Res. on Crops **19** (4) : 695-700 (2018) DOI : 10.31830/2348-7542.2018.0001.49
With two figures
Printed in India

Effect of female and male age on copulation of the banded sunflower moth, *Cochylis hospes* Walsingham (Lepidoptera : Tortricidae)

M. S. MPHOSI*

Limpopo Agro-Food Technology Station
University of Limpopo, Private Bag X1106, Sovenga, 0727, South Africa
**(e-mail : maboko.mphosi@gmail.com)*

(Received : October 16, 2018/Accepted : November 15, 2018)

ABSTRACT

Life-history strategies of insect are controlled by fitness-related trait trade-offs, in particular, the age at which either one of the two sexes copulate and this demonstrated to influence insect reproductive output and longevity. The banded sunflower moth, *Cochylis hospes* Walsingham, is one of the most important lepidopteran pests of cultivated sunflower, *Helianthus annuus* L., in North America. Larvae of *C. hospes* feed on the head of sunflower, causing extensive damage and a reduction in the number of seeds produced (Royer and Walgenbach, 1987). Individual virgin females of either species, age 1, 2, 3, 4 and 5 days, were placed with a single conspecific virgin male (2-4 days old) in a 20 ml glass vial 3 h prior to the onset of the scotophase, in a room under the same conditions as those of the respective colonies. The percentages of copulations, and percentages of females with spermatophore in their bursa copulatrix, were compared by χ^2 analyses; if there were any significant differences, each pair was compared by a χ^2 analysis. The distributions of time that copulation started for the different age females and males were compared, respectively, by chi-squared tests. The percentage of female *C. hospes* that copulated was significantly influenced by female age. Significantly higher proportions of females aged 3 and 5 days old copulated than did females of the other ages. The percentage of female *C. hospes* that copulated that had spermatophores in their bursa copulatrix was also significantly influenced by female age.

Key words : *Cochylis hospes* Walsingham, copulation, *Helianthus annuus* L., sunflower moth

INTRODUCTION

The banded sunflower moth, *Cochylis hospes* Walsingham, is one of the most important lepidopteran pests of cultivated sunflower, *Helianthus annuus* L., in North America. Larvae of *C. hospes* feed on the head of sunflower, causing extensive damage and a reduction in the number of seeds produced (Royer and Walgenbach, 1987). The banded sunflower moth larvae are mostly restricted to feeding on *Helianthus* spp. and other species of Compositae (Wilson, 1996). Banded sunflower moths have one generation in North Dakota and the upper Great Plains. More than one generation is possible in the southern regions. Adults emerge about mid-July and are present in the field until mid-August. An individual moth lives between seven and 10 days, but moths are present for a total of about eight weeks each season because of the extended emergence period. Although some moths rest in the sunflower field during the day, many are found in vegetation (especially broadleaf vegetation) along field margins.

At twilight, females move into the field to lay eggs. Within a week after emergence, the moths begin to lay eggs on the bracts of the sunflower heads. Females preferentially deposit more eggs on midsized buds than smaller or larger buds. Very few eggs are laid on plants at pollen shed and later. Most eggs are laid singly or in small clusters and are deposited on the outer whorl of bracts. A few eggs are placed on the inner bracts and the

underside of the sunflower head. Eggs are present through early August and hatch about five to eight days after being deposited. Larvae develop through five instars, or stages, in about 10 to 14 days and are present in sunflower heads from mid-July to mid-September. Newly emerged larvae initially are found on and between the bracts and later move to the disk flowers and feed on pollen. Third and later instars tunnel through the disk flowers and feed on young, developing seeds. Each larva penetrates and consumes the contents of several seeds. After feeding to maturity, larvae drop to the ground and spin cocoons in the soil, where they pass the winter. The silken cocoons become coated with soil and are difficult to detect. Pupation takes place in late June or early July the following year. The pupal period lasts for about 12 days. There is no information on *C. hospes* copulation to date. The study investigated the effects of female and male age on copulation patterns.

MATERIALS AND METHODS

Insects

Banded sunflower moths were generously supplied (by Sharon Grugel) as pupae from a laboratory colony maintained at USDA-ARS Northern Crop Science Laboratory, Fargo, North Dakota. The colony was originally established from larvae collected in North Dakota. Regular introductions of wild insects and tests for selectivity towards sunflower were carried out to ensure that the colony was representative of the wild population. Rearing procedures for the insects have been reported elsewhere. Briefly, eggs were laid on synthetic material, placed atop synthetic diet, covered with sterilized wood chips, and left for approximately 2-5 days at 27±1°C and 60-70% humidity under a 15 : 9 L : D light cycle. Because it was difficult to separate the pupae from the wood chips, the combined mass was placed in emergence containers. Each day, newly emerged adults were collected and the sexes separated and placed in containers, with a 10% sugar solution for food, until used in the experiments.

Effect of Female Age on Time of Copulation

Individual virgin females of either species, age 1, 2, 3, 4 and 5 days, were placed with a single conspecific virgin male (2-4 days old) in a 20 ml glass vial 3 h prior to the onset of the scotophase, in a room under the same conditions as those of the respective colonies. Pairs were observed every 15 min, from three hours prior to the onset until the end of the scotophase, and the time at which copulation started and finished for each pair of insects noted. At the conclusion of the observations, females were dissected for the presence or absence of a spermatophore in the bursa copulatrix. If a female was not observed to mate, but a spermatophore was found inside her bursa copulatrix, she was not included in the data set. Each treatment (age of female) was replicated at least 30 times.

Effect of Male Age on Periodicity of *C. hospes* Copulation

Males 1, 2, 3, 4 and 5 days old were placed in a vial with a 2-4 day virgin female. Mating was observed as above.

Data Analysis

The percentages of copulations, and percentages of females with spermatophore in their bursa copulatrix, were compared by χ^2 analyses; if there were any significant differences, each pair was compared by a χ^2 analysis. The distributions of time that copulation started for the different age females and males were compared, respectively, by chi-squared tests. For the analysis, copulation times were categorized as "Before" (=commenced in the last three hours of the photophase), "Early" (= commenced in hours 1-4 of the scotophase) or "Late" (= commenced in hours 5-8 of the scotophase). χ^2 tests for the different aged insects compared the distributions of copulations across each of these three categories (Analytical Software, 1998).

RESULTS AND DISCUSSION

The percentage of female *C. hospes* that copulated was significantly influenced by female age (Table 1). Significantly higher proportions of females aged 3 and 5 days old copulated than did females of the other ages. The percentage of female *C. hospes* that copulated that had spermatophores in their bursa copulatrix was also significantly influenced by female age (Table 1). In particular, a higher percentage of younger females that copulated tended to have spermatophores in their bursa copulatrix than did older females. Overall, across 150 observations, the mean time of copulation was 129±3 min. Male age had a significant effect on the percentage of *C. hospes* copulations, with a significantly greater percentage of 3 d-old males copulating than for all other ages (Table 2). High percentages of all copulations with males of different ages resulted in transfer of a spermatophore to females; the only significant difference was that for 1-d males in which a significantly smaller percentage of spermatophores were transferred than for all other male ages (Table 2). Overall, across the 150 observations, the mean time of copulation was 120 ± 4 min. Female age significantly (χ^2= 43.0330, P<0.0001) influenced the time that copulation commenced in *C. hospes* (Fig. 1). The most apparent difference was that all the youngest females (1 d) mated late (scotophase), whereas the older (2-5 d) females mated predominantly early (in the scotophase). Male age also significantly (χ^2 =60.6042, P<0.0001) influenced the time that copulation commenced in *C. hospes* (Fig. 2). Similar to that found for the effect of female age, 1-d males commenced copulation late in the scotophase, whereas older (2-5 d males) commenced copulation predominantly in the early part of the scotophase.

Table 1. The percentage of copulation, and the percentage of these copulations in which females had a spermatophore inside the bursa copulatrix, in relation to age of female *C. hospes*

Female age (days)	Per cent copulation[1]	Per cent female copulated with spermatophore in bursa copulatrix
1	40b[2]	75b
2	43b	92a
3	70a	76b
4	53b	56c
5	63a	58c
χ^2	5.54	42.01
P	0.018	0.0001

[1]N = 30. [2]Percentages in each column followed by the same letter are not significantly different (P>0.05) by least significant difference (chi-squared analysis).

Table 2. The percentage of copulation, and the percentage of these copulations in which females had a spermatophore inside the bursa copulatrix, in relation to age of male *C. hospes*

Male age (days)	Per cent copulation[1]	Per cent females copulated with spermatophore in bursa copulatrix
1	47b[2]	71b
2	57b	88a
3	83a	80ab
4	53b	69b
5	17c	80ab
χ^2	29.805	20.943
P	<.0001	0.0003

[1]N = 30. [2]Percentages in each column followed by the same letter are not significantly different (P>0.05) by least significant difference (chi-squared analysis).

The timing of sexual maturity of female moths varies greatly across species, with some species being ready to mate almost immediately when their genitalia are accessible following eclosion (Seabrook *et al.*, 1987; Kvedaras, 2002) and other species taking several days to reach maturity (Wenninger and Averill, 2006). Our study demonstrates that *C. hospes* females are not sexually mature upon eclosion and, on an average, are not at peak sexual maturity for at least one day following eclosion, as evidenced by their respective peak copulation percentages; peak copulation percentage of female *C. hospes* was at 3-d following eclosion. Given that competency to produce and release pheromone mediates sexual maturity in female moths (Raina and Stadelbacher, 1990), this delay in reaching peak copulation percentage likely results from females, in the day following eclosion, producing and/or releasing a relatively small amount of pheromone which fails to elicit appropriate sexual responses from males. An inability to produce and/or release sex pheromone by recently eclosed females has been reported in numerous other species

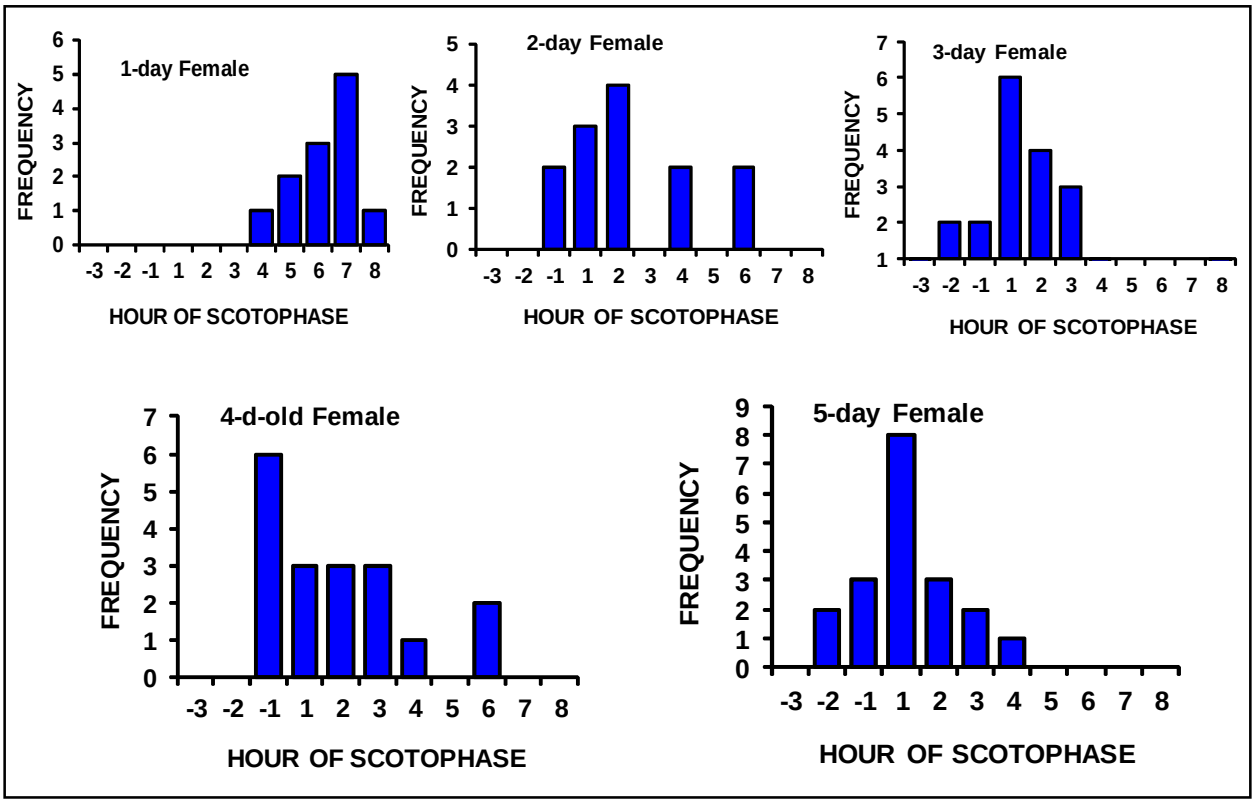

Fig. 1. Number and distribution of copulations that commenced at a particular hour of the scotophase for females of different ages of *C. hospes*.

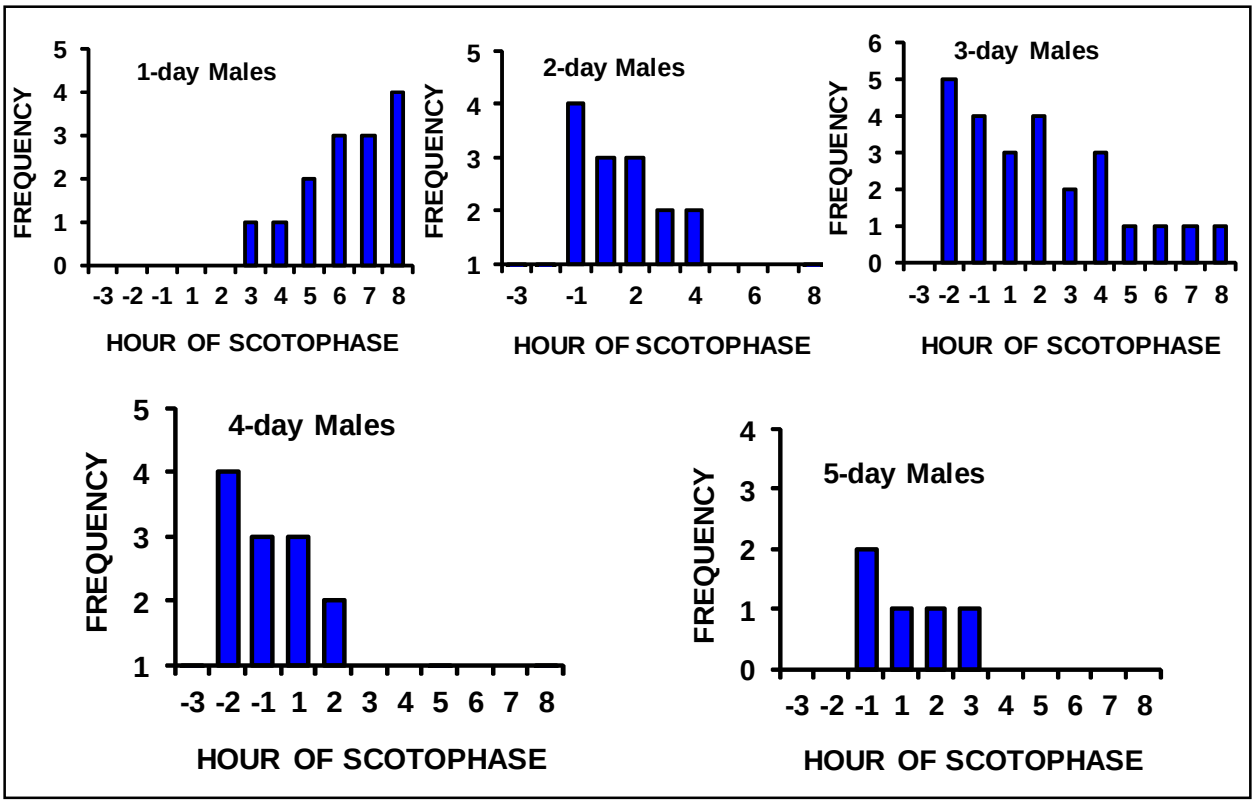

Fig. 2. Number and distribution of copulations that commenced at a particular hour of the scotophase for males of different ages of *C. hospes*.

(Collins and Cardé, 1985; Raina *et al.,* 1986; Raina and Stadelbacher, 1990; Delisle, 1992). Whether such a delay in pheromone production/release corresponded with the maturation of other female reproductive characters (e. g. oocyte maturation) has not been determined in *C. hospes* species.

Similar to that found for females, copulation measures of males of both species were not maximal for 1-day-old males, suggesting that males also need a short period following eclosion to reach maximal sexual maturity. In the case of *C. hospes*, a smaller percentage of 1-d-old males than that of older males copulated with females, suggesting that the 1-d-old males may not be as responsive to sex pheromone as older males (Bergh and Seabrook, 1986).

The diel periodicity of copulation *C. hospes* tended to occur at the beginning of the scotophase (i. e. crepuscular). The period of sexual activity was synchronized between males and females of *C. hospes* species and the diel periodicity of copulation showed strong age-related effects in both males and females *C. hospes*. Two effects were apparent. Firstly, both one-day old females and males commenced copulation significantly later in the scotophase than did their respective older counterparts. This is likely caused by young females not producing and/or releasing pheromone, and young males not being responsive to pheromone (Bergh and Seabrook, 1986). Secondly, as females aged, they tended to commence copulation earlier in, or prior to the start of, the scotophase. This observation of females mating earlier as they age is likely due to older females commencing release of pheromone ("calling") earlier. This phenomenon has been noted previously in other species e. g. *Choristoneura rosaceana* (Harris), *Mamestra brassicae* (L.) (Noldus and Potting, 1990) and *Heliothis armigera* (Hüber) (Hou and Sheng, 2000). It has been suggested that this is an adaptive behaviour that increases the probability of older females attracting a mate through reduced competition, early in the scotophase, with younger females (Swier *et al.,* 1977).

REFERENCES

Analytical Software (1998). *Statistics for Windows : Users Manual Analytical Software.* Tallahassee, Florida.

Bergh, J. C. and Seabrook, W. D. (1986). A simple technique for indexing the mating status of male spruce budworm, *Choristoneura fumiferana* (Lepidoptera : Tortricidae). *Canadian Entomologist* **18** : 37-41.

Collins, R. D. and Cardé, T. R. (1985). Variation in and heritability of aspects of pheromone production in the pink bollworm moth, *Pectinophora gossypiella* (Lepidoptera : Gelechiidae). *Ann. Entomol. Soc. Am.* **78** : 229-34.

Delisle, J. (1992). Age related changes in the calling behaviour and the attractiveness of obliquebanded leafroller virgin females, *Choristoneura rosaceana* under different constant and fluctuating temperatures. *Entomol. Exp. Appl.* **63** : 55-62.

Hou, M. L. and Sheng, C. F. (2000). Calling behaviour of adult female, *Helicoverpa armigera* (Hubner) (Lepidoptera : Noctuidae) of overwintering generation and effects of mating. *J. App. Entomol.* **124** : 71-75.

Kvedaras, O. L. (2002). The influence of host plants on the mating behaviour of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera : Noctuidae). Ph. D. thesis, School of Rural Science and Agriculture. The University of New England, Armidale, NSW Australia.

Noldus, L. P. J. J. and Potting, R. P. J. (1990). Calling behaviour of *Mamestra brassicae* (Lepidoptera : Noctuidae) : Effect of age and photoperiod. *Ent. Exp. Appl.* **56** : 23-30.

Raina, A. K., Klun, J. A. and Stadelbacher, E. A. (1986). Diel periodicity and effect of age and mating on female sex pheromone titer in *Heliothis zea* (Lepidoptera : Noctuidae). *Ann. Entomol. Soc. Am.* **79** : 128-31.

Raina, A. K. and Stadelbacher, E. A. (1990). Pheromone titer and calling in *Heliothis virescens* (Lepidoptera : Noctuidae) : Effect of mating with normal and sterile backcross males. *Ann. Entomol. Soc. Am.* **83** : 987-90.

Royer, T. A. and Walgenbach, D. D. (1987). Impact of sunflower moth (Lepidoptera : Pyralidae) larval infestations on yield of cultivated sunflowers. *J. Econ. Entomol.* **80** : 1297-1301.

Seabrook, W. D., Linn, C. E., Dyer, L. J. and Shorey, H. H. (1987). Comparison of

electroantennograms from female and male cabbage looper moths (*Trichoplusia ni*) of different ages and for various pheromone concentrations. *J. Chem. Ecol.* **13** : 1443-53.

Swier, S. R., Rings, R. W. and Musick, G. J. (1977). Age-related calling behaviour of the black cutworm, *Agrotis ipsilon*. *Ann. Entomol. Soc. Amer.* **70** : 919-24.

Wenninger, E. J. and Averill, A. L. (2006). Effects of delayed mating on reproductive output of female oriental beetle, *Anomala orientalis* (Coleoptera : Scarabaeidae). *Agric. Fores. Entomol.* **8** : 221-31.

Wilson, R. L. (1996). The biology and management of the sunflower moth, *Homoeosoma electellum*–Past, present and future. pp. 60-66. In : Proc. Ninth Great Plains Sunflower Insect Workshop, L. D. Charlet (ed.). USDA, ARS.