EFFECT OF WIND DIRECTION AND HANGING LEVEL OF ATTRACTIVE SEPTA PHEROMONE CAPSULES ON BLACK CUTWORM, *AGROTIS IPSILON.*

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(Manuscript received 14 August 2005)

Abstract

The present work was carried out to evaluate the residues and catches of Black cutworm pheromones (Attractive septa) of *Agrotis ipsilon*. The pheromone capsules were hanged in three directions of the cotton experimental field (North, South and West) and at three different levels of the cotton plant stems (lower, medium and upper). Gas-liquid chromatographic analysis indicates that the total levels of the pheromone three isomers Z-7 dodecanyl-1-acetate (12 acetate), Z-9 tetradecanyl-1-acetate (14 acetate) and Z-11 hexadecanyl-1-acetate (16acetate) at a ratio of 1:1:2 (w/w/w) decreased much faster after 9, 15, 21 and 27 days from the commencement of the experiment. The loss percentages of the pheromone isomers (87.71 %) were much higher after 27 days from application at the upper hanging level compared with lower and medium levels (69.40 % and 78.14 %, respectively). The maximum loss percentages of pheromone isomers (81.29 %) were found after 27 days from treatment at North direction compared with the unhanging control. The catches of black cutworm males in the North direction were higher than that of the other directions.

INTRODUCTION

Pheromones are specific, non-toxic substances and play a good role in managing the insect populations by reduction of mated females through trapping of male moths (Tamaki, 1980). The sex pheromone components of most lepidopterous species are similar in structure (Tamaki, 1977). They have a straight chain of 10-18 carbon atoms with one or more double bonds in the chain and an oxygen- bearing functional group at one end (typically acetate, alcohol, or aldehyde). Hill *et al.* (1979) identified two sex pheromone components (Z)-7-dodecenyl acetate and (Z)-9-tetradecenyl acetate of the females Black cutworm moth *Agrotis ipsilon* which are

emitted in an approximate ratio of 5:1 and these components were effective in attract *Agrotis ipsilon* males.

Berg, *et al.* (2005) studied the mode of action of pheromone on *Helicoverpa assulta.* They found that, the macroglomerular complex in the primary olfactory center of male moths receives information from numerous pheromone-detecting receptor neurons housed in specific sensilla located on the antennae. Liu and Meng (2003) characterized the female sex pheromones of yellow tortrix, *Acleris fimbriana* Merick (Lepidoptera: Tortricidae) as (E)-11,13-tetradecadienal (E11, 13-14:Ald), (E)-11,13-tetradecadienyl acetate (E11, 13-14:Ac) and (E)-11-tetradecenyl acetate (E11-14: Ac). Trapping effect of synthetic chemicals E11, 13-14:Ald, alone and in combination with E11, 13-14:Ac or/and E11-14: Ac to *A. fimbriana* males was tested in Beijing Xishan Orchard. E11, 13-14:Ald on its own was much attractive to *A. fimbriana* males. Neither E11, 13-14:Ac nor E11-14: Ac alone caught any moths. The catches markedly increased by adding E11, 13-14:Ac to E11, 13-14:Ald and the optimum ratio of E11, 13-14:Ald and E11, 13-14:Ac was 6:4 to 5:5.

The discovery and synthesis of sex pheromone of Black cutworm has renewed the possibility of utilizing it in the insect management programs. The effectiveness of a pheromone in the field application depends, among other factors on its purity and chemical stability (Badr-El-Dein, 1999). The most problematic chemical transformations of insect pheromone are oxidation, hydrolysis and double bond ratio and stereoisomerization (Hoda, 1989). These reactions can be thermal and /or photochemical, either catalyzed or uncatalyzed. Yushima (1984) identified Z-11 hexadecanyl-1-acetate (16-acetate) and its activity was evaluated in the field for three years in two different locations. Their results indicate that the best ratio of the three isomers, i.e., Z-7 dodecanyl-1-acetate (12-acetate): Z-9 tetradecanyl-1-acetate (14-acetate): Z-11 hexadecanyl-1-acetate (16-acetate) was 1:1:2 (w/w/w).

In the present study, attractive septa contain three isomers of pheromone, Z-7 dodecanyl-1-acetate (12-acetate), Z-9 tetradecanyl-1-acetate (14-acetate) and Z-11 hexadecanyl-1-acetate (16-acetate) at a ratio 1:1:2(w/w/w) was used to attract to black cutworm males, *Agrotis ipsilon.* This insect is widely distributed in cotton growing areas of the world, causing both qualitative and quantitative losses. Gas-liquid chromatographic technique was conducted to evaluate the residual black cutworm sex pheromones in the cotton field. In addition the effect of wind direction and the level of attractive septa hanging on the cotton plant stems during 2003 season at Bani- Sewif governorate, were studied.

MATERIALS AND METHODS

1. **Black cutworm pheromone.** The test sex pheromone formulation (attractive septa) of Agrotis ipsilon was a mixture of the three isomers, i.e., Z-7 dodecanyl-1-acetate (12-acetate), Z-9 tetradecanyl-1-acetate (14-acetate) and Z-11 hexadecanyl-1-acetate (16-acetate), at a ratio of 1:1:2(w/w/w), was purchased from Sigma Chemical Co. (St. Louis. USA).
2. **Antioxidant.** The antioxidant 2,6-ditert-butyl-4-methyl phenol (butylated hydroxytoluene, BHT) was purchased from Sigma Chemical Co. (St. Louis. USA). BHT was added to stabilize the pheromone isomers at a ratio of 1:1(w/w).
3. **Preparation of lures.** An aliquot from pheromone solution (0.1 ml) was added into a rubber septum and the solvent was allowed to evaporate at room temperature. The obtained lures were packaged in an aluminum foil and stored in a deep freezer (-20°C) until use.
4. **Field Experiment.** An experiment was carried out during 2003 season in cotton field (2 feddans) at Bani- Sewif governorate cultivated with Giza 75 variety. The cotton plants received the normal agricultural practices and were divided into three directions (North, South and west) depending on the wind direction. Water traps were used which consisted of circular tray (34-cm diam.) made of galvanized metal covered with a flat top and partially filled with water containing a small quantity of soap (as a killing agent). A pheromone dispenser was hanged in the surface center of the trap top approximately 5-cm above the surface of the water. The pheromone application started when the cotton plants had an average of 5 fruiting branches per plant in the field as recommended by the Ministry of Agriculture. The recommended dose was 2 mg/capsule. The spacing between capsules was arranged to hang at different levels on the cotton plant stems: low (30 cm), medium (60 cm) and upper (100 cm). The capsules were hanged around the main stem and secured with a double twist according to the method of (Flint *et al.*1990).
5. **Sampling.** Three replicates were performed per each treatment. Each replicate was obtained from one geographic direction, which represents hanging level of pheromone on cotton plants (lower, medium and upper). The samples were also collected at different intervals after at 0, 3, 9, 15, 21 and 27 days from application. Two capsules were collected for analysis from different traps (or directions). After sampling, the capsules of pheromones were kept in closed glass tubes and stored in a deep freezer (-20°C) until analysis.
6. **Determination of pheromone isomers.** The method of Butler and McDonough (1981) was used for the qualitative and quantitative determination of Black cutworm pheromone isomers. The remaining black cutworm pheromone in each capsule was mixed with 10 ml of internal standard (ISTD) solution (Ac-15, 0.2%).

An aliquot of this solution (1ul) was injected onto Hewlett Packard series 5890 (GLC) equipped with flame ionization detector (FID) and capillary column HP-25M (carbowax 25M x 0.32mm x 0.3um film thickness). The oven initial temperature was 150°C fixed for 2 min then increased to 200°C at the rate of 10 °C/min and fixed for 3 min. The split injection and FID temperatures were 250°C and 270°C, respectively. Gases flow rates of nitrogen as carrier gas, hydrogen and air were 1.8 ml / min, 40 ml / min and 350 ml / min, respectively. The response factor was assigned by dividing the peak area of the total pheromone isomers over the peak area of the ISTD. The amount of pheromone in the lure was calculated as follows:

$$\frac{\text{Total pheromone peak area}}{\text{Ac-15 peak area (ISTD)}} \times \text{response factor}$$

7. Statistical analysis. The least significant difference (LSD) test was applied to compare treatment differences according to Snedecor and Citron, (1973).

RESULTS AND DISCUSSION

1-Effect of hanging level. Results in Table (1) and Figure (1) indicate that the levels of black cutworm pheromone isomers were almost stable within three days from the start of the experiment, followed by a rapid decrease in the upper hanging level. The loss percentages of pheromone were significantly different throughout the whole experiment at upper hanging level and were 75.42%, 80.45 % and 84.63% after 9, 15, and 21 days from the treatment, respectively. The upper level had the highest loss of pheromone being 87.71 % after 27 days from application. At lower and medium hanging levels, the data show slight losses of the pheromone levels however, there was significant different in the pheromone concentration induced within the first three days of the experiment. After 9, 15, 21 and 27 days from treatment the loss of pheromone levels were significant different at the low and medium hanging levels (34.42, 49.18, 58.46 and 69.40 % at low and 37.70, 49.73, 63.93 and 78.14 % at medium level, respectively) compared with the relative concentration of the beginning of experiment.

Table (2) and Figure (2) indicate the catch numbers of *Agrotis ipsilon* males at different hanging levels (upper, medium and lower). In general, extending the experimental period irrespective of hanging level significantly increased the number of black cutworm male catches. In the mean time, the hanging level of pheromones had profound effects on the number of black cutworm male catches. The number of catches at the various hanging levels was in the decreasing order: upper > medium > lower. It is worth noting that the number of black cutworm male catches at the upper level was about 1.33 and 1.85 times as high as in medium and lower levels,

respectively, at the end of the experiment. These results are in consistent with the residual pheromone level where the higher pheromone evaporation rate was occurred at the upper hanging level compared with other hanging levels.

Ortiz *et al.* (2004) investigated the potential for pheromone-based mating disruption of the Olive Pyramid Moth (OPM) *Euzophera pinguis* in olive groves during the second flight period in small-plot trials. The female of this species emits a blend of (9Z, 12E)-tetradecadien-1-ol and (9Z, 12E)-tetradecadienyl acetate, which were synthesized for field tests. Mean catches of *E. pinguis* males in pheromone traps were greatly reduced (> 95%) in pheromone-treated plots relative to similar traps placed in control plots. In addition, significant reductions were recorded (35-40%) in the ovipositor and infestation levels during pheromone treatment. The total amount of pheromone blend released from disruption dispensers during the field trials was estimated to average 5.4 mg/ha/day, over 56 days.

2-Effect of wind direction. The effect of wind direction on the evaporation of the pheromone isomers is shown in Table (3) and Figure (3). The values illustrate the average pheromone concentration remained in the capsules at a certain period of time. Each average concentration also indicates the mean value of three replicates for each hanging direction (North, South and West). The results indicate that all the three directions induced loss of pheromone levels and the evaporation rate of pheromone isomers was the highest in the North direction compared with the other directions along the experimental period. After 3 days from the beginning of the application there was significant different losses of pheromone isomer concentration at all three directions and were 24.56 %, 11.17 % and 15.22% for the North, South and West directions, respectively, compared with the unhanging control. The loss percentages of pheromone isomers were highly significant different at all wind directions and were 48.37 %, 63.04 %, 71.74 %, 81.29 %, 50.83 %, 56.98 %, 64.80 %, 75.98% and 47.95 %, 58.48 %, 69.00%, 74.45% at North, South and West directions, after 9, 15, 21 and 27 days from application, respectively.

The data herein are in agreement with Adams *et al.* (1995) who studied the relationship between the wind directions and pheromone dispersion. In addition, the numbers of moths captured were reduced at the intermediate temperature (22°C) only on windy nights.

Table (4) and Figure (4) show the relationship between the catching of *Agrotis ipsilon* males and the different hanging pheromone directions (North, South and West). There was a general trend, i.e., the number of black cutworm males increased in all directions over time. The number of catches was also dependent upon the direction of cotton plants towards the wind. The highest black cutworm male catches

induced at the North direction followed by the West and South directions. The number of black cutworm male catches at the North direction was about 1.21 and 1.23 times as high as that found in the West and South directions, at the end of the experiment. It seems that the highest catches of males were related the highest decrease of pheromone release at North direction.

Sukovata *et al.* (2004) tested attraction and kill technology for management of European pine shoot moth *Rhyacionia buoliana* in 4-6-yr-old Scots pine, *Pinus sylvestris,* plantations managed by two formulations based on ricinoleic acid and hydrocarbon fraction (petroleum jelly) in combination with (E)-9-dodecenyl acetate. Trap catches occurred in plots treated with three attracticide formulations, i.e., Rhykil-1 (with Tinuvin UV absorber), Rhykil-2 (with a new UV absorber, 3,3'-dihydroxy-2, 2'-bipyridyl), and Rhykil-3 (without the insecticide) at 3,000 droplets per hectare Significantly reduced the catches in comparison with those in control plots, suggesting that all formulations were highly effective. From the data under study, it can be recommended that the pheromone capsules have to be placed at upper level of the plant and at wind direction.

Table 1. Average concentrations (mg/capsule) of Black cutworm pheromone residues at various intervals after hanging at different levels on the cotton plant

Time after treatment (day)	Hanging level					
	Lower		Medium		Upper	
	Mean ± SE	Loss (%)	Mean ± SE	Loss (%)	Mean ± SE	Loss (%)
0	1.83±0.02	0.00	1.83±0.01	0.00	1.79±0.01	0.00
3	1.62±0.02	11.47	1.62±0.02	10.55	1.27±0.02	29.16
9	1.20±0.02	34.42	1.14±0.01	37.70	0.44±0.03	75.42
15	0.93±0.01	49.18	0.92±0.02	49.73	0.35±0.02	80.45
21	0.76±0.02	58.46	0.66±0.02	63.93	0.28±0.02	84.36
27	0.56±0.02	69.40	0.40±0.01	78.14	0.22±0.01	87.71

Data are expressed as means ± SE (n =3).
Significant different from the respective values in the beginning of the experiment p*< 0.05.

Table 2. Numbers of males of Black cutworm catched by pheromone after hanging at different levels on the cotton plant stems.

Time after treatment (day)	Hanging level		
	Lower	Medium	Upper
0	0.00a	0.00a	0.00a
3	11.66 ±1.20	11.66 ±1.45	17.00±1.73
9	15.66 ±1.76	22.33 ±2.03	35.00 ±2.31
15	20.33 ±1.45	26.33 ±1.76	37.66 ±2.03
21	24.66 ±2.02	35.00 ±1.73	42.66 ±1.76
27	27.00 ±1.73	37.33 ±1.45	50.00 ±1.73

Data are expressed as means ± SE (n =3).
Significant different from the respective values in the beginning of the experiment p*< 0.05.

Table 3. Average concentrations (mg/capsule) of Black cutworm pheromone residues at various intervals after hanging at different directions on the cotton plants.

Time after treatment (day)	Direction of hanging					
	North		South		West	
	Mean ±SE	Loss (%)	Mean ± SE	Loss (%)	Mean ±SE	Loss (%)
0	1.71±0.06	0.00	1.79±0.02	0.00	1.84±0.02	0.00
3	1.29±0.02	24.56	1.59±0.03	11.17	1.56±0.02	15.22
9	0.89±0.03	48.37	0.88±0.02	50.83	0.95±0.02	47.95
15	0.71±0.01	63.04	0.77±0.02	56.98	0.68±0.01	58.48
21	0.53±0.01	71.74	0.63±0.01	56.80	0.52±0.02	69.00
27	0.32±0.02	81.29	0.43±0.01	75.98	0.47±0.02	74.45

Data are expressed as means ± SE (n =3).

Significant different from the respective values in the beginning of the experiment p*< 0.05.

Table 4. Numbers of males of Black cutworm pheromone after hanging at different directions on the cotton plants.

Time after treatment (day)	Hanging direction		
	North	South	West
0	0.00a	0.00a	0.00a
3	20.70 ±1.20	10.70 ±1.76	14.70 ±1.45
9	34.70 ±2.03	25.00 ±1.73	27.00 ±2.31
15	40.00±2.31	27.70± 2.03	32.33 ±1.76
21	45.30 ±1.45	35.00 ±1.73	38.30 ±2.03
27	51.00 ±2.08	40.70 ±1.76	42.00 ±1.73

Data are expressed as means ± SE (n =3).

Significant different from the respective values in the beginning of the experiment p*< 0.05.

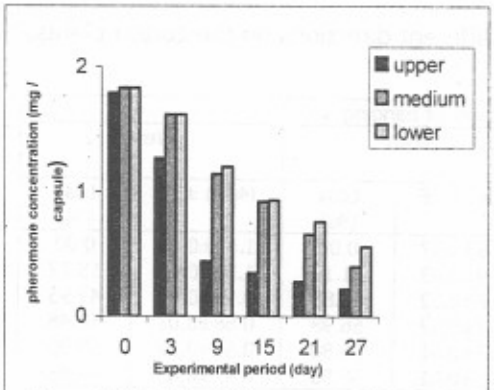


Figure (1) Residual pheromone against hanging level on the stems of cotton plants.

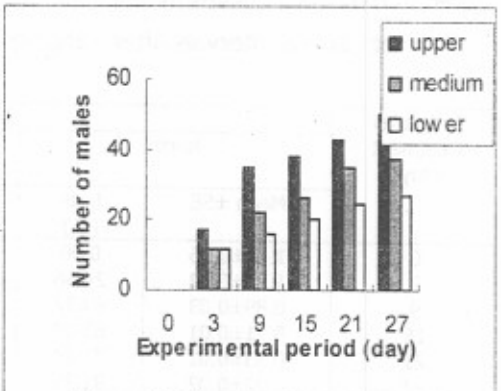
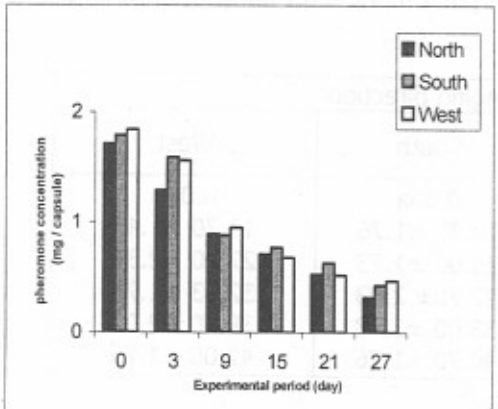


Figure (2). Number of black cutworm male catches at different hanging levels.



Figure(3) Residual pheromone concentrations against wind direction.

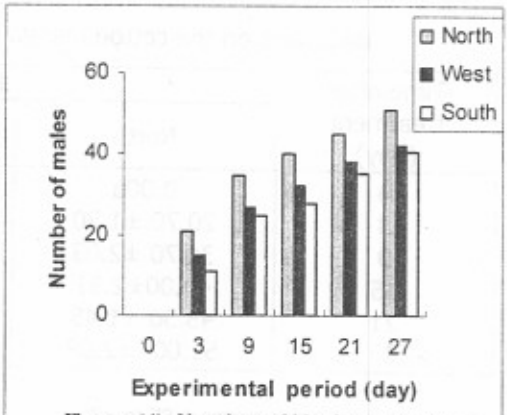


Figure (4). Number of black cutworm male catches at different hanging directions.

REFERENCES

1. Adams, C.J., C. A. Beasley, and T. J. Henneberry, 1995.Effect of temperature and wind speed on pink bollworm (Lepidoptera: Gelochiidae) moth capture during spring emergence. J. Econ. Entomol. *88* (5): 1263-70.
2. Badr-El-Dein, A. M. 1999.Persistence and residue analysis of non-traditional pesticides. M.Sc. Thesis, Faculty of Agriculture, Cairo University.
3. Berg, B. G., T. J. Almaas, J. G., Bjaalie, and H. Mustaparta, 2005. Projections of male-specific receptor neurons in the antennal lobe of the Oriental tobacco budworm moth, *Helicoverpa assulta*: a unique glomerular organization among related species. J. Comp. Neurol. *486* (3): 209-20.
4. Butler, L. I. and L. M. McDonough, 1981. Insect sex pheromone: Evaporation rates of alcohol and acetate from natural rubber septa. J.Chem. Ecol. *7:* 627-633.
5. Hill, A. S., R. W. Rings, S. R. Swier and W. L. Roelofe, 1979. Sex pheromone of the Black cutworm moth, *Agrotis ipsilon. Ipid.5*: 439- 452.
6. Hoda, A. Mohamed 1989.Studies on sex pheromone of Black cutworm, *Agrotis ipsilon* (Hufnagel). With description of related sense organs. M.Sc.Thesis, Faculty of Agriculture, Cairo University.
7. Flint, H. M., A. K. Yamamto, N. J. Parks, and A. K. Nyomura, 1990. Aerial concentration of gossipy lure, the sex pheromone of pink bollworm (Lepidoptera : Gelochiidae), in cotton filed treated with long lasting dispensers. Environ. Entomology, *19* : 1845-1851.
8. Liu, Y. and X. Meng. 2003. Trapping effect of synthetic sex pheromone of *Acleris fimbriana* (Lepidoptera: Tortricidae) in Chinese northern orchard. Naturforsch [C]. *58* (5-6): 421-5.
9. Ortiz, A., A. Quesada and A.Sanchez. 2004. Potential for use of synthetic sex pheromone for mating disruption of the olive pyramid moth, *Euzophera pinguis*. : J Chem. Ecol., *30*(5): 991-1000.
10. Snedecor, C.W. and W.C. Citron, (1973). Statistical methods, 6th edn. Ames, I. A.: Iowa State University Press.
11. Sukovata, L., A. Kolk, and M.Cieslak, 2004. Effect of attract and kill formulations and application rates on trap catches of European pine shoot moth (Lepidoptera: Tortricidae) and shoot damage in Scots pine saplings. J Econ Entomol., *97*(5):1619-23.

12.Tamaki, Y. 1977. Complexity diversity and specify of behavior modifying chemicals in Lepidoptera and dipterans. In chemical control of insect behavior (ed. Shorey, H.H. and Mckelvey,J.J.). P.253-285, Academic Press, New York.

13.Tamaki, Y. 1980. Insect sex pheromones and pest management: recent advances in Japan. Jap. Pest. Inform. 37: 22-25.

14.Yushima 1984. Sex pheromone as a powerful tool for forecasting outbreak of insect pests. Ibid. 45:12-17.

تأثير اتجاه الريح و مستوى التعليق على معدل جذب فرمون الدودة القارضة السوداء

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أجريت تجربة حقلية في عام ٢٠٠٣ بحقل قطن في بني سويف بتعليق مصايد مائية بها كبسولات فرمون الدودة القارضة السوداء في ثلاث اتجاهات مختلفة من الحقل (الشمال، الجنوب ، الغرب) مع اتجاه الرياح كما علقت على ثلاث مستويات من ساق النبات (الأعلى ، المتوسط ، المنخفض) لتوضيح معدل إطلاق الفرمون من الكبسولات مع عد الذكور المصطادة للدودة القارضة السوداء.

أوضحت نتائج التحليل على جهاز الغاز الكروماتوجراف ما يلي:

يتكون فرمون الدودة القارضة السوداء من ثلاث مشابهات و قد انخفض تركيز الفرمون سريعا بعد ٩ ،١٥ ،٢١ ،٢٧ أيام من التجربة حتى سجلت أعلى نسبة فقد للمشابهات كانت ٨٧،٧١% بعد ٢٧يوم من المعاملة عند المستوى الأعلى للتعليق مقارنة بالمستوي المتوسط والمنخفض كانت ٧٨،١٤% و٦٩،٤٠% على التوالي . اتضح أيضا أن أعلى نسبة فقد للمشابهات كانت ٨١،٢٩ % بعد ٢٧يوم من التجربة في اتجاه الشمال مع اتجاه الرياح مقارنة بالكنترول غير المعلق . كما أن الاصطياد الأعلى من الذكور كانت في اتجاه الشمال للتعليق (٥١ ذكر) عن الاتجاهات الأخرى .

من هذه النتائج يمكن التوصية بتعليق كبسولات فرمون الدودة القارضة السوداء بالمصايد المائية المستوي الأعلى و أيضا مع اتجاه الرياح لتعطي اعلي جذب للذكور.