Tropical Agricultural Research Vol. 15: 177- 187 (2003)

Host Plant Influences on the Reproductive Behaviour of Tomato Fruitworm, *Helicoverpa armigera* Hubner (Lepidoptera : Noctuidae)

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ABSTRACT. *Laboratory tests proved that tomato plant influences mating of Helicoverpa armigera Hubner either, in the behaviour sequence or in percentage of final mating. When the tomato plants and female moths of H. armigera were placed together, the attraction of male moths was higher at 6, 7 and 8 h after scotophase on the second day. The influence was highly pronounced when the females were placed in front of tomato foliage rather than placing in parallel. When the microwave assisted hexane extract of tomato foliage was combined with newly emerged female moths, the response of male moths was higher, even on the first day itself. The mating success in terms of egg hatchability was high in female moths caged with tomato plants when compared to that in moths caged without tomato plants. It was demonstrated that female H. armigera moths were more attractive to unmated males in the presence of tomato plants. However, it was not established whether such increased attractiveness was due to the stimulation of releasing of sex pheromones by host plant chemicals or due to an additive or synergistic effect of the combination of sex pheromone and host odour on attraction of males. There is a possibility of an additive or synergistic effect of tomato odour on male response to female pheromone because the air-stream carried first over tomato foliage and then over females was not more attractive than females confined along with tomato foliage.*

INTRODUCTION

The reproductive success of an insect among other things depends on maturation rate, fecundity and mating success. Larval nutrition decides the maturation rate and fecundity whereas adult nutrition decides the adult longevity and fecundity. As mating is energetically expensive and risky, investment in mating could be related to the suitability of larval hosts. Thus, females may synchronize reproductive activities through host plants, in part via plant stimuli acting on sex pheromone levels. Females may delay sexual attraction of males, courtship and mating until suitable hosts are available (Raina *et al.*, 1992; Landolt and Phillips, 1997). When stimulated by host plant odour, females release from their corpora cardiaca a neuropeptide, which stimulates the production of pheromone (Raina, 1993). Increased pheromone production in the presence of host plants in insects like *H. zea* and *Homeosoma electellum* were reported by McNeil and Delisle (1989) and Raina *et al.* (1992). Hormonal regulation of sex pheromone production in the female corn earworm is controlled by signals from the host plant. Female corn earworm moths delayed pheromone biosynthesis and pheromonal signaling in the absence of host plant material

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and released greater amounts of pheromone in the presence of corn silk. Raina (1988) demonstrated stimulation of pheromone synthesis and calling in female *Helicoverpa phloxiphaga* Grote and Robinson by the presence of a host plant, *Castilleja indivisa* Engelm. Analogous observations were also made on *Yponomeuta* species, which delayed female pheromonal calling until a host plant was found (Hendrikse and Vos-Bunnemeyer, 1987).

Males of several species of nymphalid butterflies and arctiid moths use plant compounds as pheromone precursors (Conner *et al.*, 1981; Einser and Meinwald, 1987; Schneider *et al.*, 1981) and females may also increase pheromonal signaling upon encountering host plants. Female cabbage looper moths on host plants begin calling earlier in the scotophase (dark phase) and are more attractive to males throughout the scotophase, than the females not on host plants. Landolt *et al.* (1994) found that males were more attractive to airflow when it first came over cotton foliage and passed over females than it came *vise versa*. Therefore females have been stimulated to release pheromone by the odour of cotton foliage.

It is a questional whether this pheromone applies to a highly polyphagous and mobile species like *H. armigera*. Earlier studies indicated that host plants did not significantly influence the chance of a female moth being mated (Kvedaras *et al.*, 2000 a; Kvedaras *et al.*, 2000 b). Similar results were also made on *Sesamia nonagrioides* Lefebvre in maize (Lopez *et al.*, 1999).

Previous studies have suggested that male response to female sex pheromone was increased by Z-3 hexenyl acetate in *H. zea* and by phenylacetaldehyde in *S. frugiperda*. However, Gregg *et al.* (2000) found that plant volatiles detracted *H. armigera* from responses to pheromones in the field and did not enhance them. In contrast, Xiao *et al.* (2001) reported that the presence of odours of wilted leaves of Chinese wing-nut tree (*Pterocarya stenoptera*) and virgin females had stronger attractiveness to males than that of virgin females only. Males of the cabbage looper released greater amounts of pheromone component, d-linalool when exposed to the combination of odour of cabbage foliage and the female pheromone Z-7-dodecenyl acetate, than when exposed to the female pheromone alone (Landolt and Heath, 1980). However, no observation has so far been done to establish such attraction towards tomato plant and its pests. Therefore a series of laboratory experiments were carried out to determine whether the chemicals in tomato plants affect attraction of males towards females of tomato fruitworm, *H. armigera*.

MATERIALS AND METHODS

Plant and Insect Material

Tomato, *Lycopersicon esculentum* were grown in greenhouse condition at Asian Vegetable Research and Development Center, Shanhua, Taiwan. The seeds were sown in seedling trays, filled with mixture of vermiculite (South Seavermiculite and Perlite Co., The Netherlands) and peat moss – growing media (Know – You Seed Co., Taiwan). After six weeks the seedlings were transplanted to plastic pots of 18 cm diameter and 17 cm in height. The pots were watered daily. Every 10 days, a foliar fertilizer of Nitrofoska (N : P : K : Mg at 20 : 19 : 15 : 0.5, BASF, Germany) at the rate of 3 g 100 ml^{-1} water was applied by spraying uniformly over the plants.

Tomato fruitworm. *H. armigera* was reared in the laboratory by placing several pairs of *H. armigera* adults in acrylic cylinders of 30 cm height and 15 cm diameter. Inner surface of the cylinder was wrapped with rough tissue paper. A cotton plug soaked in honey solution was placed in the bottom of the acrylic cylinder to serve as food as well as mating stimulant for adults. Eggs were collected from the tissue paper after two days. The tissue paper was cut into several small pieces around eggs, wrapped in a nylon net and disinfected by dipping in 7% formaldehyde. Then incubation was done at 27^{0}C and 70% RH for one day. The tissue papers containing eggs were then transferred to a petridish along with a moist cotton ball to maintain the humidity, and kept in an incubator. When the eggs were hatched, the neonate larvae were transferred to plastic containers with Bio-serve diet (BioServe, French Town, USA). In a stainless steel pot, 19.8 g of agar was added to 820 ml of distilled water and the mixture was heated and stirred continuously until the agar became a clear solution. When the molten agar solution became clear, it was cooled to 55^{0}C. At this stage, 160.75 g of dry mix was added into the agar solution and mixed thoroughly to form a uniform paste. The prepared diet was placed in containers and allowed to cool to room temperature. When the larvae were in fourth instar, individual larva was placed in a separate plastic container with food to avoid cannibalism. When the larvae were pupated, pupae were separated by gender (Cheng, 1970) and kept in different cages and the emerged adults were fed with honey solution until they were used for experiments or for breeding.

Extraction of tomato leaves

Leaves of tomato plants grown in an insect-free greenhouse, with natural light at Asian Vegetable Research and Development Center (AVRDC) were used in this experiment. Four to five medium sized leaves were cut from the middle portion of the 10 week old plants and cleaned thoroughly. Leaves were dipped in 600 ml hexane in a glass beaker, topped with a petridish to prevent the escape of hexane vapour, for thirty seconds. Then the extraction was aided by placing the beaker in a household microwave oven for 30 seconds at 800 W. The hexane extract was filtered through anhydrous sodium sulfate column and concentrated to 2 ml in a rotary evaporator. The extract was stored at –20^{0}C until used.

Influence of tomato plants on the mating behaviour

Three approaches were used; i.e. (1) an assessment of the temporal pattern through the scotophase of attraction of male moths to the female moths held with tomato foliage, (2) a direct comparison of the attractiveness of female moths, tomato foliage and female moths with tomato foliage, and (3) determination of the effects of extracted tomato phytochemicals on the attractiveness of female moths.

The flight tunnel used for all bioassays was an acrylic cylinder (100 cm X 15 cm) housed inside a dark, clean room devoid of any organic odours, with air from inside the room (at ambient conditions) pulled through the cylinder and vented to the outside. Moths were tested for attraction in response to an air-stream passed through: (1) a cylinder holding female moths, (2) a cylinder holding cut end of tomato foliage, (3) a cylinder holding female moths and tomato foliage in parallel, and (4) two cylinders in series with the first holding tomato foliage followed by female moths (Figure 1). A 30 cm long cylinder, drilled in the centre served as holding cylinder for moths and foliage. One end of the holding cylinder was covered with aluminium foil

whereas the other end was connected with two long cylinders of 100 cm long and 15 cm in diameter. The other end was closed with nylon net.

In the first set of experiment, three freshly emerged, unmated females which were not previously exposed to any organic odour, were caged in a small net cage and kept inside the holding cylinder. Five virgin males were released through the opposite end and it was closed with nylon net.

In the second set of experiment, fresli cut tomato foliage was inserted in a conical flask containing autoclaved, sterile distilled water and kept inside the holding cylinder. Five virgin males were released through the opposite end and it was closed with nylon net.

In the third set of experiment, three freshly emerged, unmated females which were not previously exposed to any organic odour, were caged in a small net cage and kept along with cut tomato foliage parallely inside the holding cylinder. Five virgin males were released through the opposite end and it was closed with nylon net.

In the fourth set of experiment, two cylinders in series with the first holding cut tomato foliage followed by three freshly emerged, unmated females which were not previously exposed to any organic odour were kept in a small net cage.

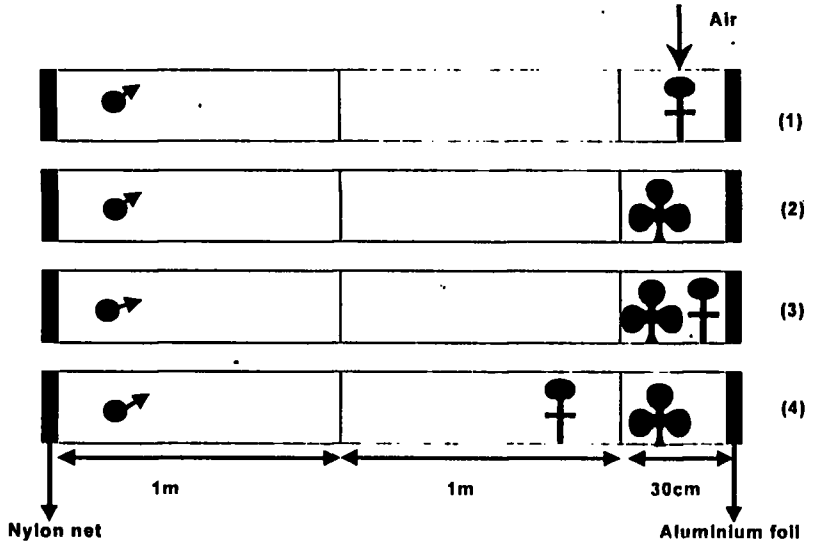

Fig. 1. Illustration of wind tunnel bioassays to study the influence of tomato plants on mating behaviour of tomato fruitworm, *H. armigera*.

A small aquarium pump provided airflow. Air was purified by passage through a 10 cm X 1 cm column of activated charcoal before entering the cylinders holding moths or tomato foliage. Airflow entered the flight tunnel in the centre of the holding cylinder and carried the odours emanated either from the tomato foliage or from the female moths or from the both to the opposite end, where the male moths were kept. During each hour of the scotophase, male moths were observed for their attraction

to the cage of female moths or to the tomato foliage or to the both, placed at the upwind end of the flight tunnel. These experiments were conducted over two days for each experimental set and each experimental set was replicated for four times. Percentage response rates for different treatments were subjected to analysis of variance (ANOVA) and significant differences among means were determined using Duncan's multiple range test (DMRT).

Synergism between the tomato plant odourants and sex pheromones

Microwave assisted extract of tomato foliage was applied to a Whatman no. 1 filter paper at the rate of 0.5 ml and, it was used to wrap the cage of the female moth. It was kept in the centre of the holding cylinder and the attraction of male moths was tested as explained in the previous experiment. Percent male attraction was calculated and the data were statistically analyzed using Student's t test.

Tomato plants on the oviposition behaviour

Fresh cut tomato foliage was inserted in a conical flask containing autoclaved, sterile distilled water and, kept inside the holding cylinder. Freshly emerged, unmated female moths were anaesthetized with Co_2 and tethered with the tomato foliage in the holding cylinder. The female moths were tethered with small glass rod and kept inside the holding cylinder without any tomato foliage as the control. Five male moths were released from the opposite end and airflow was provided by a small aquarium pump through the centre of the holding cylinder. After 48 h, the female moths were removed from the cylinders and allowed to lay eggs. The observations were made on the total number of eggs laid and the number of eggs hatched (Tethered female technique). The difference in mean number of eggs laid and per cent egg hatching were calculated and the data were statistically analyzed using Student's t test.

RESULTS AND DISCUSSION

When the tomato plants and female moths of *H. armigera* were placed together, there was no significant influence on the first day (Figure 2). The attraction of male moths towards this combination was high at 6, 7 and 8h after scotophase on the second day (Figure 3). However, there was no significant influence upto 5h after scotophase. It increased after 6h of scotophase and reached a maximum of 85.00% at 8h after scotophase on day 2. The influence was highly pronounced where the females were placed in front of tomato foliage rather than placing in parallel.

Based on these observations, the microwave-assisted extract of tomato foliage was combined with newly emerged female moths of *H. armigera* to investigate the influence on change of pheromone levels of females and on the attraction of males. The response of male moths was higher to the combination than to females alone. Also, the response was observed on the first day itself. The attraction was 75.00 and 85.00% at 6 and 7h after scotophase on day 1, respectively (Figure 4) and was significant higher than the females alone ($p < 0.05$). However, the percent attraction was declining at 8h after scotophase but the difference in percent attraction remained significant. On day 2, significant attraction was observed from 4h after scotophase and it gradually increased to 80.00% at 7h after scotophase (Figure 5). However, the attraction was not significantly different from females alone, at 6 and 8 h after scotophase.

Though the above results were ambiguous and non-conclusive, there could be a influence of the host plant on the behaviour of *H. armigera*. As it was necessary to examine the influence of tomato on the mating success, *i.e.*, the fertility of eggs in terms of egg hatchability, the tethered female technique (Lopez *et al.*, 1999) was used. The results are presented in Table 1. The mean number of eggs laid by female moths caged with tomato plants was 61.00 as against 19.80 by moths caged without tomato plants. Though the difference in egg laying was not significant, a significant difference ($p < 0.05$) was observed in percent egg hatching. It was 86.70% in moths caged with tomato plants whereas it was only 11.50% in moths caged without host plants.

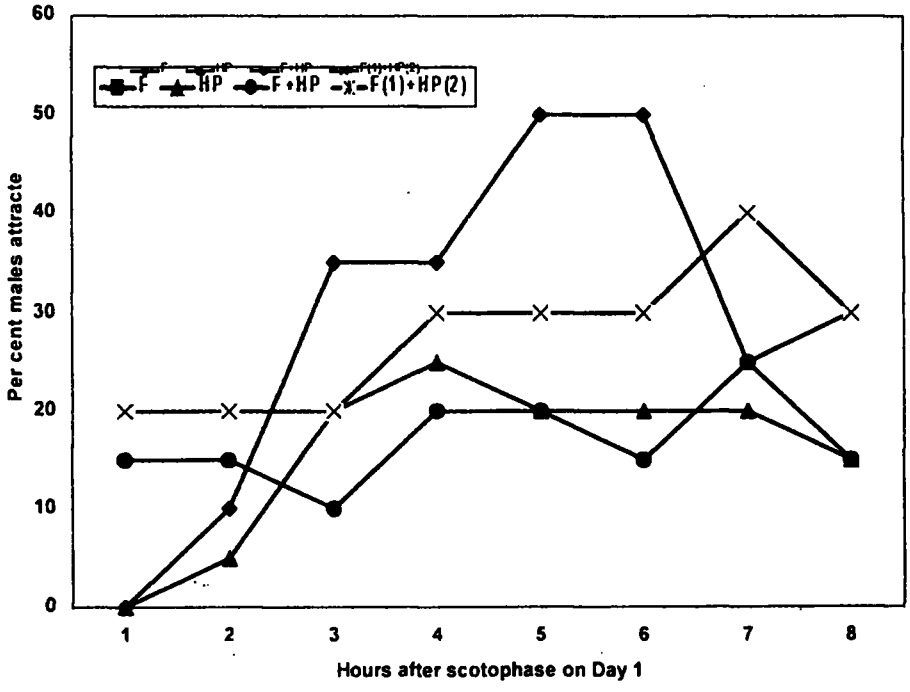

Fig. 2. Influence of tomato plants on attraction of tomato fruitworm, *H. armigera* males to females (Day 1).
F – Females , HP – Host plant , F + HP – Females + Host plant
F(1) + HP(2) – Females (First) + Host plant (Second)

In this study, it was not possible to establish any correlation between the host plant chemistry and sex pheromone production and subsequent mating in *H. armigera*. It was shown that female *H. armigera* were more attractive to unmated males in the presence of tomato plants. However, it was not established whether such increased attractiveness was due to the stimulation of releasing of sex pheromones by host plant chemicals or due to an additive or synergistic effect of the combination of sex pheromone and host odour on attraction of male.

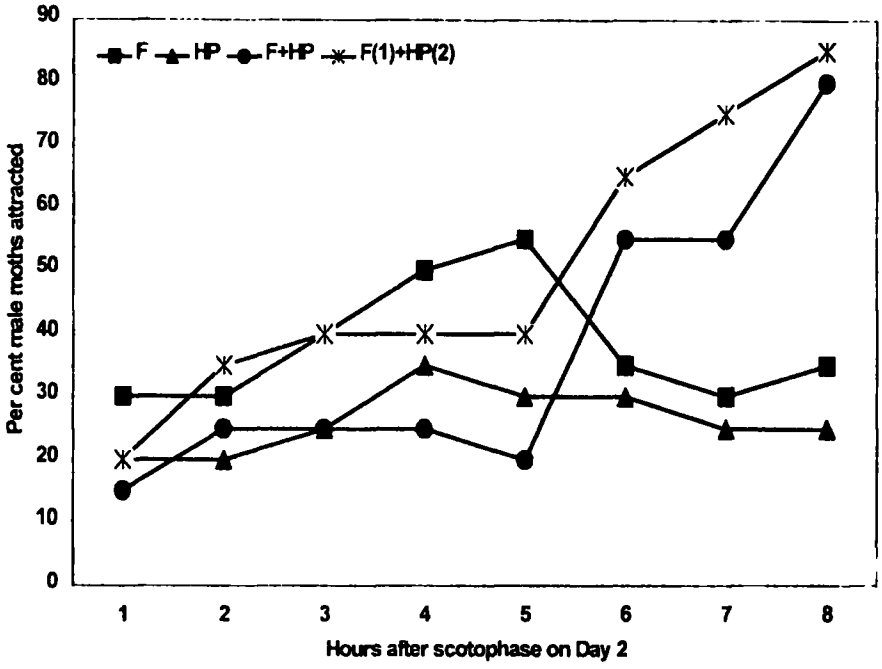

Fig. 3. **Influence of tomato plants on attraction of tomato fruitworm, *H. armigera* Males to females (Day 2).**
F - Females, HP – Host plant, F + HP – Females + Host plant
F(1) + HP(2) - Females (First) + Host plant (Second)

Table 1. **Influence of tomato plants on the oviposition and egg hatchability of tomato fruitworm, *H. armigera* (Tethered female technique).**

Treatment	No. of eggs laid	Egg hatching (%)
With host plant	61.00 ± 14.43	86.70 ± 5.86
Without host plant	19.80 ± 7.24	11.50 ± 11.45
t value	2.05^{ns}	3.91^{*}

* Significantly different at p=0.05
^{ns} non-significant

No previous studies were found in the literature on the stimulation of female pheromone release by host odour, using tomato. However, there is a possibility of an additive or synergistic effect of tomato odour on male response to female pheromone because the as the airstream carried first over tomato foliage and then over females was not more attractive than females confined parallely with tomato foliage. The results from the combination of female moths and purified phytochemicals on male attraction also added evidence to this hypothesis. The increased attraction of males to females with tomato microwave assisted extract may then be due to the additive or synergistic effect rather than stimulation of pheromone release by host odour. It was also observed the significant increase in attraction of males to the combination of females and microwave-assisted extract of tomato on day 1 itself.

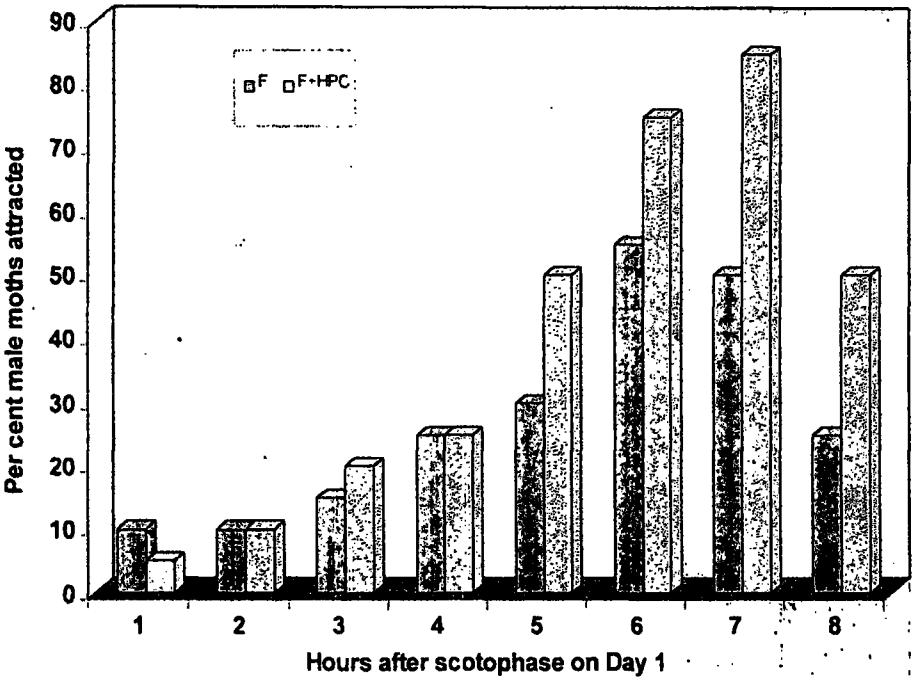

Figure 4. Influence of tomato microwave assisted hexane extract on attraction of males of tomato fruitworm, *H. armigera* to females (Day 1).
F – Females ; F + HPC – Females + Host plant chemicals

A significantly higher mating success ($p < 0.05$) was demonstrated by tethered female technique in the presence of tomato foliage. Kvedaras *et al.* (2000 a and b) showed that host plants like cotton, maize and pigeonpea did not significantly affect the mating behaviour and mating success of *H. armigera*. In contrast, Xiao *et al.* (2002) speculated that the purpose of female *H. armigera* orientation to volatiles from wilted leaves of a non-host plant, Chinese wingnut tree (VWLCT), *Pterocarya stenoptera* was to locate sites for pheromone calling and mating as the males were strongly attracted to a combination of a virgin female and VWLCT.

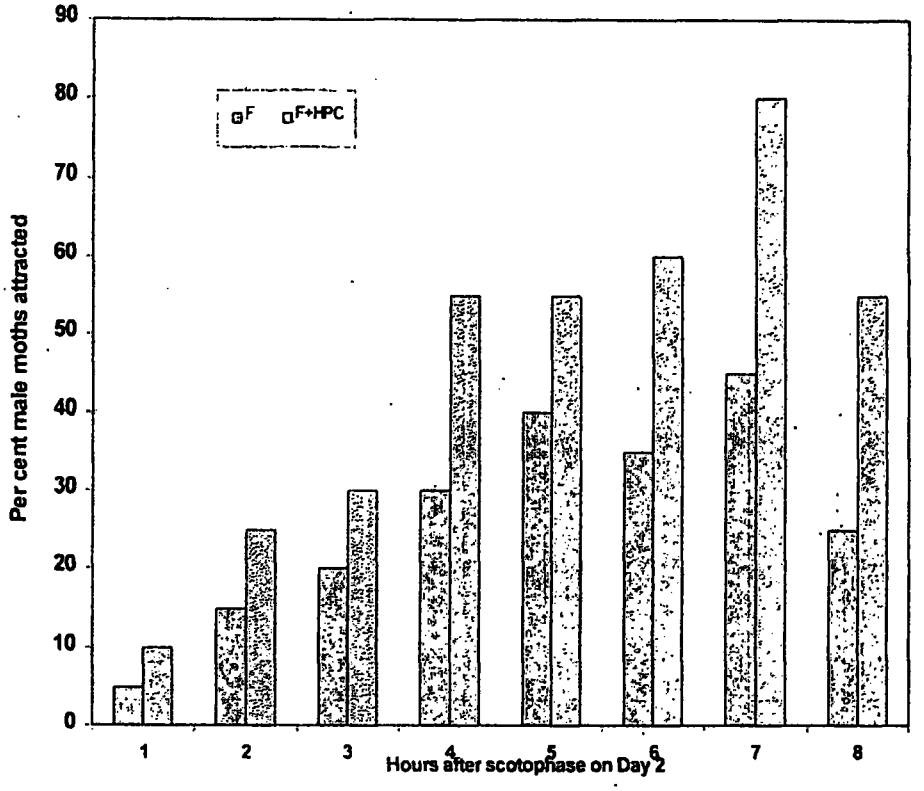

Fig. 5. **Influence of tomato microwave assisted hexane extract on attraction of males of tomato fruitworm, *H. armigera* to females (Day 2).**
F – Females; F + HPC – Females + Host plant chemicals

CONCLUSIONS

A significant increase in attraction of males to the combination of females and chemicals isolated from tomato plants was observed on day 1 itself. The increased attraction of males to females with tomato plant or chemicals isolated from tomato plant may be due to additive or synergistic effect rather than stimulation of pheromone release by host odour. The mating success was also significantly high when the mating took place in the presence of tomato foliage. Therefore, an advantage could be gained in pest control by male orientation to female pheromones in the presence of tomato plant odour.

ACKNOWLEDGEMENTS

The senior author is thankful to Jawaharlal Nehru Memorial Fund, New Delhi for providing financial assistance for taking up this research work at Asian Vegetable Research and Development Center, Taiwan.

REFERENCES

Conner, W.E., Eisner, T., Vadermeer, R.K., Guerrero, A. and Meinwald, J. (1981). Pre-copulatory sexual interaction in an arctiid moth (*Utetheisa ornatrix*): Role of a pheromone derived from dietary alkaloids. Behav. Ecol. Sociobiol. 9: 227-235.

Cheng, H.H. (1970). Characters for distinguishing the sex of pupae of the dark sided cutworm, *Euxoa messoria* (Harris) (Lepidoptera : Noctuidae). Ganad. Jour. Zool. 48 : 587-588

Eisner, T. and Meinwald, J. (1987). Alkaloid derived pheromones and sexual selection in Lepidoptera. pp. 251-269. In: G.D. Prestwich and G.J. Blomquist (eds.). Pheromone Biochemistry, Academic Press, New York. P.360.

Gregg, P.C., Kvedaras, O.L., Moore. C., Soccoro, A.P.D. and Alter, D. (2000). Mating behaviour in *Helicoverpa armigera*: Influence of host plants. Australian Cotton CRC Annual Report 2000-2001. pp. 40-41.

Hendrikse, A. and Vos-Bunnemeyer, E. (1987). Role of host plant stimuli in sexual behaviour of small ermine moths (*Yponomeuta*). Ecol. Entomol. 12: 363-371.

Kvedaras, O.L., Gregg, P.C., Soccoro, A.P.D., Alter, D. and Moore, C. (2000 a). The effects of host plant odours on the calling behaviour, egg maturation and mating success of the cotton bollworm, *Helicoverpa armigera* (Lepidoptera : Noctuidae). In: Abstract Book I – XXI – International Congress of Entomology, Brazil, August 20-26, 2000.

Kvedaras, O.L., Gregg, P.C. and Soccoro, A.P.D. (2000 b). Techniques used to determine the mating behaviour of *Helicoverpa armigera* (Hubner) (Lepidoptera : Noctuidae) in relation to host plants. Aus. J. Entomol. 39(3): 188-194.

Landolt, P.J. and Heath, R.R. (1990). Sexual role reversal in mate-finding strategies of the cabbage looper moth. Science. 249: 1026-1028.

Landolt, P.J. and Phillips, T.W. (1997). Host plant influences on sex pheromone behavior of phytophagous insects. Ann. Rev. Entomol. 42: 371-391.

Landolt, P.J., Heath, R.R., Millar, J.G., Davis-Hernandez, K.M., Dueben, B.D. and Ward, K.E. (1994). Effects of host plant, *Gossypium hirsutum* L., on sexual attraction of cabbage looper moths, *Trichoplusia ni* (Hubner) (Lepidoptera : Noctuidae). J. Chem. Ecol. 20(11): 2959-2974.

Lopez, C., Sans, A. and Eizaguirre. M. (1999). Influence of maize plant on mating of *Sesamia nonagrioides* Lefebvre (Lepidoptera : Noctuidae). Invest. Agr.: Prod. Prot. Veg. 14(3): 415-422.

McNeil, J.N. and Delisle, J. (1989). Host plant influences, calling behaviour and ovarian development of the sunflower moth, *Homeosoma electellum*. Oecologia. 80: 201-205.

Raina, A.K. (1988). Selected factors influencing neurohormonal regulation of sex pheromone production in *Heliothis* species. J. Chem. Ecol. 14: 2063-2069.

Raina, A.K. (1993). Neuroendocrine control of sex pheromone biosynthesis in Lepidoptera. Ann. Rev. Entomol. 38: 329-349.

Raina, A.K., Kingan, T.G. and Mattoo, A.K. (1992). Chemical signals from host plant and sexual behavior in a moth. Science. 255: 592-594.

Schneider, D., Boppre, M., Zweig, J., Horsley, S.B., Bell, T.W., Meinwald, J., Hansen, K. and Diehl, E.W. (1981). Scent organ development in *Creatonotos* moths: Regulation by pyrrolizidine alkaloids. Science. 215: 1264-1265.

Xiao Chun, Hu ChunHua, Du JiaWei and Zhang ZhongNing. (2001). Behavioral responses of adult *Helicoverpa armigera* to the odors of wilted leaves of Chinese wing-nut tree, *Pterocarya stenoptera*. Entomological Knowledge. 38(4): 278-281.

Xiao Chun, Gregg, P.C., Hu Wenli, Yang ZhiHua and Zhang ZhongNing. (2002). Attraction of Cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera:Noctuidae), to volatiles from wilted leaves of a non-host plant, *Pterocarya stenoptera*. Appl. Entomol. and Zool. 37(1): 1-6.