone of *S. incertulas* laid out in rice fields 0.5 km from the mating disruption trial plots in India. The half life averaged 26.8 days in both seasons' trials, with approximately 95% released over 80 days.

Stem borer damage assessments in the economically important reproductive stage of the 1995 season crop (68 and 81 DAT) suggested that larval populations in all pheromone-treated plots were significantly reduced compared with those in the local check plots treated with insecticide (Table 5). Pheromone treatments had no significant effect on WH damage levels in the local check plots as indicated by comparable levels of larval damage in distant check plots (Table 5). There was a significant effect of pheromone dose on larval damage with plots treated with 10 g a.i./ha having damage levels approximately twice those treated with 40 g a.i./ha, although there were no significant differences between damage levels in plots treated with 40 and 80 g a.i./ha (Table 5).

In order to further optimize pheromone dose for control, a second trial was conducted in 1996 with pheromone doses of between 18.6 and 36.8 g a.i./ha. Larval WH damage levels in local check plots in 1996 (Table 6) were comparable with those in 1995 (Table 5), but the level of control obtained by mating disruption was not as high as obtained in the 1995 trial. Nevertheless, WH damage levels in check plots were significantly higher than some of the pheromone-treated plots, with pheromone application rates of 32.2 and 36.8 g a.i./ha resulting in percentage WH damage levels (75 and 85 DAT) approximately half those of check plots (Table 6). Efficacy of mating disruption in the 1996 trial was also reflected in yield data; pheromone-treated plots producing on average 40% higher yields than check plots treated with insecticide (Table 6). In 1995, the yield was 11.2% higher on average for plots treated with pheromone compared with check plots, but differences in yields were not significant (Table 5).

4. Discussion

In order to develop pest monitoring and control systems based on volatile sex pheromones, efficient controlled

Table 5
Mean percentage of damaged tillers and yield in replicated pheromone- and insecticide-treated check plots, 1995 trial

Treatment (g/ha)	Percentage of damaged tillers[a]					Yield (kg/ha)
	25 DAT Mean ± SE	35 DAT Mean ± SE	45 DAT Mean ± SE	68 DAT Mean ± SE	81 DAT Mean ± SE	125 DAT Mean ± SE
10	0.74 ± 0.28ab	0.28 ± 0.28a	0.32 ± 0.16ab	9.77 ± 0.48a	8.16 ± 0.36b	6267 ± 933a
20	0.60 ± 0.07ab	0.43 ± 0.15a	0.91 ± 0.26ab	8.65 ± 3.2a	8.17 ± 1.99b	6267 ± 533a
40	0.18 ± 0.003a	0 ± 0a	0.11 ± 0.06a	6.50 ± 2.38a	4.64 ± 0.18ab	6267 ± 291a
80	0.20 ± 0.12a	0 ± 0a	0.16 ± 0.09a	5.54 ± 1.57a	3.27 ± 0.43a	5600 ± 400a
Local check	0.98 ± 0.06bc	0.77 ± 0.42a	1.13 ± 0.38c	21.77 ± 2.02b	14.88 ± 0.76c	5400 ± 305a
Distant check	1.36 ± 0.22c	1.73 ± 0.40b	1.14 ± 0.09c	21.67 ± 2.03b	9.70 ± 4.87c	5467 ± 933a

[a]Means in a column followed by the same letter are not significantly different at $P<0.05$ by Newman–Keuls test, SE = standard error.

Table 6
Mean percentage of damaged tillers and yield in replicated pheromone- and insecticide-treated check plots, 1996 trial

Treatment (g/ha)	Percentage of damaged tillers[a]					Yield (kg/ha)
	35 DAT Mean ± SE	45 DAT Mean ± SE	55 DAT Mean ± SE	75 DAT Mean ± SE	85 DAT Mean ± SE	125 DAT Mean ± SE
18.6	0.28 ± 0.28a	0 ± 0a	0.63 ± 0.15a	16.32 ± 1.56ab	19.18 ± 3.05ab	4800 ± 0c
27.6	0.14 ± 0.14a	0.18 ± 0.11a	0.69 ± 0.36a	9.26 ± 1.93a	22.01 ± 0.93ab	5533 ± 240d
32.2	0 ± 0a	0.31 ± 0.23a	0.35 ± 0.11a	8.88 ± 2.91a	16.09 ± 4.33a	5067 ± 133cd
36.8	0 ± 0a	014 ± 0.14a	0.64 ± 0.02a	11.11 ± 2.92ab	14.70 ± 4.42a	5468 ± 240d
Local check	0.2 ± 0.2 a	0.55 ± 0.41a	0.77 ± 0.05a	22.78 ± 4.63b	21.28 ± 0.92ab	3867 ± 67b
Distant check	0 ± 0a	0.75 ± 0.33a	1.24 ± 0.51a	24.09 ± 3.94b	31.73 ± 3.79b	3267 ± 67a

[a]Means in a column followed by the same letter are not significantly different at $P<0.05$ by Newman–Keuls test, SE = standard error.

release systems are essential to deliver behaviourally relevant aerial concentrations of the bio-active compounds during periods when crops are at risk of infestation. Thus, in order to achieve season-long control of a pest species using pheromones, an understanding of the physicochemical properties of formulations is essential in order to predict efficacy under field conditions. For example, Cork et al. (2001) found that the brinjal fruit and shoot borer, *Leucinodes orbonalis*, was most effectively controlled by mass trapping when the pheromone was released from polyethylene vials rather than rubber septa because of the inherently faster release rates of the pheromone, (*E*)-11-hexadecenyl acetate and the related alcohol, from vials.

Effects of changing molecular structure of volatiles on release from PVC-resin formulations reflected trends observed in related studies with rubber septa (Butler and McDonough, 1981). Thus, increasing aliphatic chain length by two methylene groups resulted in half lives that increased by a factor of approximately 7.5 at 27 °C, which was similar to that observed by McDonough et al. (1989) for rubber septa (7.0 at 25 °C) and reflected differences in heats of evaporation (ΔH) of the compounds. Nevertheless, release rates from the PVC-resin formulations tested were consistently faster than from rubber septa, typically 3.8 times at 27 °C, although half life can be modified by changing surface to volume ratios (Lloyd and Mathysse, 1966; Cork et al., unpublished). Half lives of saturated aliphatic acetates in the PVC-resin formulations tested decreased with increasing temperature but not in a linear relationship as found with rubber septa (McDonough et al., 1989).

In previous work on the PVC-resin formulations (Cork et al., 1989), release rates were measured by the collection of volatiles released (c.f. Weatherston, 1989). The alternative approach of measuring release rates by analysis of residual pheromone at intervals of exposure under laboratory or field conditions does not normally distinguish between loss by degradation and loss by release. However, in the present study, formulations included a chemically stable, saturated acetate of similar ΔH to the test compounds, e.g. unsaturated acetates, aldehydes, and their relative release rates compared. Thus, if there is no degradation the compounds will all be lost at similar rates by release (Hall et al., 1981).

In the absence of protectants, labile aldehydes and unsaturated compounds were rapidly degraded in the plasticized PVC (Sartwell et al., 1980), as indicated by the reduction in half lives compared with that of 14:Ac. However, the addition of a polymer-soluble dye, WB, to formulations was found to provide significant levels of protection of highly labile compounds, such as conjugated diene aldehydes, exposed to full sunlight under tropical conditions. Z9-14:OH was released at half the rate of Z9-14:Ald despite having a similar molecular weight, confirming that release from the plasticized polymer is better predicted by differences in ΔH. Data presented here suggest that there are practical limits to the range of compounds that the PVC-resin formulation can be used for (12- to 16-carbon compounds) and that bioactive volatile compounds can themselves act to influence release at relatively high loadings. The formulation was ideally suited for the release of 14-carbon acetates and 16-carbon aldehydes (Chamberlain et al., 2000; Felland et al., 1995), these having typical field lives of 70–100 days, although PVC-resin formulations have been successfully used with compounds of higher molecular weight (Grassi et al., 2002).

Previous research demonstrated that *S. incertulas* could be controlled by mating disruption using natural and unnatural blends of pheromone (Cork and Basu, 1996; Cork et al., 1996) but relied on relatively large plot sizes (10 ha) and a single dose (40 g a.i./ha/season) with 625 dispensers/ha (4 m spacing) to achieve control (Cork et al., 1998). Nevertheless, questions remained over the optimal application rate and the minimum area needed to achieve control. Mating disruption is considered to be most efficacious when adult populations are low and trials were conducted in isolated plots (Campion et al., 1989; Sanders, 1997). In practice, farmers have no control over either of these parameters and so field trials were conducted to establish optimal application rates under typical farming conditions in plot sizes that more closely resembled those of farmers' holdings in South Asia. Clear dose–response effects were observed in data from both seasons' field trials, suggesting that even in 1 ha plots mating disruption of *S. incertulas* could be achieved. Pheromone application levels of only 10 g a.i./ha/season resulted in larval damage levels that were significantly less than those achieved by farmers using insecticides. However, 30–40 g a.i./ha/season should be recommended to ensure control in seasons of high infestation.

Given the first-order release of bio-active compounds from the formulation, significant savings in pheromone application rates could be achieved by delaying application, because rice is not equally susceptible to stem borer attack at each crop stage (Anonymous, 1989). Area-wide adoption and further refinements in distribution of dispensers and use of additional measures to treat 'hot spots' and edge effects caused by proximity to buildings, roads or canals as achieved with other crop pests (Brown et al., 2000; Il'-ichev et al., 2002) are location specific and can only be achieved once several seasons' data have been accumulated.

Conducting mating disruption trials with different doses of pheromone should provide an insight into the mechanism of action, assuming that above a particular aerial concentration male moths would be incapable of locating potential mates, so that further increases in pheromone concentration would not result in higher levels of disruption. Combining the data from both field trials and plotting percent WH against application rates showed an inverted linear relationship ($y = yo + ax$, x = application rate, y = %WH, yo = intercept, 17.02, a = constant, −0.18) between dose and percentage WH (Tcheslavskaia et al., 2005), which suggested that a 95 g a.i./ha/season dose would result in complete suppression of larval damage, at the pest population levels experienced in these trials.

Farbert et al. (1997) showed that pheromone at behaviourally relevant concentrations could be detected 100 m from treated plots, suggesting that the effective area under mating disruption was significantly larger than that which contained dispensers. In a related study, Sharov et al. (2002) were able to demonstrate an impact of mating disruption on trap catches and, more importantly, mating success of female moths 250 m from plots treated with gypsy moth, *Lymantria dispar*, pheromone. Given that the first generation of *S. incertulas* female moths would emerge into rice that was attractive for oviposition they would not be expected to move far and risk predation after mating. Thus, by increasing the effective area under mating disruption, by increasing dose, the possibility of gravid female moths immigrating into the plots treated with pheromone could be expected to decrease, thereby assuring higher levels of control than could be achieved by controlling insects within treated plots alone.

The trials do not provide evidence to support the supposition that the mechanism of control of reservoir formulations such as the PVC-resin dispensers operated by combining the effects of false-trial-following and sensory fatigue to prevent male moths from locating receptive conspecific female moths (Sanders, 1997). Nevertheless, as only 500–625 dispensers were applied per 1 ha plot, the effectiveness of the technique might suggest that longer-term (primer) effects due to pre-exposure to pheromone during daylight hours when the formulation would have released high amounts of pheromone could well have played a part in influencing mating success (Wyatt, 1997).

Acknowledgements

We would like to acknowledge Mr D.I. Farman, NRI, for chemical analysis of field collected samples, Mr Y. Sambasiva Rao for assistance collecting mating disruption trial data and the smallholders in Medchal, Andhra Pradesh, India, for allowing us to undertake our field experiments on their rice fields. This work was commissioned by Natural Resources Institute as part of the Adaptive Research Initiative Programme (R5747CB) of the UK's Department for International Development (DFID). However, DFID can accept no responsibility for any information provided or views expressed.

References

Anonymous, 1989. IRRI Program Report. International Rice Research Institute, Manila, Philippines, pp. 48–49.

Brown, D.J., Il'-ichev, A.L., Muller, W., Polesny, F., Verheyden, C., Webster, A.D., 2000. The potential for the removal of organophosphate insecticides from stone fruit production in the Goulburn Valley, Australia. Acta Hortic. 525, 85–91.

Butler, L.I., McDonough, L.M., 1981. Insect sex pheromones: evaporation rates of alcohols and acetates from natural rubber septa. J. Chem. Ecol. 7, 627–633.

Campion, D.C., Critchley, B.R., McVeigh, L.J., 1989. Mating disruption. In: Jutsum, A.R., Gordon, R.F.S. (Eds.), Insect Pheromones in Plant Protection. Wiley, Chichester, UK, pp. 89–119.

Chamberlain, D.J., Brown, N.J., Jones, O.T., Casagrande, E., 2000. Field evaluation of a slow release pheromone formulation to control the American bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. Bull. Entomol. Res. 90, 183–190.

Cork, A., Basu, S.K., 1996. Control of the yellow stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae) by mating disruption with a PVC resin formulation of the sex pheromone of *Chilo suppressalis,* in India. Bull. Entomol. Res. 86, 1–9.

Cork, A., Hall, D.R., Mullings, J.L., Jones, O.T., 1989. A new PVC resin formulation for controlled release of insect pheromones. In: Proceedings of the 16th International Symposium on Controlled Release of Bioactive Materials, Chicago, USA, pp. 9–10.

Cork, A., De Souza, K., Krishnaiah, K., Kumar, D.V.S.S.R., Ashok Reddy, A., Casagrande, E., 1996. Control of yellow stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae) by mating disruption on rice in India: effect of unnatural pheromone blends and application time on efficacy. Bull. Entomol. Res. 86, 515–524.

Cork, A., De Souza, K., Krishnaiah, K., Ashok Reddy, A.S., Zainullabuddin, S., 1998. Season-long control of yellow stem borer, *Scirpophaga incertulas* (Lepidoptera: Pyralidae) by mating disruption with the natural ratio of pheromone components. Bull. Entomol. Res. 88, 109–116.

Cork, A., Alam, S.N., Das, A., Das, C.S., Ghosh, G.C., Phythian, S., Farman, D.I., Hall, D.R., Maslen, N.R., Vedham, K., Rouf, F.M.A., Srinivasan, K., 2001. Female sex pheromone of brinjal fruit and shoot borer, *Leucinodes orbonalis* (Lepidoptera: Pyralidae): blend optimization. J. Chem. Ecol. 27, 1867–1877.

Cork, A., Alam, S.N., Rouf, F.M.A., Talekar, N.S., 2003. Female sex pheromone of brinjal fruit and shoot borer, *Leucinodes orbonalis*: trap optimization and application in IPM trials. Bull. Entomol. Res. 93, 107–113.

El-Sayed, A.M., Suckling, D.M., Wearing, C.H., Byers, J.A., 2006. Potential of mass trapping for long term pest management and eradication of invasive species. J. Econ. Entomol. 99, 1550–1564.

Farbert, P., Koch, U.T., Farbert, A., Staten, R.T., Cardé, R.T., 1997. Pheromone concentration measured with electroantennogram in cotton fields treated for mating disruption of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). Environ. Entomol. 26, 1105–1116.

Felland, C.M., Hull, L.A., Barrett, B.A., Knight, A.L., Jenkins, J.W., Kirsch, P.A., Thomson, D.R., 1995. Small plot mating disruption trials for tufted apple bud moth, *Platynota idaeusalis*, in Pennsylvania apple orchards. Entomol. Exp. Appl. 74, 105–114.

Fitzgerald, T.D., St Clair, A.D., Daterman, G.E., Smith, R.G., 1973. Slow release plastic formulation of the cabbage looper pheromone *cis*-7-dodecenyl acetate: release rate and biological activity. Environ. Entomol. 2, 607–610.

Fitzpatrick, S.M., Troubridge, J.T., Maurice, C., 2004. Pheromone released from polyvinyl chloride dispensers disrupts mate-finding and pheromone-source location by *Rhopobota naevana* (Lepidoptera: Tortricidae) in cranberries. Can. Entomol. 136, 91–108.

Grassi, A., Zini, M., Forno, F., Witzgall, P., Mazomenos, B., Konstantopoulou, M., 2002. Mating disruption field trials to control the currant clearwing moth, *Synanthedon tipuliformis* Clerck: a three-year study. Bulletin-OILB/SROP 25, 69–76.

Hall, D.R., Nesbitt, B.F., Marrs, G.J., Green, A.St.J., Campion, D.G., Critchley, B.R., 1981. Development of microencapsulated pheromone formulations. In: Leonhardt, B.A., Beroza, M. (Eds.), Insect Pheromone Technology: Chemistry and Applications. American Chemical Society Symposium Series No. 190, pp. 131–143.

Hendricks, D.E., Shaver, T.N., Goodenough, J.L., 1987. Development and bioassay of molded polyvinyl chloride substrates for dispensing tobacco budworm (Lepidoptera: Noctuidae) sex pheromone bait formulations. Environ. Entomol. 16, 605–613.

Howse, P.E., 2004. Mechanism of the Exosex auto-confusion system of mating disruption. Int. Pest Control 45, 321–322.

Howse, P.E., Stevens, I.D.R., Jones, O.T., 1998. Insect Pheromones and Their Use in Pest Management. Chapman & Hall, London.

Il'-ichev, A.L., Gut, L.J., Williams, D.G., Hossain, M.S., Jerie, P.H., 2002. Area-wide approach for improved control of oriental fruit moth *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) by mating disruption. Gen. Appl. Entomol. 31, 7–15.

Lloyd, J.E., Mathysse, J.G., 1966. Polymer-insecticide systems for use as livestock feed additives. J. Econ. Entomol. 59, 363–367.

McDonough, L.M., Brown, D.F., Aller, W.C., 1989. Insect sex pheromones: effect of temperature on evaporation rate of acetates from rubber septa. J. Chem. Ecol. 15, 779–790.

McDonough, L.M., Aller, W.C., Knight, A.L., 1992. Performance characteristics of a commercial controlled-release dispenser of sex pheromone for control of codling moth (*Cydia pomonella*) by mating disruption. J. Chem. Ecol. 18, 2177–2189.

Sanders, C.J., 1981. Release rates and attraction of PVC lures containing synthetic sex attractant of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Torticidae). Can. Entomol. 113, 103–111.

Sanders, C.J., 1997. Mechanisms of mating disruption in moths. In: Cardé, R.T., Minks, A.K. (Eds.), Insect Pheromone Research: New Directions. Chapman & Hall, London, pp. 333–346.

Sartwell, C., Daterman, G.E., Sower, L.L., Overhulser, D.L., Koerber, T.W., 1980. Mating disruption with synthetic sex attractant controls damage by *Eucosma sonomana* (Lepidoptera: Tortricidae: Oletreutinae) in *Pinus ponderosa* plantations. I. Manually applied polyvinyl chloride formulations. Can. Entomol. 112, 159–162.

Sharov, A.A., Leonard, D., Liebhold, A.M., Clemens, N.S., 2002. Evaluation of preventative treatments in low-density gypsy moth populations using pheromone traps. J. Econ. Entomol. 95, 1205–1215.

Tcheslavskaia, K.S., Thorpe, K.W., Brewster, C.C., Sharov, A.A., Leonard, D.S., Reardon, R.C., Mastro, V.C., Sellers, P., Roberts, E.A., 2005. Optimization of pheromone dosage for gypsy moth mating disruption. Entomol. Exp. Applic. 115, 355–361.

Weatherston, I., 1989. Alternative dispensers for trapping and disruption. In: Jutsum, A.R., Gordon, R.F.S. (Eds.), Insect Pheromones in Plant Protection. Wiley, Chichester, UK, pp. 249–278.

Witzgall, P., Lindblom, T., Bengtsson, M., Tóth, M., 2004. The Pherolist. ⟨www-pherolist.slu.se/pherolist.php⟩.

Wyatt, T.D., 1997. Putting pheromones to work: paths forward for direct control. In: Cardé, R.T., Minks, A.K. (Eds.), Insect Pheromone Research: New Directions. Chapman & Hall, London, pp. 445–459.

Yamanaka, T., 2007. Mating disruption or mass trapping? Numerical simulation analysis of a control strategy for lepidopteran pests. Popul. Ecol. 49, 75–86.