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ARTHROPOD MANAGEMENT

Mating Incidence of Feral *Heliothis virescens* (Lepidoptera: Noctuidae) Males Confined with Laboratory-reared Females

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ABSTRACT

Testing insecticide susceptibility on moths captured in the field has facilitated research through access to ample samples of feral insects. Traps baited with pheromones can provide numerous males, but to conduct further testing these moths need to mate with females and produce offspring. Daily assessment of mating incidence, offspring production, and mortality of laboratory-confined moths, if they are used in experiments, can aid in understanding the effective gene pool of their offspring. This study compared these basic parameters when laboratory-reared female moths of *Heliothis virescens* Fabricius were confined with either feral or laboratory-reared males for 1 to 5 d. Overall, feral moths copulated less frequently than laboratory males (68.6% versus 98.2%). The copulation frequency of laboratory males, determined by a spermatophore marker, indicated that every male had equal access to copulate with females. Higher mortality (≥65%) was observed in laboratory males and females in the presence of the opposite sex than under same-sex confinement conditions (≤35%) at the same crowding ratio, indicating that copulation carries important consequences in longevity. The significantly highest mating incidence, fertile egg production, and lowest moth mortality occurred when females were confined with feral males for only 2 d. Female fertility was 28 to 56% when copulated by feral males, and 22 to 83% when copulated by laboratory males. This information suggests that peak genetic diversity of feral male offspring is reduced by approximately 30% when accounting for copulation frequency and female fertility. Studies using the progeny of moths (feral or laboratory-reared) confined for 2 d offer the possibility of obtaining the greatest female and male genetic representation and the most abundant number of fertile eggs.

The tobacco budworm (*Heliothis virescens* Fabricius) has historically been a major pest of cotton. A critical factor that affects the status of *H. virescens* as a pest has been the development of resistance to a wide range of synthetic insecticides (Sparks, 1981; Luttrell et al., 1987; Hardee et al., 2001; Terán-Vargas et al., 2005). Since 1996, *H. virescens* can be effectively controlled by commercial cultivars of cotton (*Gossypium hirsutum* L.) genetically engineered to express the insecticidal Cry proteins from the bacterium *Bacillus thuringiensis* Berliner (Bt). The possibility of developing resistance to these proteins is an important issue. The delay of resistance evolution to insecticidal Bt proteins in important pests is considered in 'the public good' by the United States Environmental Protection Agency (EPA) (Matten & Reynolds, 2003). As a consequence, companies that want to register Bt crops must provide an insect resistance management plan to the EPA. Part of this plan must include resistance monitoring for targeted pests of the transgenic crop.

To detect changes in tobacco budworm susceptibility to Bt proteins, a Bt resistance monitoring program has been developed and implemented since 1995. The USDA-Agricultural Research Service (ARS) at Stoneville, Mississippi, in collaboration with multiple research institutions in North America, received males of *H. virescens* captured in pheromone traps in the field to test the response of F1 and F2 generations to Bt proteins. Although mating feral males with laboratory females only represents part of the genetics of this pest, this method facilitated the capture of feral moths in space and time. Because it has become increasingly more difficult to collect

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tobacco budworm moths (Parajulee et al., 2004; Blanco et al. 2005), it is important to maximize efforts to make the best use of samples and achieve an ample genetic representation of males collected in the field. Because the frequency of resistance alleles to the Bt protein Cry1Ac in field populations of *H. virescens* is believed to be 1.5 x 10^{-3} (Gould et al., 1997), adequate testing for this protein should involve large numbers of insects to assess any changes on resistance frequency. With a limited sample, the probability of 'missing' those rare resistance alleles in any given sample is high. To meet the goal of obtaining the maximum genetic representation from field samples, it is critical to understand the reproductive interactions among moths that take place under laboratory conditions.

Under the current USDA-ARS Stoneville Bt resistance monitoring protocol, 0 to 66% of the field-captured males die during overnight delivery transit to the ARS-Stoneville laboratory (Blanco, unpublished data). Once the survivors from the samples are used to establish colonies, mating success under laboratory conditions can be influenced by a number of factors. Temperature and relative humidity influence moth longevity and fecundity, and larval hatch (Ellington and El-Sokkari, 1986) and reproductive maturity (Colvin et al., 1994). In addition, the ratio of females to males in mass mating conditions directly affects fecundity and oviposition patterns (Guerra et al., 1972; Ellington and El-Sokkari, 1986). Tobacco budworm adults collected directly from the field as larvae show low mating frequency and low oviposition patterns when first introduced into laboratories. In addition, it may take three generations for the reproductive behavior of feral insects to resemble that of a laboratory strain (Raulston, 1975). Male age (Henneberry and Clayton, 1985; LaMunyon and Huffman, 2001) and the number of generations that females have been kept in the laboratory (Laster and Stadelbacher, 1991) may also play an important role in maximizing the number of copulations. All of these obstacles have the potential for decreasing both genetic diversity and the number of offspring resulting from field-collected males.

With the introduction of new proteins from *B. thuringiensis* into some cotton cultivars, susceptibility to Bt testing will require larger numbers of insects, increasing the need for greater field samples, higher mating frequency, and more progeny per individual moth. Because data generated under this method are presented to regulatory agencies and industry, a better understanding of the actual genetic diversity tested may be an important component in understanding Bt-resistance frequency over time. The goals of this study were to assess reproductive incidence of *H. virescens* based on temporal dynamics of mating and oviposition patterns and longevity. Mating data generated will be used to develop a better protocol that will maximize the genetic contribution of males collected in the field in bioassays. Data will also assist in calculating the genetic diversity in our Bt-resistance monitoring tests.

MATERIALS AND METHODS

Males of *Heliothis virescens* were captured in pheromone traps near agricultural fields (referred to as feral moths) in Florida (Gasden County), Georgia (Decatur, Mitchell, Tift, and Appling counties), and Texas (Brazos County). Moths captured over a 1- to 2-d period were shipped by overnight delivery in 0.4 L (referred to as pint-size) carton containers (8.5 cm Ø; Neptune; Newark, NJ) to the USDA-ARS facility in Stoneville, Mississippi. Upon arrival, males from different geographies were immediately confined with 24- to 36-h-old laboratory-reared (King et al., 1985) females at a ratio of 30♀ : 30♂.

Confinement arenas were 3.7l L containers (carton, 17.0 cm Ø, Neptune) capped with cheese cloth (Batist; Zweigar; Piscataway, NJ) and a sponge (4 x 6 x 1.5 cm; Janitors Supply, Inc.; Erie, PA) saturated with 10% (w:v) sucrose solution. The cheese cloth was changed and the sponge was re-wetted daily. Confinement arenas were maintained in an incubator at 27 ± 1 °C, 75 ± 10% RH, and 14:10 h L: D for 1 to 5 d. After a fixed number of confinement days ("exposure time" 1 to 5 d), each arena was placed in a refrigerator (3 °C) for a few minutes to diminish moth activity. Living and dead moths were counted, males were discarded, and 20 to 25 females (depending on female mortality) were individually transferred to pint-size containers with 15 mL of sucrose solution in a 30-mL plastic cup with a tissue (Kimwipe EX-L, Kimberly-Clark, Roswell, GA) stuffed into the cup to prevent the moths from drowning. The container was capped with a piece of cheese cloth. Pint-size containers were randomly moved daily between shelves in an incubator under the above described environmental conditions. Cheese cloths were changed daily for days 1 through 6, and the number of eggs deposited and adult female mortality were assessed. Cheese cloths with eggs were

individually placed in sandwich-size plastic bags and maintained for 2 d in an incubator at the same environmental conditions. The number of fertilized eggs (dark-brown eggs to hatched larvae) produced by each female was recorded for 6 consecutive days. At the end of the trial, all females were dissected to determine the number of spermatophores present in the bursa copulatrix.

Mass mating and mortality. To assess the role that crowding and/or mating had on moth mortality, 30♀ : 30♂ or 60 same sex laboratory-reared moths that were 24- to 36-h-old were maintained for 5 d under the same previously described manner, except they were not placed into pint-size containers. Mortality was assessed daily. This procedure was repeated 4 times on different dates.

In a separate experiment, laboratory males were fed a 10% sucrose solution or 0.1% (w : v) rhodamine B (95%; Sigma-Aldrich Co.; Saint Louis, MO) dissolved in 10% sucrose solution for 2 d. These males were confined with 2-d-old females (30♀ : 30♂) so that 2, 5, 8, 11, 14, or 17 of the 30 males in each confinement arena were rhodamine-fed males. Moths were left to copulate for 2 d and spermatophores were dissected from each female.

The use of rhodamine as a marker for mating frequency. In another set of experiments, 30 laboratory males fed either sucrose or rhodamine were confined for 2 d with 30 2-d-old laboratory females to assess the potential effect that rhodamine may have had on mating frequency. Spermatophores were obtained from each copulated female. This experiment was repeated four times in different dates.

Another set of experiments investigated into the potential contamination between a rhodamine-marked and an unmarked spermatophore when in contact inside a bursa copulatrix. A 2-d-old female was introduced for 1 d into a pint-size container with a rhodamine-fed male. The marked male was removed and an unmarked male then introduced for 1 d. The opposite treatment (an unmarked male introduced first) was concurrently done. After exposure to males, spermatophores were extracted. This experiment was repeated 4 times in different dates.

Spermatophores of experiments that included rhodamine solution were examined on a fluorescent microscope (Axioscop 2; Zeiss; Thornwood, NJ) with an illuminator (N HBO 103; Zeiss) and a rhodamine filter (absorption 540 nm / emission 625 nm). The filter optimized visualization of rhodamine-treated (positive resolution) compared with untreated (negative resolution) spermatophores virtually invisible in this light. Images of positive and negative resolution were electronically captured utilizing the AxioVision (version 2.05; Zeiss) computer program (Blanco et al., in press).

Data analyses. Data analyses were performed as randomized complete blocks. Each block was a temporal replicate of the experiment. Independent variables were the origin of males (laboratory versus feral) and the length of time males and females were exposed to one another (exposure time). Restricted-maximum likelihood estimates of sources of variance were used to perform two-way analysis of variance (ANOVA). Analyses were performed in Proc Mixed of SAS (version 8.2; SAS Institute; Cary, NC) (Littel et al., 1996). Fixed sources of variance included origin of males, exposure time, and interactions between these effects. Block effects were considered as a random source of variance. Least-squared means and their standard errors are represented in the text and graphs. Differences between feral versus laboratory males were tested for each level of exposure time via the slice option of the lsmeans statement. Differences are presented within graphs.

Dependent variables measured included number of spermatophores per living female, proportion of living females that were mated, number of fertile eggs per living female, and percentage of dead females.

Because mortality measures were taken during each mating arena over five consecutive days (cumulative mortality), a repeated-measures design was used in the ANOVA (Proc Mixed). A heterogeneous, first-order autoregressive structure best modeled the covariance among subjects. Effects in the model included the four treatments described above, time (day 1 through day 5), and their interaction. Significant differences for the treatment effects were partitioned into three orthogonal contrasts: (1) comparison of cumulative percentage mortality in confinement arenas with the opposite sex versus without the opposite sex, (2) comparison of cumulative percentage mortality for 30 moths versus 60 moths in single confinement arenas, and (3) comparison of cumulative percentage mortality in confinement arenas containing feral males versus laboratory males. Time effects were also completely partitioned into polynomial functional responses (i.e., linear, quadratic, cubic, and remaining effects).

Comparisons of rhodamine detection and the effect it had on copulations were made with single-paired t-test using a binomial distribution (version 9.1; SAS Institute) to compare proportion of marked males.

RESULTS

Mating dynamics. The proportion of females that mated varied with the origin of the males (F = 68.17; df = 1, 30; $P < 0.0001$), the amount of time males and females were exposed to each other (F = 4.77; df = 4, 30; $P = 0.0042$), and the interaction between origin and exposure time (F = 3.02; df = 4, 30; $P = 0.0331$). The percentage (mean ± SE) of mated laboratory females was greater when females had access to laboratory males (98.2 ± 1.6%) than when exposed to feral males (68.4 ± 2.6%). The proportion of females that mated with laboratory males did not vary during the experiment (contrast $F = 0.13$; df = 4, 30; $P = 0.9689$) (Fig. 1). Feral males did not mate as readily (<50% females were mated on day 1), but by day 4 approximately 86% of females were mated. The number of spermatophores per living female varied significantly with the origin of males ($F = 107.65$; df = 1, 30; $P < 0.0001$), amount of time males and females were exposed to one another (F = 27.66; df = 4, 30; $P < 0.0001$), and interaction between origin and exposure time (F = 10.55; df = 4, 30; $P < 0.0001$). Females in confinement arenas containing laboratory males had significantly more spermatophores (2.58 ± 0.10) than in confinement arenas with feral males (1.06 ± 0.10). The average difference in the number of spermatophores when females had access to laboratory versus feral males increased with exposure time (Fig. 2). When males and females both originated from the laboratory, multiple mating increased dramatically with exposure time. For confinement arenas containing feral males, multiple matings were less frequent.

Egg production. The number of fertile eggs per living female was significantly affected by the amount of time females were exposed to males (F = 3.70; df = 4, 27; $P = 0.0158$). Although feral and laboratory males were different in mating incidence, the origin of males ($F = 0.06$; df = 1, 27; $P = 0.8134$) and its interaction with exposure time (F = 1.26; df = 4, 27; $P = 0.3086$) did not significantly affect the number of fertile eggs per female (Fig. 3). Egg production was greatest after 2 to 3 d of exposure between males and females (exposure at day 2 and day 3 compared with other exposure times) (F = 12.45; df = 1, 27; $P = 0.0015$). There were no other differences among exposure times for egg production (day 2 versus day 3; F =1.41; df = 1, 27; $P = 0.2461$; nor differences among days 1, 4, and 5; $F = 0.48$; df =2, 27; $P = 0.6261$) (Fig. 4).

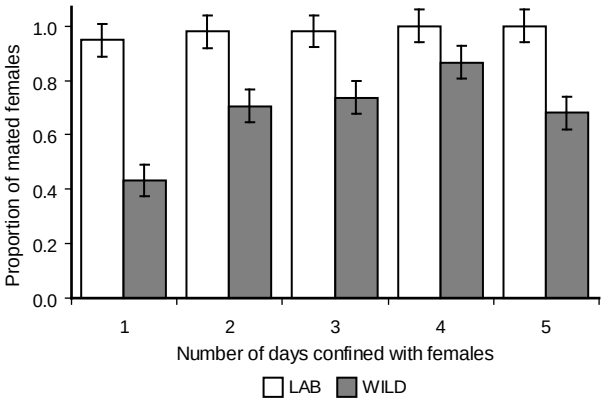

Figure 1. Proportion of females containing one or more spermatophores in their bursa copulatrix (mated) after confinement for 1-5 days with laboratory-reared or feral (wild) males. Bars represent ± SEM.

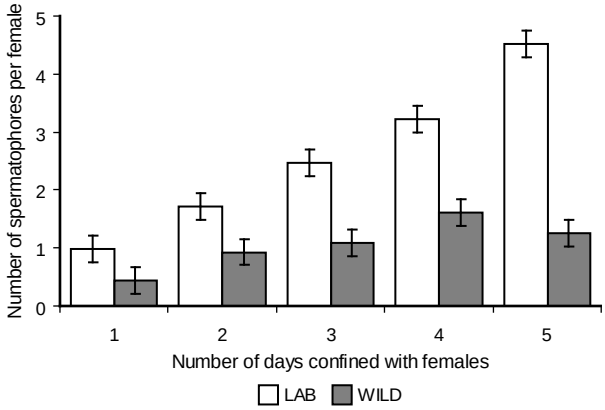

Figure 2. Average number of spermatophores in the bursa copulatrix of females confined for 1-5 days with laboratory-reared or feral (wild) males. Bars represent ± SEM.

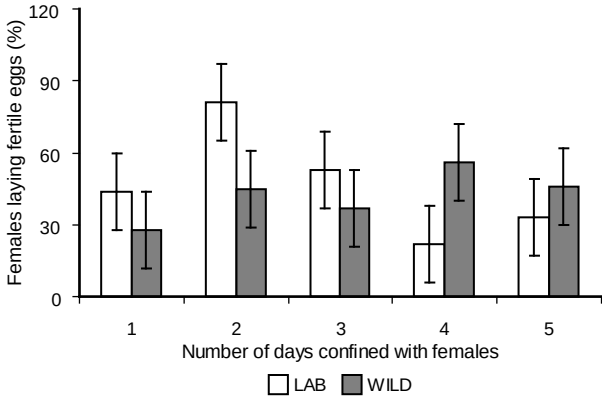

Figure 3. Percentage of females laying fertile eggs after they were confined for 1-5 days with laboratory-reared or feral (wild) males. Bars represent ± SEM.

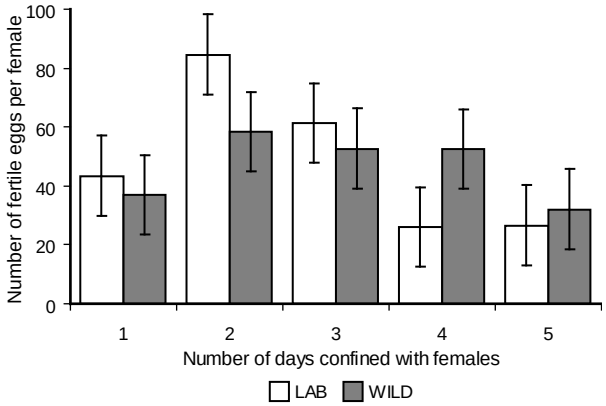

Figure 4. Average number of fertile eggs oviposited over a 6-d period by females confined for 1-5 days with laboratory-reared or feral (wild) males. Bars represent ± SEM.

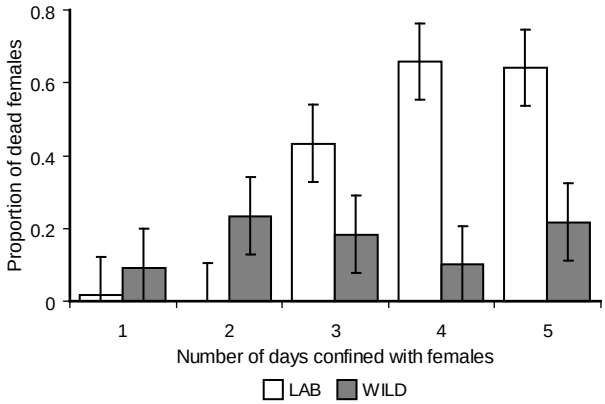

Figure 5. Female mortality when confined for 1-5 days with laboratory-reared or feral (wild) males. Bars represent ± SEM.

Mortality. Female mortality in these mating studies varied with the origin of males ($F = 10.13$; df = 1, 27; $P = 0.0037$), amount of time males and females were exposed to one another ($F = 6.39$; df = 4, 27; $P = 0.0009$), and the interaction between origin and exposure time ($F = 6.56$; df = 4, 27; $P = 0.0008$) (Fig. 5). Female mortality was greater in mating arenas containing laboratory males (35.0 ± 6.7%) than arenas with feral males (16.5 ± 6.7%). Differences in female mortality when laboratory males were present were especially salient after a 2-d exposure period (Fig. 5). Mortality of females in the presence of feral males did not significantly differ with exposure time ($F = 0.51$; df = 4, 27; $P = 0.7280$).

Crowding and mating effects on adult mortality. Cumulative mortality of females of *H. virescens* was affected by confinement arena treatment, time, and the interaction between them (Table 1). Females not exposed to males suffered less mortality than females exposed to males (Fig. 5; Table 1). In addition, there was greater cumulative mortality averaged over time when females were combined with laboratory males (39.3 ± 4.0%) versus feral males (24.2 ± 4.0%). In the absence of males, there was also greater mortality averaged over time in confinement arenas with 30 females (15.5 ± 4.0%) versus 60 females (3.5 ± 4.0%). The cumulative increase in mortality across time was best described by a linear

Table 1. Repeated-measures ANOVA for cumulative percentage mortality of *H. virescens* females

Source[z]	Ndf	ddf	*F*	*P*
Confinement arena	**3**	**16.1**	**14.07**	**<0.0001**
With versus without males	**1**	**16.1**	**30.65**	**<0.0001**
Without males: 30 versus 60 females	**1**	**16.1**	**4.46**	**0.0507**
With males: Feral versus laboratory males	**1**	**16.1**	**7.12**	**0.0167**
Time	**4**	**18.3**	**32.62**	**<0.0001**
Linear	**1**	**22.9**	**109.08**	**<0.0001**
Quadratic	**1**	**37.0**	**0.70**	**0.4079**
Cubic	**1**	**17.2**	**0.62**	**0.4415**
Remaining	**1**	**19.4**	**4.92**	**0.0386**
Confinement arena x time	**12**	**22.2**	**7.64**	**<0.0001**
(With versus without males) x time	**4**	**18.3**	**14.60**	**<0.0001**
(Without males: 30 versus 60 females) x time	**4**	**18.3**	**1.91**	**0.1517**
(With males: feral versus lab males) x time	**4**	**18.3**	**6.91**	**0.0014**

[z] Effects of confinement arena treatment, time, and their interaction were partitioned into a priori orthogonal contrasts to further explore causes of significance.

response (Table 1). The dependence of treatment differences and time was due to different temporal responses in confinement arenas with males versus without males, and different temporal responses in mating arenas with feral versus laboratory males (Table 1; Fig. 5).

Cumulative mortality of males of *H. virescens* was also affected by confinement arena treatment, time, and their interaction (Table 2). Males not having access to females suffered less mortality than males exposed to females (Fig. 6; Table 2). There were no differences between feral (41.2. ± 4.4%) and laboratory males (33.2 ± 4 .4%) in cumulative mortality averaged over time when they had access to females. In the absence of females, there was no significant difference in mortality averaged over time in mating arenas with 30 males (14.7 ± 4.4%) versus 60 males (4.6 ± 4.4%). The cumulative increase in mortality across time was best described by a linear response (Table 2). The dependence of treatment differences and time was due to different temporal responses of confinement arenas with males versus without males, and different temporal responses in confinement arenas with feral versus laboratory males (Table 2; Fig. 6).

Effect of rhodamine on mating parameters and proportion of marked males under confinement conditions. The proportion of rhodamine-marked spermatophores closely followed the theoretical binomial distribution. Expected versus observed marked spermatophores fit into ≥30% confidence interval (Table 3). The use of rhodamine B as spermatophore marker did not affect the average number of marked (1.73 ± 0.07) and unmarked (1.54 ± 0.07) spermatophores per female when confinement conditions included equal number of marked and unmarked males (P = 0.18, t = 1.71, df = 3). No significant differences were found between the number of marked and unmarked spermatophores per female when a rhodamine-fed male was first introduced to the container with a female (0.83 ± 0.09 versus 0.70 ± 0.09. P= 0.38, t = 1.02, df = 3) or when it was introduced as a second male (0.82 ± 0.09 versus 0.92 ± 0.09. P = 0.51, t = 0.74, df = 3).

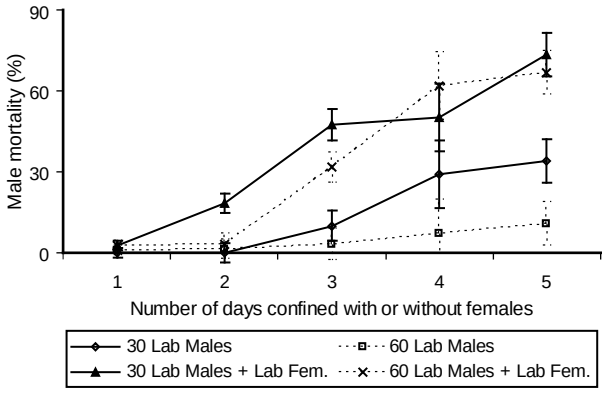

Figure 6. Male mortality when confined for 1-5 days with laboratory-reared females. Bars represent ± SEM.

Table 2. Repeated-measures ANOVA for cumulative percentage mortality of *H. virescens* males

Source[z]	Ndf	ddf	*F*	*P*
Confinement arena	3	13.2	14.53	0.0002
With versus without females	1	13.2	39.31	<0.0001
Without males: 30 versus 60 males	1	13.2	2.62	0.1294
With females: feral versus laboratory males	1	13.2	1.66	0.2196
Time	4	19.9	35.23	<0.0001
Linear	1	21.1	135.92	<0.0001
Quadratic	1	39.3	0.12	0.7284
Cubic	1	18.0	16.33	0.0008
Remaining	1	25.6	0.01	0.9440
Confinement arena x time	12	24.5	4.49	0.0002
(With versus without males) x time	4	19.9	11.91	<0.0001
(Without males: 30 versus 60 females) x time	4	19.9	1.35	0.2876
(With males: feral versus lab males) x time	4	19.9	3.57	0.0238

[z] Effects of confinement arena treatment, time, and their interaction were partitioned into a priori orthogonal contrasts to further explore causes of significance.

Table 3. Percentage of rhodamine-marked spermatophores extracted from females exposed to a known proportion of males that produce rhodamine-marked spermatophores

Marked males introduced	Total # of spermatophores recovered	Expected number	Observed number	30 % confidence interval[z]
2 of 30	39	2.6	2	1 to 3
5 of 30	44	7.3	8	6 to 8
8 of 30	42	11.2	11	10 to 12
11 of 30	41	15.0	15	13 to 16
14 of 30	28	13.0	11	11 to 14
17 of 30	47	26.6	23	25 to 28

[z] Based on binomial distribution.

DISCUSSION

Understanding the mating incidence of males captured in pheromone traps (feral) under laboratory conditions can enhance our understanding of the genetic representation of their progeny. This in turn could aid in the estimation of rare allele representation of tested samples in bioassays. One way of assessing male genetic representation in an established colony is by determining mating frequency. Dissecting the bursa copulatrix of females and counting spermatophores (Henneberry and Clayton, 1985) after they have been exposed to males for a fixed number of days is a reliable technique for determining mating frequency. Because a laboratory-adapted female can copulate daily, the increment of these male structures inside the bursa copulatrix gives an indication of the mating frequency. Knowing male copulation frequency can also help in assessing the genetic diversity of offspring.

Copulations by feral males of *H. virescens* peaked on the second day (71%), while laboratory males peaked on the first day (95%) (Fig. 1). Although the age of feral males was not known, it is assumed that they were at least 2-d-old (assuming the moth got captured the same day of emergence plus another day during shipment makes it at least 2-d-old), while laboratory males were 2- to 3-d-old at the time they were introduced with females. The fact that larger proportion of copulations in laboratory moth matings nearly reached its maximum capacity, demonstrates that females used in this study were highly sexually receptive. Laboratory-reared moths of *H. virescens* copulated almost daily for 5 d, and there was a constant increment in the average number of spermatophores per female (Fig. 2). The high frequency of copulations observed in laboratory moths might indicate that genetics (paternity) switches daily in the laboratory culture if sperm precedence, the act of the subsequent sperm to predominate over previous insemination(s), acted constantly (LaMunyon, 2000; LaMunyon and Huffman. 2001). Because this phenomenon allowing the switch of paternity occurs only in two out of three females (LaMunyon, 2001), it is possible that only a fraction (≈33%) of the males were 'represented' in the progeny by more than one female under this intense copulation frequency.

Rhodamine-marked laboratory males at known proportions (6.6 to 56.6%) were confined with females for 2 d. The resulting proportion of rhodamine-marked spermatophores closely reflects the expected proportion based on the theoretical frequency of a binomial distribution. This indicates that all laboratory males had an equal probability of copulating with females in this experiment (Table 3).

Because the transfer of sperm without spermatophores was documented by Henneberry and Clayton (1985), counting only spermatophores in females may not be an entirely accurate way of assessing mating incidence in males. Therefore, a third parameter that helps in understanding the genetic diversity under mating conditions is the proportion of females that laid fertile eggs (Fig. 3). Although this is not a direct assessment of copulation incidence, the proportion of gravid females with or without spermatophores increased the knowledge of mating interactions in this insect. Although sperm transfer without spermatophores was confirmed by the proportion of females laying fertile eggs without spermatophores, it only occurred in 3.4% of the females that mated with feral tobacco budworm males. In comparison, only 0.2% of fertile females mated with laboratory males did not contain spermatophores. The high proportion of fertile females with spermatophores (Fig. 1) indicates that

counting these structures, as suggested by Henneberry and Clayton (1985), is a reliable method.

There are other factors that also contribute to maximum genetic representation in bioassays. In addition to maximum copulation diversity, maximum oviposition of fertile eggs (number of fertile eggs per female) assists in understanding the genetic diversity of an offspring sample (Fig. 4). Here again, the proportion of copulated females (Fig. 1) and the proportion of females laying fertile eggs (Fig. 3) closely corresponds with the mean number of fertile eggs oviposited per female that was observed during the second confinement day (Fig. 4). Considering egg fertility and mating incidence together, a more robust trend is apparent for considering 2 d of confinement as the optimum time to obtain the highest number of fertile eggs with the greatest genetic diversity. Furthermore, females that are inseminated earlier in their life may use a bigger part of their fat reserves for egg production (Proshold et al., 1982), which might be reflected in increased fertile eggs in sufficient amount to result in reliable laboratory tests.

Another important aspect of the reproductive biology of this insect is the effect that copulation has on decreasing longevity (Henneberry and Clayton, 1985). Sperm competition and secondary chemicals introduced by males during copulation have been proven to have a negative effect on female longevity in other insects (Brookes, 2001). Figures 5 and 6 represent the trend of higher mortality in both sexes as the number of copulations increases. Laboratory moths at the same crowding ratios (30 or 60 in a confinement arena), but in the absence of the opposite sex, lived longer than those exposed to copulations.

Given that only 68% of the females are copulated and 45% of these are fertile, the feral male genetic representation is reduced to approximately 30%. Based on findings from this study, progeny of feral tobacco budworm males and laboratory females confined for 2 d is suggested to provide the maximum genetic diversity for bioassays. Maintaining and studying ways of enhancing this diversity is critical when using laboratory-reared individuals in screening programs.

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DISCLAIMER

Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA or the Centro de Investigaciones y Estudios Avanzados (CINVESTAV).

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