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**MATING FREQUENCY OF EUROPEAN CORN BORER  
(LEPIDOPTERA: CRAMBIDAE) IN MINNESOTA, KANSAS, AND TEXAS**

J. L. Hinton<sup>1</sup> and D. A. Andow<sup>1</sup>

**ABSTRACT**

The frequency of mating and polyandry in natural populations are important parameters for understanding evolutionary dynamics. Mating frequency among natural populations of *Ostrinia nubilalis* (Hübner) [Lepidoptera: Crambidae] are quite variable. Showers et al. (1974) found 91.1, 73.8, and 71.3% of females had mated during the second flight over 1971-3 at one location in Iowa. During 1971, only 10% mated multiple times, with lower levels of polyandry in subsequent years. In an earlier study in Iowa, Pesho (1961) found that 65-100 % of females had mated and up to 43% had mated more than once. A population in southwestern Ontario averaged 73% mating and 37% polyandry for the 5-year period from 1971-5, a higher rate of polyandry than during the same period in Iowa (Elliot, 1977). In this note, we amplify these previously published results by reporting the mating status of female *O. nubilalis* captured in light traps in Minnesota, Kansas and Texas. We also provide evidence that some females in natural populations may be sperm-limited.

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Female *O. nubilalis* were collected alive using BioQuip black light traps throughout the entire second flight period during the summers of 1999 and 2000. Traps were suspended above grassy areas next to corn fields at least 200 m from another trap. Collection intensity varied among sites, but was constant within sites. At Rosemount, MN samples were collected 3-5 nights a week from 4 traps, at Lambertson, MN samples were collected 2-3 nights a week from 6 traps, while in Garden City, KS and Edmonson, TX, samples were taken 1-3 nights a week from 1 or 2 traps. In most samples,  $\approx$  4 adults were caught per trap-night, and the highest densities were at Rosemount during 2000. While it is possible that females mated in the traps before we collected them during the morning, we note that the highest captures of unmated females were from the Rosemount 2000 samples (see results).

Moths were placed in individual cages (8.5 cm diam.  $\times$  6.5 cm) in a walk-in growth chamber, 16L:8D, 29:15 °C and 80% RH, and provided water (1999) or both water and sugar/agar diet (2000) (Leahy and Andow 1994). Females were allowed to oviposit on waxed paper sheets until the female died. Dead moths were dissected to determine the number of spermatophores in the bursa copulatrix, and the presence or absence of eggs in the ovaries. Spermatophore counts are commonly used to study mating frequency in *O. nubilalis* (Drecktrah and Brindley 1967; Showers et al. 1974; Fadamiro and Baker 1999). Abdomens were placed in 10% KOH for 1.5 hours to soften the tissue. Although KOH can clear insect cuticle, at the low concentration and short time exposure we used, it probably did not clear or digest existing spermatophores from our samples, because abdomens removed from KOH retained pigmented abdominal cuticle, ovarian tissue, some fat body tissue, and a pigmented, intact bursa copulatrix. The presence of eggs in the ovaries was noted, and the number of spermatophores counted in the bursa copulatrix. Occasionally clear, completely unsclerotized spermatophores were observed. Dissection results for each moth were matched with her oviposition record to determine if she laid eggs and if those eggs were viable. Mating history

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was scored as the number of spermatophores in the bursa. Oviposition history was scored as no, sterile, or fertile eggs laid. Ovary condition in abdomens were scored as either having mature eggs or not.

Each locality and year combination was treated as a different collection. The number of females was the response variable, and the effects of mating history, ovary condition, oviposition history and collection were analyzed by log-linear contingency table analysis (SAS 1999). We pooled the data by combining the 2 and 3 spermatophore categories into one "multiply-mated" category. Only 9 females were observed to have 3 spermatophores.

There was significant variation among collections in mating history ( $g^2 = 18.38$ ,  $df = 8$ ,  $P = 0.0186$ ) (Table 1). Polyandry varied from 7.0% (Texas 2000) to 15.1% (Lamberton 2000), and females without a detectable spermatophore ranged from 17.1% (Kansas 2000) to a high of 38.3% (Rosemount 2000) (Table 1). The range of variation is similar to that previously reported (Pesho 1961, Showers et al. 1974, Elliot 1977). A higher proportion of females were mated at Lamberton during 2000 than during 1999 (Table 1). The observed variation in spermatophore number may have been related to differences in male density or sex ratio (Elliot 1977) with lower mating associated with a relative scarcity of males, but it was probably also related to other factors, such as female age (Fadamiro and Baker 1999), egg load and spermatophore size (Royer and McNeil 1993).

The only other statistically significant effect was the relation between ovary condition and mating history ( $g^2 = 20.35$ ,  $df = 2$ ,  $P < 0.0001$ ) (Table 1). Most females without eggs in their ovaries at death had a spermatophore (85.6%), while 50.9% of the females with eggs had no spermatophore. It is not surprising that many unmated females did not lay any eggs, while mated females laid all of their eggs before dying. Male ejaculate in many Lepidoptera stimulates oocyte development and oviposition (Gillott 2002).

We observed that 30.0% (158/529) of all field-collected females with detectable spermatophores did not lay fertile eggs in the laboratory environment (Fig. 1). Of these, 28.5% (45/158) had no eggs in their ovaries at death, which implies that they either resorbed their eggs or had no eggs to lay (perhaps having laid them all prior to capture). However, 43.0% (68/158) laid only infertile eggs, which suggests that some females are sperm-limited in the field. In addition, 28.5% (45/158) had eggs in their ovaries but refused to lay any eggs. We had supplied similar females additional mates in the laboratory, but most still did not oviposit (Bourguet et al. 2003), which indicates that these females may have refused to oviposit under laboratory conditions. It is also possible that these females had mated with poor quality males and had not been induced to mate (Gillott 2002).

We also observed 23 females that had no detectable spermatophore that laid viable eggs. As we noted in our methods, we are unlikely to have destroyed or missed observing a spermatophore in the bursa copulatrix. We observed considerable variation in the degree of sclerotization of the spermatophores, so we suggest that a small proportion of spermatophores are completely digested by some females.

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Table 1. Percent of *Ostrinia nubilalis* females (number in parentheses) categorized by the number of spermatophores present in the bursa (Multiple = 2+ spermatophores), location and year, and absence or presence of eggs in the ovaries.

Number of Spermatophores	Location and Year					Eggs in Ovaries			Total
	Lamberton 1999	Kansas 2000	Lamberton 2000	Rosemount 2000	Texas 2000	Absent	Present		
	0	24.0 (44)	17.1 (20)	17.8 (13)	38.3 (116)	19.3 (11)	14.4 (67)	50.9 (137)	
1	67.8 (124)	71.8 (84)	67.1 (49)	48.2 (146)	73.7 (42)	70.3 (326)	44.2 (119)	60.7 (445)	
Multiple	8.2 (15)	11.1 (13)	15.1 (11)	13.5 (41)	7.0 (4)	15.3 (71)	4.8 (13)	11.5 (84)	
Total	100 (183)	100 (117)	100 (73)	100 (303)	100 (57)	100 (464)	100 (269)	100 (733)	

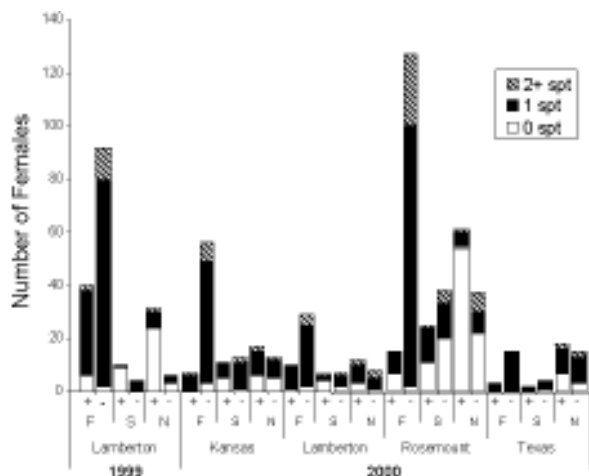


Figure 1. Number of *Ostrinia nubilalis* females (raw data) for each spermatophore class (0, 1, 2+ spermatophores (spt) in the bursa), categorized by collection (location and year), oviposition history in the laboratory (F = fertile eggs laid, S = sterile eggs laid, N = no eggs laid), and presence (+) or absence (-) of eggs in the ovaries at the end of life.

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