



Pergamon

Journal of Insect Physiology 45 (1999) 385–392

Journal
of
Insect
Physiology

Reproductive performance and longevity of female European corn borer, *Ostrinia nubilalis*: effects of multiple mating, delay in mating, and adult feeding

Henry Y. Fadamiro *, Thomas C. Baker

Department of Entomology, Iowa State University, Ames, IA 50010-3222, USA

Received 6 April 1998; accepted 31 July 1998

Abstract

In a recent study on the pheromone-mating disruption of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), we recorded a significant reduction in mating frequency, as well as a marked delay in mating in feral females captured in disruptant-treated fields. In order to be able to accurately interpret the results in terms of effective population control, the current study was undertaken on the effects of multiple matings and a delay in mating on reproductive performance. Female *O. nubilalis* that mated at least twice had significantly higher fecundity and fertility, compared with once-mated females. In addition, multiple-mated females deposited a significantly larger portion of their egg complement, relative to single-mated or unmated females. Females that experienced a 3-day delay in mating showed a significant reduction in fecundity compared with females that mated soon after emergence. A 1-week delay in mating resulted in a further reduction in fecundity and a near zero fertility. The effect of sugar feeding on reproduction was not significant. In general, unmated females lived longer than mated females, and sugar-fed mated females had a higher longevity than water-fed mated females. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Multiple mating; Reproductive output; Fecundity; Fertility; Spermatophores

1. Introduction

Polyandry, a reproductive strategy in which insects acquire several mates during their reproductive life is widespread in the Lepidoptera (Drummond, 1984; Royer and McNeil, 1993; Ward and Landolt, 1995; Delisle and Hardy, 1997; Landolt, 1997). Several possible reasons have been proposed for multiple matings in female moths. The most important potential benefits to the female include: enhancement of fecundity and longevity by obtaining non-sperm resources from males; achievement of enough sperm supply to fertilize her full egg complement; or increase in the genetic diversity of progeny (Drummond, 1984; Marshall and McNeil, 1989; Ramaswamy et al., 1997).

The European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) is a major pest of corn, *Zea*

mays L. in the United States. Although the female has been classified as normally monandrous (Caffrey and Worthley, 1927; McNeil et al., 1997), several workers have reported multiple matings in feral and laboratory-reared *O. nubilalis* females. Pesho (1961) recorded 8–43% multiple matings in feral females, while Showers et al. (1974) reported that 10% of females captured in light traps had mated twice. This was in the same range as the 11% multiple matings recorded by Loughner (1971). Drecktrah and Brindley (1967) recorded a higher proportion (46.5%) of multiple mated females, and Elliot (1977) reported that 29–37% of a feral population in southwestern Ontario had mated twice, and up to 13% three times. In their study on the reproductive performance of females reared on different diets, Onokogu et al. (1980) recorded a maximum of six spermatophores with multiple matings in the range of 30–70%.

In a recent study conducted in Iowa on the potential of pheromone mating disruption of the European corn borer, we recorded a maximum of four matings per female (Fadamiro et al., unpublished data). Of the 327 feral free-flying females net-captured in untreated

* Corresponding author. Present address: Department of Entomology, University of Minnesota, St Paul, MN 55108. E-mail: fadam001@tc.umn.edu

(check) plots during the first flight, about 70% had mated more than once. Also, 75% multiple matings ($n = 274$) was recorded in the check plots during the second flight. The incidence of multiple mating was more preponderant during the peak than at the beginning of each flight. While the mean number of spermatophores averaged about two per female in the check plots, a significant reduction in mating frequency was recorded in the treated plots (1.33–1.58 spermatophores per female) (Fadamiro et al., unpublished data). Furthermore, our data showed that a higher level of mating disruption was achieved during the beginning of the first flight: about 50–60% of females captured in pheromone-treated plots were unmated during the first 10 days of the first flight, compared with the 90–100% mated females captured in the check plots during the same period.

In order to be able to accurately interpret our results, we need to know what influence mating frequency, as well as a delay in first mating might have on the reproductive success of *O. nubilalis* females. Because we are not aware of any studies conducted to directly address these pertinent questions, the current study was commenced to try to begin to understand why European corn borer females mate more than once.

In addition, previous studies on the effect of adult feeding on reproductive fitness in European corn borer have achieved variable results. Kira et al. (1969) suggested that only drinking water was essential for egg production by females. In recent studies, however, sugar-containing diets were shown to increase fecundity and longevity (Miller, 1988; Leahy and Andow, 1994). Thus, our experiment was designed to test the effect of sugar feeding on reproductive output and longevity of female European corn borer, as well as possible interactions with mating frequency.

2. Materials and methods

2.1. Insect rearing

The Iowa strain of *O. nubilalis* is bivoltine, with a female sex pheromone blend of the (*Z*) and (*E*) isomers of 11-tetradecenyl acetate in ratio 97:3 (Klun et al., 1973). Adults used in this experiment were from wild (1996) populations reared in a laboratory for 19 generations. Adults were either sexed at the pupal stage, or on the first day of emergence to minimize labor. Sexing of adults on the first day of emergence was sufficient for this study, since no matings by European corn borer were recorded during the first 12 h of emergence (Royer and McNeil, 1991; personal observation). An adult was considered 0 day old on the day of eclosion.

2.2. Experimental protocol

In order to maximize oviposition, the experiment was conducted under cyclic photoperiod and temperatures (L:D, 16:8; 27:23 \pm 1°C; 90 \pm 5% relative humidity) (Binder and Robbins, 1996). Newly emerged virgin females were placed in individual 7.6 cm \times 7.6-cm-diameter cylindrical screen cages made of 18-mesh copper wire (Kira et al., 1969). The cages were then placed in plastic trays containing a thick layer of white sterile absorbent cotton (Acco Absorbent Cotton Co., MI) dampened with water or 8% sucrose-water. The cotton was replaced every 5 days.

There were six groups of females:

- (i) female supplied with no males throughout its entire life;
- (ii) female paired with a fresh male for 24 h only on day 1 post-emergence;
- (iii) female paired with a fresh male each day for 3 days starting from day 1 post-emergence;
- (iv) female paired with a fresh male each day for up to 5 days starting from day 1 post-emergence;
- (v) female paired with a fresh male each day for up to 5 days, starting from day 4 post-emergence;
- (vi) female paired with a fresh male each day for up to 5 days starting from day 7 post-emergence.

Females in groups (iv), (v) and (vi) were paired with fresh males for up to 5 days, or until death, whichever came first. Early mortality of adult European corn borer was minimal under these experimental conditions. Twenty-eight females were placed in each group.

To determine the effect of food on reproductive output and longevity, half of the females in each group were fed on water while the other half were fed on 8% sucrose-water (Flavorite Sugar, Preferred Products Inc., MN). Where applicable, a fresh (1 or 2-day-old) virgin male was paired with a female. The male was removed after 24 h, and where applicable, replaced with another fresh virgin male.

A sterile disposable polystyrene plastic Petri dish (100 mm \times 15 mm upper lid part, Fisher Scientific, PA) was placed on top of the cylindrical cage as an oviposition site, and was changed daily. Eggs were incubated for 5 days on the oviposition site, under the experimental conditions described above.

To determine the fecundity of a female, the total area of an egg mass deposited on a Petri dish was measured on a digital analyzer (Decagon Industries Monochrome Ag Vision Imaging System, Pullman, WA), a standard method of estimating fecundity in European corn borer (Binder and Robbins, 1996). Measurement of egg mass was done on the second day following deposition. Areas measured were later converted to number of eggs using a calculated conversion factor of 0.20 mm per egg. Singly-laid eggs were counted manually. Preoviposition

Table 1
Mating patterns of female *O. nubilalis* under different conditions

Female category	No. females paired ^a	% mated	No. females with 1–3 spermatophores			% multiple matings
			1	2	3	
Paired for 1 day on day 1 post-emergence	25	28.0	7	0	0	0
Paired for 2–3 days from day 1 post-emergence	25	68.0	12	5	0	29.4
Paired for 4–5 days from day 1 post-emergence	27	77.8	13	7	1	38.1
Paired for 3–5 days from day 4 post-emergence	28	67.9	16	2	0	10.5
Paired for 3–5 days from day 7 post-emergence	24	20.8	5	0	0	0

An insect was considered 0 day old on the day of emergence. A female was paired daily with a fresh 1- or 2-day-old virgin male. With the exception of the first two categories of females that were paired with males for a maximum duration of 1 to 3 days, a female was paired with a fresh virgin male every day for a maximum of 5 days or until death, whichever came first.

^aThe difference between the starting sample size for each group (28 females per group) and the number of females reported reflects female loss due to early mortality or escape.

Table 2
Effect of mating frequency on reproductive performance and longevity of female *O. nubilalis* categorized by: (A) total number of spermatophores in the bursa, and (B) number of ruptured spermatophores in the bursa

Female category	No. females observed ^a	Fecundity (no. of eggs deposited)	% fertility	Pre-oviposition period (days)	Longevity (days)	Index of remaining eggs
<i>A. Total number of spermatophores</i>						
Unmated	53	163 ± 182c	0 ± 0c	7.1 ± 3.6a	15.1 ± 3.8a	3.8 ± 1.1a
Single-mated	48	540 ± 292b	64.9 ± 34.8b	3.9 ± 1.6b	13.0 ± 3.6b	1.7 ± 1.4b
Multiple-mated	15	630 ± 181a	77.5 ± 29.9a	3.3 ± 1.5b	11.8 ± 2.6b	0.7 ± 0.9c
<i>B. Number of ruptured spermatophores</i>						
0	32	133 ± 170c	0 ± 0c	7.0 ± 3.4a	14.8 ± 3.7a	4.0 ± 0.9a
1	55	538 ± 274b	63.9 ± 34.5b	3.7 ± 1.6b	12.8 ± 3.6b	1.6 ± 1.4b
2	7	742 ± 193a	96.3 ± 4.7a	3.3 ± 1.8b	11.4 ± 1.3b	0.4 ± 0.5c

Values (means ± SD) in the same column, under the same classification, having no letters in common are significant at $P < 0.05$.

^aFemales with a 7-day delay in mating were excluded from the analysis.

fecundity ($P = 0.005$), fertility ($P = 0.002$), preoviposition period ($P = 0.0001$), and longevity ($P = 0.005$) were recorded (Table 3). Females that had access to a male on their first day of emergence had a higher reproductive output and a shorter preoviposition period than females that were paired with a male for the first time on the fourth or seventh day of emergence. Four-day-old virgin females mated with fresh males had a significantly greater fertility and a shorter preoviposition period than 7-day-old virgin females paired with fresh males for the same length of time. A significantly higher mean longevity (16.6 days) was recorded for females that did not gain access to a male until 7 days post-emergence, com-

pared with females that had access to male on the first day (12.9 days), or fourth day (13 days) of emergence. No significant differences were recorded for the index of remaining eggs among the three categories of females (Table 3).

3.4. Effect of food

In order to detect possible interactions among food, mating status and the different fitness parameters, unmated and mated females were grouped separately and the effect of food was determined. Single-mated females with access to sugar-water showed a slight but not sig-

period was calculated as the number of days from female eclosion to first oviposition, regardless of a female's mating status. Fertility of eggs was determined visually under the microscope, examining for the presence of yolk after 2 days of egg incubation. A Petri dish containing eggs and its bottom part was later taped using a parafilm tape (American National CanTM, IL) on the 4th day of incubation to prevent escape by hatched larvae. Eggs were incubated for 5 days and allowed to fully hatch as larvae. Each Petri dish was double-checked for fertility of eggs at the end of incubation.

Each female was observed daily to determine longevity, and at death was preserved in appropriately-labeled glass vials containing 70% ethanol. She was later dissected under the microscope at $10\times$, to determine the presence, number, and condition of spermatophores. Upon dissection, females were classified on the basis of either the total number of spermatophores, or the number of ruptured spermatophores in their bursa. The latter classification was based on the observation that some of the multiple-mated females had at least one spermatophore fully intact at death. Since a spermatophore is assumed to be depleted of sperm only when completely ruptured (Showers et al., 1974), a classification based on the number of ruptured spermatophores could be a more appropriate measure of spermatophore depletion.

The 'index of remaining eggs' was estimated by grading the number of eggs remaining in the abdomen of a dead female, using a range of 0 to 5 (0, none, as observed for females that deposited all their eggs before death; 5, complete full, as observed for females that did not deposit any eggs throughout their life).

Analysis of data was performed using ANOVA followed by the LSD test for comparing means (SAS Institute Inc., 1985).

3. Results

3.1. Mating patterns

The general mating pattern of female European corn borer under different conditions is shown in Table 1. Although 28 females were placed in each mating group at the start of the experiment, only females that made it to the seventh day post-emergence 'cut-off' were included in the data (Table 1). Thus, the difference between the starting sample size (28 females per group) and sample size reported in Table 1 reflects loss of females due to early mortality or escape.

Only 28% of the 1-day-old virgin females paired with a male for 24 h had mated, and none more than once. A higher percentage of mating (68%) was recorded for females paired with a fresh male each day for 3 days, with multiple matings of about 29%. Of the 27 1-day-old virgin females paired with a fresh male each day for

up to 5 days, 78% had mated at least once. This is in the same range as the 68% mating recorded for the females who gained their first access to male on their fourth day of emergence. Virgin females whose first access to a male was delayed for about a week mated the least (20.8% mating), and none more than once (Table 1). About 38% of the 1-day-old virgin females paired with a fresh male each day for up to 5 days mated more than once, compared with 10.5% multiple mating recorded for 4-day-old virgin females with the same length of access to males. Only one female mated three times, and this was in the treatment where newly emerged females had access to a fresh male for up to 5 days. Very few females that experienced a 7-day delay in mating mated (20.8%), and consequently this group was excluded from statistical analyses of the effects of mating frequency and food on reproductive performance.

3.2. Effect of mating frequency

Significantly higher fecundity ($P = 0.0001$) and fertility ($P = 0.0001$) were recorded for multiple-mated females, compared with single-mated, or unmated females (Table 2A). Multiple-mated females laid virtually all of their egg complement and had a significantly lower index of remaining eggs. Unmated females had a significantly lower fecundity, longer preoviposition period, higher longevity, and higher index of remaining eggs ($P = 0.001$) than both classes of mated females (Table 2A). Most of the eggs deposited by unmated females were laid as scattered, single eggs, rather than as egg masses, and all were infertile. No significant interactions were recorded among mating frequency, food and all the fitness parameters ($P > 0.05$).

Similar results were obtained when females were classified according to the number of ruptured spermatophores in their bursa (Table 2B), with the differences in reproductive output among the three groups of females becoming more pronounced. Females that had two ruptured spermatophores in their bursa had significantly higher fecundity ($P = 0.001$), fertility ($P = 0.0001$) and a lower index of remaining eggs ($P = 0.0001$), than females that had one or no ruptured spermatophores (Table 2B). Paired females having no ruptured spermatophores in their bursa had a longer preoviposition period ($P = 0.001$), and a higher longevity ($P = 0.005$) than females that had one or two ruptured spermatophores (Table 2B).

3.3. Effect of delay in first mating

Since few multiple matings were recorded in this study, and virtually none for females that had delayed access to males, comparisons were made among once-mated females with their first access to a male on 1-, 4-, or 7-days post-emergence. Significant differences in

clear whether the increased fecundity recorded for multiple-mated *O. nubilalis* females in the current study was due to an increase in egg production in vivo, or simply an increase in egg deposition by multiple-mated females.

The significant reduction in index of remaining eggs recorded for multiple-mated females suggests that remating enhanced egg deposition as Watanabe (1988) proposed for the yellow swallowtail butterfly, *Papilio xuthus*. Herman and Barker (1977) reported that male-derived factors may induce oogenesis through hormonal effects. Our results, therefore, suggest that male-derived factors may also be playing a role in reproduction by inducing or enhancing oviposition (egg deposition), perhaps through similar hormonal effects.

Male-derived nutrients can contribute to egg production only in species that eclose as adults with little or no eggs yolked (Boggs, 1990). For these species also, adult feeding may play an important role in egg production (Boggs, 1990). Adult European corn borers have been observed to exhibit feeding-related behaviors in the field (Showers et al., 1976; Cantelo and Jacobson, 1979). However, results of work on the effect of adult feeding on the reproductive performance of female *O. nubilalis* have not been consistent. Caffrey and Worthley (1927) first reported that sugars in imbibed water did not improve reproduction, and Kira et al. (1969) concluded that only drinking water was essential for egg production by female European corn borer. Miller (1988) reported that females that were fed on 15% honey-water showed a significant improvement in four reproductive performance attributes, but not in total fecundity, than females that were fed on water alone. In contrast, Leahy and Andow (1994) recorded a significant increase in fecundity for European corn borer females that were fed on diets containing sugar. Although direct comparisons cannot be made among the different studies due to differences in experimental design and moth colonies, it is possible that the differences in these results could be due to the different classes and concentrations of sugar used in the different studies. While Miller (1988) utilized 15% fructose-water in his experiment, Leahy and Andow (1994) used both fructose (42% solution) and sucrose at very high concentrations. Since only 8% sucrose-water was presented to adults in the current study, it is possible that the use of a higher concentration of sucrose may lead to a significant improvement in fecundity. That adult feeding may contribute to egg production in *O. nubilalis*, as shown for some closely related pyralid species (Waldbauer et al., 1980; Taylor and Sands, 1986), seems not unlikely in light of the report that adult European corn borer females eclose with an average of only 90 mature oocytes (Miller, 1988).

Although multiple-mated females generally produced significantly more progeny than once-mated females in the current study, some (about 10%) of the females that mated once had over 90% of their eggs fertilized by the

single mating. It thus appears that a female's need to remate in order to acquire enough sperm to fertilize her full complement of eggs may depend on the size of her egg complement, as well as the size of the spermatophore transferred by her first mate. Indeed, Royer and McNeil (1993) have recorded a negative correlation between successive matings and the size of spermatophores transferred by male *O. nubilalis*. This decrease in spermatophore volume with subsequent matings translated to a decrease in the fertility of successive mates of a given male. A study in which both the size of a European corn borer female's full egg complement (potential fecundity) and the volume of spermatophores transferred by males are controlled may be necessary to examine possible interactions among these factors and remating tendency/frequency.

In the current study, a 3-day delay in mating was shown to significantly reduce fecundity, while a 1-week delay resulted in near zero fertility. This considerable reduction in fertility in female *O. nubilalis* that experienced a delay in mating would offset any adaptive value of the ability of these females to prolong their preoviposition period. Knight (1997) recorded a 40% reduction in fecundity for *Cydia pomonella* females that experienced a 2-day delay in mating. No significant reduction in reproductive output, however, was recorded for female Spiny bollworm, *Earias insulana* whose mating was delayed up to about the 9th night (Kehat and Gordon, 1977). It thus appears that whether or not a delay in mating would affect reproduction depends on the biology of the species. For instance, in the case of *E. insulana*, the preoviposition period was greatly prolonged in unmated females, enabling the female to delay oviposition until after mating. Also, female *E. insulana* had a higher natural longevity and longer oviposition period (Kehat and Gordon, 1977) than female *O. nubilalis* (present study). It is, perhaps, reasonable to suggest that longer-lived females with a longer oviposition period might also have a longer period of receptivity to the male, compared with short-lived species. Invariably, species that possess an efficient mechanism of this type that ensures reproductive success, will be relatively unaffected by a delay in mating. The present results suggest that the female European corn borer had either stopped calling, or perhaps was no longer receptive to the male after the first week of emergence.

Female secretions are known to be involved in the liberation of sperm from spermatophores (Mordue et al., 1980). It may, therefore, be that the mechanism(s) associated with the process of spermatophore depletion/sperm utilization, including the production of the necessary female secretions, was no longer functional after the 7th day of emergence, since the few females that mated at this age (about 20%) did not deplete spermatophores transferred by males.

The lower multiple matings (30–38%) recorded in the

Table 3
Effect of delay in mating on reproductive performance and longevity of single-mated female *O. nubilalis*

Timing of first exposure to male (days after emergence)	No. females observed	Fecundity (no. of eggs deposited)	% fertility	Pre-oviposition period (days)	Longevity (days)	Index of remaining eggs
1	32	591 ± 316a	65.4 ± 35.4a	3.2 ± 1.0c	12.9 ± 3.8b	1.59 ± 1.4
4	16	437 ± 209b	63.8 ± 34.7a	5.2 ± 1.8b	13.0 ± 3.5b	1.81 ± 1.2
7	5	342 ± 229b	0.19 ± 0.42b	6.8 ± 1.3a	16.6 ± 5.0a	2.40 ± 1.9

Values (means ± SD) in the same column having no letters in common are significant at $P < 0.05$

nificant improvement in all reproductive performance parameters, compared with water-fed single-mated females ($P > 0.05$; Table 4A). However, a significantly higher mean longevity ($P = 0.05$) was recorded for sugar-fed (13.8 days), than for water-fed single-mated females (11.6 days; Table 4A).

For unmated females, only the 1- and 4-day-old unmated (paired and unpaired) females that deposited at least one egg were considered for statistical analysis of the effect of food on reproduction and longevity. No significant effect of food was recorded on the fecundity, preoviposition period, or longevity of unmated females ($P > 0.05$; Table 4B).

Owing to the low proportion of multiple-mated females in this study, a statistical analysis was not conducted to test possible effects of food on the tendency of females to remate. However, remating rates of water-fed females (9/27) tended to be higher than for sugar-fed females (4/34).

4. Discussion

In addition to enhancing fecundity, multiple mating was shown to increase the fertility of female European corn borer in this study. Results from other studies on various lepidopteran species have recorded similar positive correlation between mating frequency and fecundity

(Wilkund et al., 1993; Svård and McNeil, 1994; Ward and Landolt, 1995; Landolt, 1997), fertility (Marks, 1976; Delisle and Hardy, 1997), or both (Svård and McNeil, 1994; Delisle and Hardy, 1997). Simmons (1988) also recorded increased fecundity and hatching success for multiple-mated female crickets, *Gryllus bimaculatus*. Alternative hypotheses that have been proposed regarding the role of multiple mating in female insects include: increase in genetic diversity of offsprings, sperm competition, prevention of possible genetic defects due to long-term storage of sperm, minimization of time and energy costs of resisting males, and achievement of factors that stimulate further egg development (Parker, 1970; Drummond, 1984; Halliday and Arnold, 1987; Marshall and McNeil, 1989; Ramaswamy et al., 1997).

The observed increase in the fecundity of multiple-mated females has been attributed to the role in egg production, of male-derived nutrients transferred along with sperm during mating (Boggs, 1981; Svård and McNeil, 1994). Male accessory gland secretions transferred with spermatophore during copulation have been shown to play an important role in reproduction (Leopold, 1976; Boggs and Gilbert, 1979; Watanabe, 1988; Boggs, 1990). Female insects may acquire resources necessary for egg production (Boggs, 1990), or achieve factors that stimulate egg maturation (Ramaswamy et al., 1997), from the male at the time of mating. However, it is not

Table 4
Effect of sugar feeding on reproductive output and longevity of female *O. nubilalis*: (A) single-mated females; (B) unmated females

Food category	No. females observed	Fecundity (no. of eggs deposited)	% fertility	Pre-oviposition period (days)	Longevity (days)	Index of remaining eggs
<i>(A) Single-mated females</i>						
Water-fed	18	498 ± 278	55.3 ± 38.3	4.0 ± 1.9	11.6 ± 3.2b	1.56 ± 1.42
Sugar-fed	30	564 ± 312	70.7 ± 31.8	3.8 ± 1.5	13.8 ± 3.7a	1.73 ± 1.36
<i>(B) Unmated females</i>						
Water-fed	31	149 ± 166	0 ± 0	—	14.9 ± 3.5	3.84 ± 1.09
Sugar-fed	21	185 ± 209	0 ± 0	—	15.4 ± 4.2	3.81 ± 1.12

Values (means ± SD) in the same column, under the same classification, having no letters in common are significant at $P < 0.05$.

- borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), in Southwestern Ontario. Canadian Entomologist 109, 117–122.
- Halliday, T., Arnold, S.J., 1987. Multiple matings by females: a perspective from quantitative genetics. Animal Behavior 35, 939–941.
- Herman, W.S., Barker, J.F., 1977. Effect of mating on monarch butterfly oogenesis. Experientia 33, 688–689.
- Kehat, M., Gordon, D., 1977. Mating ability, longevity and fecundity of the spiny bollworm, *Earias insulana* (Lepidoptera: Noctuidae). Entomologia Experimentalis et Applicata 22, 267–273.
- Kira, M.T., Guthrie, W.D., Huggans, J.L., 1969. Effect of drinking water on production of eggs by the European corn borer. Journal of Economic Entomology 62, 1366–1368.
- Klun, J.A., Chapman, O.L., Mattes, K.C., Wojtkowski, P.W., Beroza, M., Sonnet, P.E., 1973. Insect sex pheromones: minor amount of opposite geometrical isomer critical to attraction. Science 181, 661–663.
- Knight, A.L., 1997. Delay of mating of codling moth in pheromone disrupted orchards. In: Witzgall, P., Arn, H. (Eds.), Technology Transfer in Mating Disruption. IOBC wprs Bulletin, vol. 20, Montpellier, France, pp. 203–206.
- Landolt, P.J., 1997. Cabbage looper (Lepidoptera: Noctuidae) fecundity maximized by a combination of access to water and food, and remating. Annals of Entomological Society of America 90, 783–789.
- Leahy, T.C., Andow, D.A., 1994. Egg weight, fecundity, and longevity are increased by adult feeding on *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Annals of Entomological Society of America 87, 342–349.
- Leopold, R.A., 1976. The role of male accessory glands in insect reproduction. Annual Review of Entomology 21, 199–221.
- Loughner, G.E., 1971. Precopulatory behavior and mating success of the European corn borer under controlled conditions. Iowa State Journal of Science 46, 1–6.
- Marks, R.J., 1976. Mating behavior and fecundity of the red bollworm *Diparopsis castanea* Hmps. (Lepidoptera: Noctuidae). Bulletin of Entomological Research 66, 145–158.
- Marshall, L.D., McNeil, J.N., 1989. Spermatophore mass as an estimate of male nutrient investment: a closer look in *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae). Functional Ecology 3, 605–612.
- McNeil, J.N., Delisle, J., Cusson, M., 1997. Regulation of pheromone production in Lepidoptera: the need for an ecological perspective. In: Cardé, R.T., Minks, A.K. (Eds.), Insect Pheromone Research: New Directions. Chapman and Hall, New York, pp. 31–41.
- Miller, W.E., 1988. European corn borer reproduction: effects of honey in imbibed water. Journal of the Lepidopterists Society 42, 138–143.
- Mordue, W., Goldsworthy, G.J., Brady, J., Blaney, W.M., 1980. Insect Physiology. Blackwell Scientific Publications, Oxford.
- Onokogu, F.A., Guthrie, W.D., Awadallah, W.H., Robbins, J.C., 1980. Hatchability of eggs and mating success of European corn borer cultures reared continuously on a meridic diet. Iowa State Journal of Research 54, 347–355.
- Parker, G.A., 1970. Sperm competition and its evolutionary consequences in the insects. Biological Review 45, 525–567.
- Pesho, G.R., 1961. Female mating patterns and spermatophore counts in the European corn borer, *Pyraustae nubilalis* (Hübner). Proceedings of the North Central Branch of Entomological Society of America 16, 43.
- Ramaswamy, S.B., Shu, S., Park, Y.I., Zeng, F., 1997. Dynamics of juvenile hormone-mediated gonadotropism in the Lepidoptera. Archives of Insect Biochemistry and Physiology 35, 539–558.
- Royer, L., McNeil, J.N., 1991. Changes in calling behaviour and mating success in the European corn borer (*Ostrinia nubilalis*), caused by relative humidity. Entomologia Experimentalis et Applicata 61, 131–138.
- Royer, L., McNeil, J.N., 1993. Male investment in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae): impact on female longevity and reproductive performance. Functional Ecology 7, 209–215.
- SAS Institute Inc., 1985. SAS User's Guide: Statistics, Version 5 ed. SAS Institute Inc., Cary, NC.
- Showers, W.B., Reed, G.L., Oloumi-Sadeghi, H., 1974. Mating studies of female European corn borers: relationship between deposition of egg masses on corn and captures in light traps. Journal of Economic Entomology 67, 616–619.
- Showers, W.B., Reed, G.L., Robinson, J.F., DeRozari, M.B., 1976. Flight and sexual activity of the European corn borer. Environmental Entomology 5, 1099–1104.
- Simmons, L.W., 1988. The contribution of multiple mating and spermatophore consumption to the lifetime reproductive success of female field crickets (*Gryllus bimaculatus*). Ecological Entomology 13, 57–69.
- Svärd, L., McNeil, J.N., 1994. Female benefit, male risk: Polyandry in the true armyworm *Pseudaletia unipuncta*. Behavioral Ecology and Sociobiology 35, 319–326.
- Taylor, M.F.J., Sands, D.P., 1986. The effects of aging and nutrition on the reproductive system of *Samea multiplicalis* Guenée (Lepidoptera: Pyralidae). Bulletin of Entomological Research 76, 513–517.
- Taylor, O.R., 1967. Relationship of multiple mating to fertility in *Atteva punctella* (Lepidoptera: Yponomeutidae). Annals of Entomological Society of America 60, 583–590.
- Waldbauer, G.P., Marciano, A.P., Pathak, P.K., 1980. Life-span and fecundity of adult rice leaf folders, *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae), on sugar sources, including honeydew of the brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). Bulletin of Entomological Research 70, 65–71.
- Ward, K.E., Landolt, P.J., 1995. Influence of multiple matings on fecundity and longevity of female cabbage looper moths (Lepidoptera: Noctuidae). Annals of Entomological Society of America 88, 768–772.
- Watanabe, M., 1988. Multiple matings increase the fecundity of the Yellow swallowtail butterfly, *Papilio xuthus* L., in summer generations. Journal of Insect Behavior 1, 17–29.
- Wilkund, C., Kaitala, A., Lindfors, V., Abenius, J., 1993. Polyandry and its effect on female reproduction in the green-veined white butterfly (*Pieris napi* L.). Behavioral Ecology and Sociobiology 33, 25–33.

current laboratory study, compared with our field study (70–75%) (Fadamiro et al., unpublished data) may be due to the fact that females were only supplied with virgin males in the current study. In light of the decrease in spermatophore volume with subsequent matings in male *O. nubilalis* (Royer and McNeil, 1993), we suggest that pairing of females with virgin males, as done throughout the present study, might have reduced the tendency of females to remate. Furthermore, females were allowed continuous access to males in this study, only for up to 5 days. It is, therefore, possible that some females after their first mating were still in their refractory period during the remaining window of access to male, and only became receptive again after the 5-day window. Thus, it is possible that such females could have remated had they been allowed longer access to a male.

Another interesting observation in the current study was that some females depleted only one spermatophore, even though they had two spermatophores in their bursa at death. It therefore seems unlikely that *O. nubilalis* females have been selected to mate multiply in order to increase the genetic diversity of their progeny, since one would expect all available spermatophores in the bursa to be utilized equally if genetic diversity was being enhanced. Taylor (1967) reported that multiple matings did not contribute significantly to genetic diversity in *Atteva punctella*.

Unmated females generally lived longer than mated females, and multiple-mated females tended to show reduced longevity compared with single-mated *O. nubilalis* females. A similar decrease in longevity was recorded for multiple-mated female cabbage looper moth, *Trichopulsia ni*, compared with once-mated females (Ward and Landolt, 1995). The widespread reduction in the longevity of mated female insects may likely be related to a cost associated with the utilization of female's resources during reproduction. Pheromone production, calling, courtship and oviposition are processes that require significant energy consumption. The increase in the longevity recorded for sugar-fed mated female *O. nubilalis* compared with water-fed mated females in the present study is in agreement with the results of Leahy and Andow (1994), and suggests that sugar feeding may significantly replenish females from the depletion of resources associated with reproduction. The higher longevity recorded for females that experienced a 1-week delay in mating was probably due to the fact that such females still retained within them at least half of their estimated total egg complement at death. Hence, their longevity was similar to that of females that had no access to males throughout their lifetime. These results suggest that the resources necessary for somatic maintenance are, perhaps, being utilized during oviposition. Ramaswamy et al. (1997) hypothesized that the increase in longevity recorded for virgin female moths

may be due to egg resorption caused by the absence of a male-derived endogenous gonadotropic signal.

In summary, our results suggest that *O. nubilalis* females may mate multiply in order to enhance reproduction. Therefore, it seems reasonable to expect a significant reduction in the deposition and fertility of eggs with a pheromone-mating disruption program in which a significant decrease in mating frequency of females is achieved. Furthermore, the significant reduction in reproduction in females that experienced a 3- or 7-day delay in mating suggests that an economic control of *O. nubilalis* population may be achieved by effectively disrupting mating at the beginning of the season. Whether or not such mating reductions can lead to significant economic suppression of damage from this species remains to be seen.

Acknowledgements

We thank Jean Dyer for insect rearing, James Robbins for technical assistance and use of facility, and Brad Binder and Richard Hellmich for helpful suggestions. This study was undertaken with the funds provided (to TCB) by the Leopold Center for Sustainable Agriculture, Iowa.

References

- Binder, B.F., Robbins, J.C., 1996. Age- and density-related oviposition behavior of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Journal of Insect Behavior* 9, 755–769.
- Boggs, C.L., 1981. Nutritional and life-history determinants of resource allocation in holometabolous insects. *The American Naturalist* 117, 692–709.
- Boggs, C.L., 1990. A general model of the role of male-donated nutrients in female insects' reproduction. *The American Naturalist* 136, 598–617.
- Boggs, C.L., Gilbert, L.E., 1979. Male contribution to egg production in butterflies: evidence of transfer of nutrients at mating. *Science* 206, 83–84.
- Caffrey, D.J., Worthley, L.H.A., 1927. A progress report on the investigations of the European corn borer. *US Department of Agriculture Bulletin No. 1476*.
- Cantelo, W.W., Jacobson, M., 1979. Phenylacetaldehyde attracts moths to bladder flowers and blacklight traps. *Environmental Entomology* 8, 444–447.
- Delisle, J., Hardy, M., 1997. Male larval nutrition influences the reproductive success of both sexes of the spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Functional Ecology* 11, 451–463.
- Drecktrah, H.G., Brindley, T.A., 1967. Morphology of the internal reproductive systems of the European corn borer. *Iowa State Journal of Science* 41, 467–480.
- Drummond, B.A., III, 1984. Multiple mating and sperm competition in the Lepidoptera. In: Smith, R.L. (Ed.), *Sperm Competition and the Evolution of Animal Mating Systems*. Academic Press, New York, pp. 291–370.
- Elliot, W.M., 1977. Mating frequency of the female European corn