

## Laboratory and field evaluation of floral odours from African marigold, *Tagetes erecta*, and sweet pea, *Lathyrus odoratus*, as kairomones for the cotton bollworm *Helicoverpa armigera*

Toby J. Bruce, Alan Cork, David R. Hall and Ezra Dunkelblum<sup>1</sup>

Natural Resources Institute, Chatham Maritime, ME4 4TB Kent, United Kingdom

<sup>1</sup> Volcani Centre, Bet Dagen, Israel

**Abstract:** Significant increases in upwind flight of *H. armigera* in a wind-tunnel were obtained with air entrained headspace samples of African marigold, *Tagetes erecta*, flowers ( $P=0.014$ ), and sweet pea, *Lathyrus odoratus*, flowers ( $P=0.047$ ). Identification of the compounds contained in the Porapak Q extracts was done by GC-MS. Direct EAG was used to screen for the most electrophysiologically active natural extracts and GC-EAG was used in screening for compounds to test in the wind-tunnel. Two 4-component synthetic kairomonal blends were identified which caused significant increases in upwind flight in the wind-tunnel ( $P<0.001$  and  $P=0.014$  for marigold and sweet pea blends respectively). Funnel traps baited with floral odours were tested for their ability to catch *Helicoverpa armigera* and other insects in the field in Israel. Significantly more *H. armigera*, another noctuid pest, *Autographa gamma*, the honeybee *Apis* spp., a wasp *Halictus* spp. and lacewings (Chrysopidae) were caught in traps baited with synthetic marigold, *Tagetes erecta*, and sweet pea, *Lathyrus odoratus*, floral volatiles than in unbaited control traps. The marigold blend contained benzaldehyde, ( $\pm$ )-linalool, phenylacetaldehyde and (S)-(-)-limonene, and the sweet pea blend (-)-linalool, phenylacetaldehyde, benzyl alcohol and diacetone (4-hydroxy-4-methyl-2-pentanone) in the natural ratio. Although the target specificity and level of attraction obtained with the floral traps was too low for mass trapping, the floral traps could possibly be used for monitoring female *H. armigera* populations. These findings are discussed in relation to the potential use of floral kairomones in integrated control of *H. armigera*.

**Key words:** *Helicoverpa armigera*, kairomone, floral odour, insect-host plant interaction

### Introduction

*Helicoverpa armigera* is a serious pest of cotton, chickpea, pigeonpea, maize and other crops in many parts of the old world tropics and subtropics. Use of larvicides has been the predominant management strategy for several decades (King, 1994; Wilson, 1982). However development of insecticide resistance has caused major control problems. There are also safety and environmental contamination considerations

associated with the large quantities of insecticide often used in cotton (Menn 1991) giving impetus to finding new control methods or improved pest monitoring to allow need based insecticide application. It was within this context that the possibility of using floral compounds for trapping *H. armigera* was tested. Since female insects can be caught with a floral lure in theory a floral baited trap could have more impact on reducing oviposition levels than a pheromone trap which only captures male insects, or when used for monitoring purposes it could give a better reflection of oviposition levels within a crop. However this depends on a reasonably high level of attraction to the floral baited trap occurring under field conditions.

## Materials and methods

*Headspace sampling.* Samples of floral headspace volatiles were collected by air entrainment using freshly cut *Tagetes erecta* and *Lathyrus odoratus* flowers. Four to 55 flowers were placed in clean glass quickfit flasks (typically 500 ml capacity, although flask size was varied with the number of flowers available). Charcoal filtered air was drawn through the flask at 2 l/min for typically eight hours. On exiting the container the air was drawn through a Porapak Q filter (200mg, 60-80 mesh size, Phase Separations, UK). The entrained volatiles were eluted from the Porapak Q filters with 2 ml of dichloromethane and stored at -20°C.

*Experimental insects.* A laboratory strain of *H. armigera* was reared on a semi-synthetic chickpea-based diet. Adult moths were fed a 10% sucrose solution. The culture was maintained at 25°C, with a relative humidity of 50% and 14 : 10 h light-dark regime.

*Electroantennography and GC-EAG analyses.* Electroantennography and GC-EAG were carried out using standard methods as described in Cork *et al.* (1990). Linked GC-EAG analyses of entrained flower volatiles were replicated five times using different insects and carried out on three different Porapak Q extracts. GC retention times of compounds identified were converted into Retention Indices by comparison with the retention times of saturated, straight-chain hydrocarbons, thus *n*-tetradecane = 1400. These analyses are reported in Bruce and Cork (in press). Direct EAG using a 1µg dose on filter paper was used with synthetic compounds to confirm their electrophysiological activity.

*GC-MS analyses.* EAG-active compounds observed in the GC-EAG analyses were subjected to analysis by gas chromatography (Carlo Erba 5160 Mega Series) linked to a mass spectrometer (ITD 700, Finnigan MAT, Hemel Hempstead, U.K.). The EAG-active compounds were identified by comparing the electron impact MS with library spectra (Adams, 1995) and confirmed by comparing the GC retention times and EI-MS with synthetic standards. A cyclodextrin B column (50m x 0.22mm ID) on a Varian 3700 GC was used to identify which enantiomer(s) of chiral compounds were present in the Porapak Q extracts.

*Wind-tunnel bioassay.* A wind-tunnel (225 x 60 x 60 cm, 50 cm/sec airspeed) was used to investigate behavioural responses of female *H. armigera* to natural and synthetic blends of putative kairomone components as described by Bruce & Cork (in press). Bioassays were carried out under reduced lighting (0.8 lux), at 25°C and 50% R.H., during the first 2 - 3 hrs of scotophase which corresponded with the natural time of nectar foraging and oviposition of female *H. armigera* (Roome, 1975). Samples of floral volatiles were applied to Whatman No. 4 filter paper strips in 50 µl aliquots from stock solutions. The filter paper was then immediately clipped to a vertical support and positioned at the centre of the cross-sectional view, height 30cm. Test moths were released individually 200 cm downwind of the odour source. Maximum distance flown upwind and number of approaches to within 20 cm of the odour source were scored during a 12 min period.

*Field trapping experiments.* Compounds used in lures, benzaldehyde, (±)-linalool, phenylacetaldehyde, (S)-(-)-limonene, were purchased from Sigma Israel Chemicals Ltd. 2,6-di-*tert*-butyl-4-methyl-phenol (BHT) was used (10% of the a.i.) as an antioxidant in lures that contained benzaldehyde and phenylacetaldehyde. Dioctyl phthalate (85%) was used to slow the release of (-)-limonene (15%) which had to be formulated in separate double-thickness sachets because of its high volatility. Lures were made by pipetting these compounds into polyethylene sachets cut from a roll of polyethylene tubing (500 gauge, 125\_µm thickness, Transatlantic Plastics, Southampton, UK) and sealed using a 'Futura' electrical heat sealing device (Audion Elektro, Holland). Unitrap' (IPS, S. Wirral, UK) cone traps were baited with floral lures and set up 50 cm above crop height. Distance between traps was 12m. Traps were situated in a chickpea field for the first month (10/5/99-31/5/99) and in a cotton field for the second month (1/6/99-29/6/99). Both fields had a uniform crop. To obtain an independent measure of the *H. armigera* population density during the field experiments, four pheromone traps (Unitraps) and a light trap were also set up. They were located in the same field as the traps baited with floral lures but approx. 60 m away from the nearest floral trap to avoid interference with the main experiment. Female moths caught in the floral traps were dissected and examined for the presence of spermatophores to determine their mated status.

## Results

Electroantennography and GC-EAG analyses (Tables 1, 2)

Wind-tunnel bioassay

Wind-tunnel results are summarised in Table 3. Initially tests were conducted with aliquots of air entrained samples that had elicited EAG responses in GC-EAG analyses. Subsequently, once EAG active compounds had been identified, synthetic

kairomonal blends, using the same ratio and concentration of compounds as in the natural sample were used.

Table 1. Female *H. armigera* EAG responses to Synthetic Compounds (n = 9) and the GC Retention Time Data Used for their Identification from *Tagetes erecta* Samples

Compound (1 µg)	Mean EAG re- sponse (-mV) ± SE	P-value <sup>a</sup>	Retention Index (polar column)		Retention Index (non-polar column)	
			Natural	Synthetic	Natural	Synthetic
(-)-limonene <sup>b</sup>	0.47 ± 0.02	0.012	1200	1200	1017	1019
Benzaldehyde	0.61 ± 0.07	0.001	1522	1522	925	926
(±)-linalool	0.59 ± 0.11	0.032	1539	1541	1085	1088
Phenylacetaldehyde	0.75 ± 0.08	<0.001	1642	1642	1007	1011

<sup>a</sup> Paired *t*-test comparing treated and dichloromethane control means.

Table 2. Female *H. armigera* EAG responses to Synthetic Compounds (n = 9) and the GC Retention Time Data Used for their Identification from *Lathyrus odoratus* Samples

Compound (1 µg)	Mean EAG re- sponse (-mV) ± SE	P-value <sup>a</sup>	Retention Index (polar column)		Retention Index (non-polar column)	
			Natural	Synthetic	Natural	Synthetic
Diacetone	0.48 ± 0.03	<0.001	1366	1369	821	818
(-) -Linalool	0.59 ± 0.11	0.032	1545	1547	1086	1088
Phenylacetaldehyde	0.75 ± 0.08	<0.001	1652	1654	1014	1015
Benzyl Alcohol	0.62 ± 0.03	0.001	1887	1888	1022	1022

<sup>a</sup> Paired *t*-test comparing treated and dichloromethane control means.

### Field trapping experiments

In the first week of trapping floral baits containing the synthetic *T. erecta* and *L. odouratus* blends were compared with each other and a combined blend containing volatiles from both *T. erecta* and *L. odouratus*. As shown in Fig. 1 there was very little difference between the floral baited treatments and combining volatiles from both plant sources did not increase catches of *H. armigera*.

Due to the low *H. armigera* catches per trap per night in floral odour baited traps, different (weekly) experiments including the standard 4-component marigold trap bait (M1) and the unbaited control treatment were treated as different replicates instead of separate experiments. *H. armigera* catches were summed for these two treatments for each weekly experiment and statistical analysis comparing catches was carried out. There was a significant increase in *H. armigera* catches in traps with the standard 4-component marigold lure compared with the unbaited control trap catches over the whole season (Table 4),  $P=0.0023$  (Mann Whitney 'U' test).

Table 3. Wind-tunnel Responses of female *H. armigera* to air-entrained samples and synthetic blends of *T. erecta* and *L. odoratus* flowers in the wind-tunnel

Treatment	Furthest Flown Upwind (cm) ± S.E.	Mean No. of Upwind Ap- proaches	No. of Repli- cates	<i>P</i> -value <sup>a</sup>
Control	98.9 ± 12.0	0.80 ± 0.23	69	
<i>T. erecta</i> extract	135.5 ± 12.7	1.86 ± 0.38	63	0.014
Control	89.4 ± 21.0	0.35 ± 0.21	17	
<i>L. odoratus</i> extract	109.7 ± 26.7	2.59 ± 1.15	17	0.047
Control	77.1 ± 14.0	0.54 ± 0.28	41	
<i>T. erecta</i> synthetic blend <sup>b</sup>	147.6 ± 15.4	4.00 ± 0.96	35	0.0008
Control	67.9 ± 20.8	0.53 ± 0.53	17	
<i>L. odoratus</i> synthetic blend <sup>c</sup>	132.2 ± 18.0	2.94 ± 1.24	18	0.014

<sup>a</sup>Result of Mann Whitney 'U' test comparing treated and control medians for number of upwind approaches

<sup>b</sup> 0.23µg phenylacetaldehyde, 0.5 µg benzaldehyde, 0.73 µg (±)-linalool, 8.6 µg (+)-limonene

<sup>c</sup> 5.9 µg diacetone, 3.3µg (±)-linalool, 3.1µg phenylacetaldehyde, 0.44µg benzyl alcohol

As shown in Table 5 floral odour baited traps were less effective than pheromone baited or light traps. However they had the advantage of catching female *H. armigera* and did not require a power source. Of 48 female *H. armigera* from the floral traps that were examined, 36 contained spermatophores i.e. 25% were unmated. Floral baited traps had little selectivity and other flower visiting insects were also captured. As well *H. armigera*, significantly more *Autographa gamma*, *Apis mellifera*, *Halictus* spp. and *Chrysopa carnea* were caught in traps baited with synthetic marigold, *T. erecta*, and sweet pea, *L. odoratus*, floral volatiles than in unbaited control traps. A large proportion of the insects caught were beneficial pollinators (*Apis*, *Halictus*).

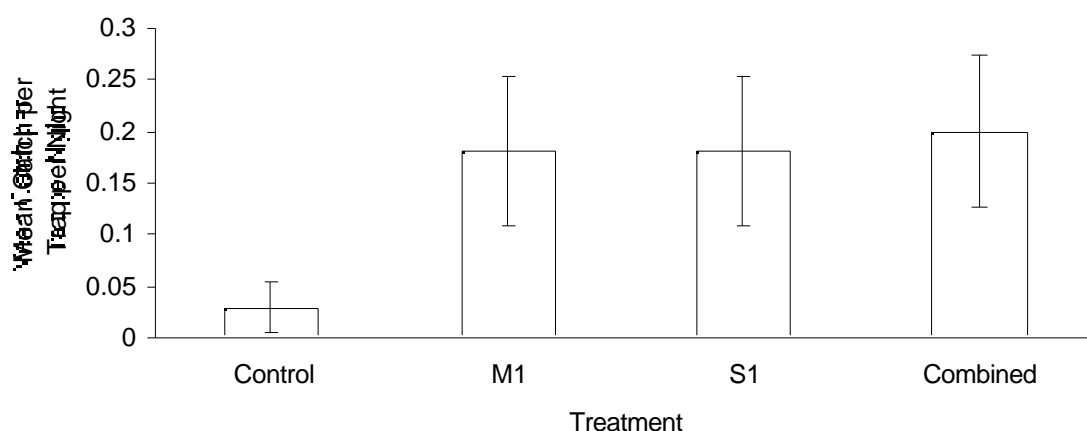


Figure 1. *H. armigera* catch during the first week of trapping. M1 = *T. erecta* blend: 1.5 x 5cm sachet containing 0.5ml of a 40:1:1 mixture of phenylacetaldehyde, benzaldehyde and ( $\pm$ )-linalool +10%BHT; 2.5 x 2.5cm sachet containing 0.5ml of (-)-limonene in dioctyl phthalate (15% (-)-limonene) inside a 4 x 5cm outer sachet. S1 = *L. odouratus* blend: 1.5 x 5cm sachet containing 0.5ml of a 1:1:1:1 mixture of diacetone, benzaldehyde, (-)-linalool and benzyl alcohol +10%BHT. Combined = 1.5 x 5cm sachet containing 0.5ml of a 1:1:1:1 mixture of benzaldehyde, benzyl alcohol, diacetone, ( $\pm$ )-linalool and phenylacetaldehyde +10%BHT and the (-)-limonene sachet as in M1.

Table 4. Mean *H. armigera* Catch in Standard Marigold Baited Traps and Unbaited Control Traps in Israel Trial (both sexes, per trap, per night)

Week	Unbaited Control	M1 <sup>a</sup>
1	0.025	0.175
2	0.000	0.208
3	0.000	0.028
4	0.000	0.056
5	0.000	0.111
6	0.000	0.111
Mean	0.004	0.115

<sup>a</sup> lure as in legend of Fig. 1

Floral baited traps had little selectivity and other flower visiting insects were also captured. As well *H. armigera*, significantly more *Autographa gamma*, *Apis mellif-*

era, *Halictus* spp. and *Chrysopa carnea* were caught in traps baited with synthetic marigold, *T. erecta*, and sweet pea, *L. odoratus*, floral volatiles than in unbaited control traps. A large proportion of the insects caught were beneficial pollinators (*Apis*, *Halictus*).

Table 5. Mean Catches in Different Types of Traps over the Whole Season

Trap Type	Mean No. <i>H. armigera</i> per trap per night
Unbaited Control	0.0004
Floral Odour Baited	0.115
Light Trap	1.35
Sex Pheromone	8.80

## Conclusions

Experimental evidence for a role of olfaction in host-plant selection behaviour by *H. armigera* has been obtained in the current study. Natural selection would be expected to favour searching mechanisms in which a preliminary assessment of the host-plant could be made prior to alighting because this would mean that time and risk could be concentrated on plants more likely to provide an oviposition site (Damman & Feeny, 1988). It seems probable that there is a sequence of cues leading to acceptance of a host-plant and that olfaction is more important in the earlier stages prior to contact with the plant (Miller & Strickler, 1984, Hsiao; 1985). Host-plant odours stimulate searching behaviour and make foraging moths such as *H. armigera* more responsive to other cues associated with host-plants (Brantjes, 1978; Hurtel & Thiéry, 1988; Bell, 1990; Bernays & Chapman, 1994).

For mass trapping purposes the floral lure used in the field trials reported here has too low a trap performance in terms of moth capture per night (on average 0.11 per night with the standard marigold blend in Israel) and was insufficiently selective. With a low level of attraction into the trap there is too much of a risk that a mated female moth could lay eggs in the crop before being attracted into the trap. One female can lay in excess of 1000 eggs (Fitt, 1989). Also the number of traps required per hectare would need to be higher and this might be uneconomic. A large number of traps could interfere with other cultivation practices where a tractor requires access to the crop.

Monitoring requires the trap catch to give a good indication of potential threat to a crop. Numbers caught per trap per night need not be high so long as they correlate well with the likelihood of crop damage by the larvae developing from the eggs of the female moths. Since a floral trap can catch female insects on which oviposition

depends it is possible that they could be useful for monitoring purposes. Maini & Burgio (1999) found a good correlation between female European corn borer, *Ostrinia nubilalis*, captures in phenylacetaldehyde baited traps and ensuing crop damage. Another important consideration for monitoring is the sensitivity of the trap to early infestation. If the trap does not catch moths until there has already been considerable oviposition in the crop the warning given could be too late for applying a pesticide spray. The economic threshold can be exceeded by fewer than 5 females per acre (Lingren *et al.*, 1982). Large larvae are a more difficult spray target than small larvae because they require a higher dose of active ingredient to be killed (Lingren *et al.*, 1982). This means that monitoring should give as much warning as possible so that arrangements for an early insecticide application can be made. Because *H. armigera* is so mobile any improvements in monitoring its populations by keeping track of populations of adult female moths in an area could lead to better informed pest management decisions about pesticide applications. Catches of female moths might give a better reflection of egg-laying in the local crop.

### Acknowledgements

The authors thank Dr K Srinivasan, Nagarjuna Agricultural Research and Development Institute, India, for suggesting use of African marigold and provision of seed material, Daniels Ltd. for a sample of synthetic piperitone, and ICRISAT, Patancheru, India for supplying the pupae used to start the *H. armigera* culture. We thank Channan Black for help with looking after sweet pea and marigold plants. The research was funded by a Natural Resources Institute Research Studentship.

### References

- Adams, R.P. (1995) Identification of Essential Oil Components by GC-MS. Allured Publishing Corporation.
- Bell, W.J. (1990) Searching behaviour patterns in insects. *Ann Rev. Entom.* **35**: 447-467
- Bernays, E.A. & Chapman, R.F. (1994) "Host-Plant Selection by Phytophagous Insects. Contemporary Topics in Entomology 2." Chapman & Hall, New York.
- Brantjes, N.B.M. (1978) Sensory responses to flowers in night-flying moths. *In* "Pollination of Flowers by Insects" Ed. AJ Richards (1978) Academic Press.
- Bruce, T.J. & Cork, A. (in press with Journal of Chemical Ecology) Electrophysiological and behavioral responses of female *Helicoverpa armigera* (Lepidoptera, Noctuidae) to compounds identified in flowers of African marigold, *Tagetes erecta*.
- Cork, A., Beevor, P.S., Gough, A.J.E. & Hall, D.R. (1990) Gas chromatography linked to electroantennography: a versatile technique for identifying insect semiochemicals. *In* "Chromatography and Isolation Chromatography and Isolation of Insect Hormones and Pheromones". Eds. A.R. McCaffery and I.D. Wilson. Plenum Press, New York and London.
- Damman, H. & Feeny, P. (1988) Mechanisms and consequences of selective oviposition by the

- zebra swallowtail butterfly. *Animal Behaviour* **36**: 563-573
- Fitt, G.P. (1989) The Ecology of *Heliothis* species in relation to agroecosystems. *Ann. Rev. Entomol.* **34**: 17-52
- Hsiao, T.H. (1985) Feeding Behaviour. In "Comprehensive Insect Physiology and Biochemistry. Vol. 9 Behaviour." Ed. G.A. Kerkut & L.I. Gilbert. Pergamon Press.
- Hurtrel, B. & Thiery, D. (1999) Modulation of flight activity in *Lobesia botrana* Den. & Schiff. (Lepidoptera: Tortricidae) females studied in a wind tunnel. *J. Insect Behav.* **12**: 199-211
- King, A.B.S. (1994) *Heliothis/Helicoverpa* (Lepidoptera: Noctuidae). In "Insect Pests of Cotton." Ed. G.A. Mathews & J.P. Tunstall. CABI International, UK
- Lingren, P.D., Sparks, A.N. & Raulston, J.R. (1982) The potential contribution of moth behavior research to *Heliothis* management. In "Proceedings Int. Workshop *Heliothis* Management, Patancheru, India, 1981. ICRISAT" Ed. Reed, W & Kumble, V. ICRISAT
- Maini, S. & Burgio, G. (1999) *Ostrinia nubilalis* (Hb.) (Lep., Pyralidae) on sweet corn: relationship between adults caught in multibaited traps and ear damages. *J. Appl. Entom.* **123**: 179-185
- Menn, J.J. (1991) Prospects and status for development of novel chemicals for IPM in cotton. *Crop Prot.* **10**: 347-353
- Miller, J.R. & Strickler, K.L. (1984) Finding and accepting host plants. In "Chemical Ecology of Insects" Ed. W.J. Bell & R.T. Cardé. Chapman & Hall.
- Roome, R.E. (1975) Activity of adult *Heliothis armigera* (Hb) (Lepidoptera, Noctuidae) with reference to flowering of sorghum and maize in Botswana. *Bull. Ent. Res.* **65**: 523-530
- Wilson, A.G.L. (1982) Past and future *Heliothis* management in Australia. In "Proceedings Int. Workshop *Heliothis* Management, Patancheru, India, 1981. ICRISAT" Ed. Reed, W & Kumble, V. ICRISAT