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MATING ARENA DYNAMICS FOR *OSTRINIA NUBILALIS* (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

Many bioassays of insect species are dependent on the use of laboratory-reared insects. If the purpose of the research is to assess the genetic variance present for an insect trait, e.g., insecticide-resistance monitoring, it is imperative to understand the potential mating dynamics and genetic contributions of adults to the larvae evaluated in bioassays. We report the results of a study utilizing a laboratory-reared colony of *Ostrinia nubilalis* (Hübner). The changes in the population dynamics (e.g., numbers of males, females, fertile egg masses, mated females) were evaluated. Although the numbers of emerging females, living females, mated females and fertile egg masses changed during the experiment, the percentage of total females that were mated did not change (~54%). The first of the females to emerge were beginning to die as later-emerging females were mating. Results suggest that experimental designs that rely on laboratory-reared *O. nubilalis* will need to test larvae from several nights of oviposition to better ensure that the total genetic composition of the population is sampled.

Key Words: European corn borer, mass mating, egg production, insect rearing, bioassays

RESUMEN

Muchos de los bioensayos de especies de insectos dependen del uso de insectos criados en el laboratorio. Si el propósito de la investigación es el evaluar la variación genética presente para una caracteristica del insecto, por ejemplo, el monitoreo de resistencia a insecticidas, es imperativo entender la dinámica potencial del apareamiento y la contribución genética de los adultos hacia las larvas evaluadas en los bioensayos. Reportamos los resultados de un estudio utilizando una colonia de *Ostrinia nubilalis* (Hübner) criada en el laboratorio. Los cambios en la dinámica de población (por ejemplo, el número de machos, hembras, masas de huevos fértiles, hembras apareadas) fueron evaluados. Aunque el numero de las hembras que emergieron, las hembras apareadas y las masas de huevos fértiles cambiaron durante el experimento, el porcentaje de las hembras totales que se aparearon no cambió. Las primeras hembras que emergieron empezaron a morir durnante el peeriodo en que las hembras que emergieron tarde empezaron a aparearse. Los resultados sugieren que los diseños experimentales que dependen sobre individuos de *O. nubilalis* criados en el laboratorio necesitaran probar las larvas de varias noches de oviposición para asegurar que la muestra representa la composición genética total de la población.

In order to provide the best information to agencies that regulate the use of insecticides, bioassays that measure changes in genetic susceptibility to insecticides from field collections need to be sensitive in their ability to distinguish among genotypes at loci affecting the resistance trait. Great efforts have been made to develop reliable doses and diet methods for use in resistance monitoring programs for ECB (Siegfried et al. 1995; Marcon et al. 1999; Marcon et al. 2000). Because of the focused efforts to develop assays for Bt resistance monitoring, we also need to emphasize protocols for rearing and sampling of mating arenas to ensure that the bioassays have the opportunity to discriminate among all of the genetic variability present in field populations. Otherwise, even if a bioassay is designed to do its job, resistant genotypes may be over-represented or completely missed due to laboratory artifacts having nothing to do with resistance.

For insect species mass reared in the laboratory where each female can oviposit hundreds of eggs, the contribution of individual females to the larval population is generally unknown. This may be an especially critical issue in bioassay results from field-collected individuals where the genetic variance presence for a trait is being evaluated, e. g., assays measuring resistance to insecticides. If eggs are sampled from a date when a small proportion of the total genetic variance from the field

population is represented by the eggs collected within the laboratory, then the genetic interpretations will be biased (Blanco et al. 2006). Adequately sampling the genetic variance from a field population will require an understanding of the mating and oviposition patterns of adults within a laboratory setting.

Resistance monitoring protocols involving collections of field populations do not generally include a sampling strategy for the eggs produced by the insect-rearing system. Blanco et al. (2006) reported the need for such sampling strategies for field-collected adults of *Heliothis* virescens used to monitor resistance to Bt proteins. The purpose of this project was to ask the question whether eggs collected from mass-mating arenas of the European corn borer (ECB), *Ostrinia nubilalis* (Lepidoptera: Crambidae), on different dates represent the same subset of genetic individuals. Egg production, adult emergence, adult death, and whether females had mated were measured to examine if these variables changed during the typical period of egg collection. We also examined how these variables affected one another. Because we used a newly established laboratory colony (maintained within the laboratory <5 generations), our results are more typical of expectations from field-monitoring studies than results from a long-established colony. However, the results offer a first approximation of whether mating dynamics, egg production, and mortality will change over time for ECB within a mass-mating arena.

Materials and Methods

Insect Rearing and Colony

The ECB colony originated from collections of >1000 mated females collected from light traps during Jul and Aug 2003. Colony numbers were maintained at numbers >1000 and experiments were initiated after the colony had been treated and sanitized for *Nosema pyrausta* infections (Bruck et al. 2001). The standard, semi-meridic diet was used to sustain larval development (Guthrie 1979). Mating arenas were maintained with a photoperiod of 14:10 (L:D) and a temperature of 28°C.

The following experimental design was completed during 7 temporal replicates. For each temporal replicate, 7 mating arenas with exactly 50 randomly-selected pupae were established. Mating arenas were randomly assigned to one of 7 exposure-time treatments, Time 1-Time 7. These treatments represent differing amounts of time in which males and females were exposed to one another and also different windows into the population dynamics within the mating arenas. Exposure time treatments began the day after the first egg mass was observed from any of the Time 1-Time 7 mating arenas. On sequential mornings, females from the remaining treatments (Time 2-Time 7) were removed and individually placed into smaller oviposition arenas and treated as described above.

For each exposure-time treatment, data on the number of dead individuals and their sex, the number of unemerged adults ("Pupae") and their sex, the number of living adults and their sex, and the number of fertile egg masses were recorded (Fig. 1). Fertile egg mass was defined as greater than 50% eggs that were black-head stage after 4 d of incubation at 27°C. Living, adult females were dissected to determine if spermatophores were present in order to characterize them as mated and unmated.

Statistical Analyses

All statistical analyses were executed with the R statistical package (R Development Core Team 2008) available from http://cran.r-project.org. Counts of the numbers of living adults, living pupae, and dead individuals vs. exposure time were assessed by generalized linear models (function "glm" from the base package of R). Separate analyses were performed for males and females. Data were analyzed with a quasi-Poisson error distribution. The quasi-Poisson distribution provided the best link function for the error terms to account for over-dispersion in the data. Counts of the different life history stages were regressed on exposure time. The interaction between exposure time and life-history status tests whether the slopes were the same for the numbers of adults, pupae, and dead individuals. The fit of this model was compared with a model including all effects of

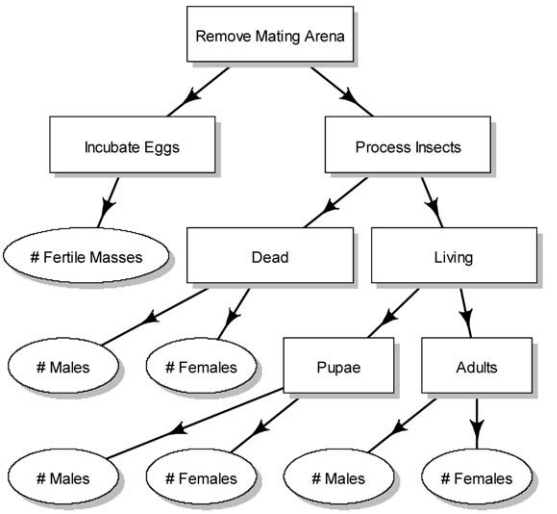

Fig. 1. Schematic of data collection performed each day for exposure time treatments. Elliptical objects represent dependent variables used in analyses.

exposure time to determine if the linear model was sufficient. Fligner-Killeen tests of homogeneity of variances determined that deviances were comparable among exposure times (Females χ^2 = 6.38, df = 6, P = 0.3822; Males χ^2 = 1.48, df = 6, P = 0.9606).

Logistic regression was used to examine whether the proportion of living females that were mated differed with exposure time ("glm" function within R). A model with exposure time as a quantitative predictor of the proportion of females that were mated was assessed and compared with a model including exposure time as a categorical variable. The difference in fit between the 2 models provides a test of the sufficiency of the logistic model in describing the ability of exposure time to predict the proportion of mated females.

Results

Mating Arena Dynamics

Dynamics within the mating arenas changed during the course of the experiment. Tests of linear responses revealed significant heterogeneity among slopes for the life-history categories vs. exposure time for females (Fig. 2, F = 30.37, df = 2, 141, P < 0.0001) and males (Fig. 2; F = 35.40, df = 2, 141, P < 0.0001). Tests for goodness-of-fit found the linear models for exposure time to sufficiently fit data for females (F = 0.49, df = 15, 126, P = 0.9409) and males (F = 0.48, df = 2, 126, P = 0.9467). Numbers of adults within mating arenas remained constant during the oviposition period as indicated by estimates of slopes (Table 1). The relative constancy in the adult numbers was a result of the counterbalancing effects from decreasing and increasing numbers of pupae and dead individuals, respectively (Fig. 2).

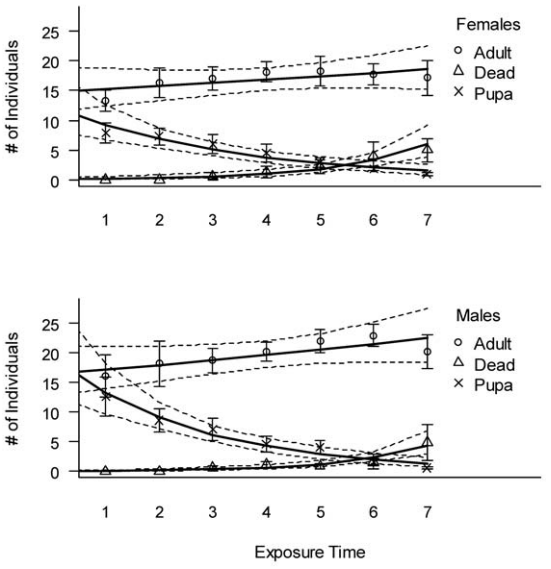

Fig. 2. Mean (±SE) numbers of individuals per mating arena for adults, pupae, and dead individuals for females and males vs. exposure time. Lines within plots represent the expected values (solid) and 95% confidence intervals (dashed) for predictions from regression analyses with quasi-Poisson errors.

The proportion of living, adult females that had mated during the egg-collection period gives an upper limit of the number of females that were ovipositing egg masses. The proportion of mated females significantly increased with time (Fig. 3; Slope t = 4.74, df = 47, P < 0.0001). However, the intercept was not significantly different from zero (t = -0.30, df = 47, P = 0.7666). The linear effect in the logistic regression sufficiently explained differences among time treatments (Goodness-of-fit χ^2 = 4.92, df = 5, P = 0.5743).

Oviposition

The numbers of fertile egg masses significantly differed among exposure time treatments (Fig. 4, F = 3.37, df = 6, 42, P = 0.0083). The model of exposure time effects could be further reduced. Groupings of exposure time treatments 1 and 2 vs. exposure time treatments 3-7 sufficiently explained differences among time effects for fertile egg mass counts (test of model reduction F = 0.37, df = 5,47, P = 0.8676). Females ovipositing during exposure time treatments 3-7 produced approx. 30 more fertile egg masses compared with the females ovipositing during time treatments 1 and 2 (Fig. 4, F = 20.58, df = 1, 47, P <0.0001).

Discussion

During this study, fertile egg production dramatically increased during the first 4 d after the first egg mass was observed. After the fourth day, the production of fertile egg masses remained stable until the end of the experiment. However, other population parameters that were measured indicate that the sampled pool of genetic variance was continually changing. For example, by 4 d after the first egg mass was observed, approx. half of the males and less than half of the females had emerged. By the 7-d exposure treatment, most of the males and females had emerged, but adult mortality was increasing. As a consequence, the numbers of fertile females remained constant from exposure treatments 4-7 d. The result that best demonstrates the impact of changing population dynamics within the mating arena is the constant percentage of females that were mated. The first of the females to emerge were beginning to die as later-emerging females were mating. Slope estimates within Table 1 indicate that the magnitudes of change during the experiment were com-

TABLE 1. RESULTS OF POISSON REGRESSION FOR COUNTS OBSERVED VS. EXPOSURE TIME FOR MALES AND FEMALES (LN (CNT) = INTERCEPT + SLOPE*EXPOSURE TIME, *DF* = 141).

	Intercept	SE	*t*	*P*	Slope	SE	*t*	*P*
FEMALES								
Adult	2.688	0.132	20.35	<0.0001	0.033	0.029	1.16	0.2482
Dead	-2.416	0.752	-3.21	0.0016	0.600	0.124	4.83	<0.0001
Pupa	2.524	0.211	11.96	<0.0001	-0.303	0.062	-4.85	<0.0001
MALES								
Adult	2.795	0.129	21.60	<0.0001	0.045	0.028	1.63	0.1061
Dead	-3.141	1.020	-3.08	0.0025	0.660	0.166	3.97	<0.0001
Pupa	2.961	0.194	15.28	<0.0001	-0.380	0.062	-6.15	<0.0001

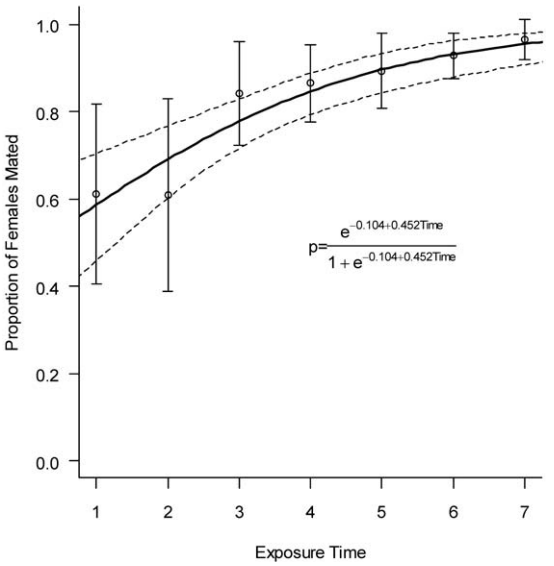

Fig. 3. Proportion of living females that had mated vs. exposure time. Error bars represent 95% CI for observed values. Lines within plots represent the expected values (solid) and 95% confidence intervals (dashed) for predictions from logistic regression analysis with quasi-binomial errors.

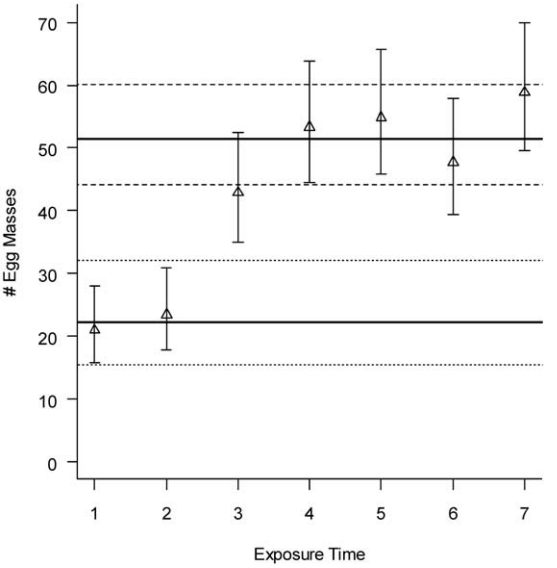

Fig. 4. Numbers of observed fertile egg masses vs. exposure time. Error bars represent 95% CI for observed values. The solid and dashed lines within the figure represent the predicted and 95% CI from a reduced model grouping exposure times 1-2 and exposure times 3-7 (see text).

parable but in opposite directions for the numbers of dead females and the numbers of unemerged females. A similar pattern seems plausible for ECB males. The number of living males did not significantly change during the experiment even though the numbers of dead males and the numbers of unemerged males significantly increased and decreased, respectively.

If bioassays are performed from only 1 or 2 egg dates, there is a great potential for the total genetic variance of the field-collected population to be under-represented. Several strategies would help to improve the sampling of the total genetic variance. Andow et al. (1998) describe a method that generates inbred F_2 isolines from mated females collected from the field ("F_2 screen"). The advantage of this approach is that the researcher knows exactly how many females contribute to the population assayed. F_2 screens generally rely on mass matings of F_1 sibs, and may also be susceptible to patterns observed in this study, i.e., the genetic variance in the family may be under-represented. This would only be critical if the female or the male that mated was heterozygous for alleles at resistance loci. Paired matings of F_1 sibs would also help improve the chance of sampling the total genetic variance, but also provide logistical hurdles.

If the resources are unavailable or limited for performing F_2 screens, and mass matings are

utilized, it may also be possible to sample several egg dates for bioassays. This would require screening more individuals, but may decrease the Type II error (concluding that resistance alleles are absent when they are present). The best strategy for sampling mating arenas would depend on the general mating and oviposition habits of ECB in the laboratory. Our initial approach was to use population dynamics as an upper limit of potential bias. A female that has mated and still living at a single point during the experiment may not have contributed eggs to the oviposition substrate for that date. In addition, we could not determine if individual males had mated. To evaluate actual changes in the genetic variance sampled, paternity testing would be necessary. Until studies of this type are completed, results from this study indicate that a good sampling strategy will be to sample egg masses on the 5th (exposure time = day 3) - 8th (exposure time = d 6) days after the emergence of the first adult. During this time period egg production is sufficient to allow ample larvae for testing. Adult mortality is low at the beginning of this sample period therefore improving the chance of sampling the genetic contributions of earlier emerging females and males. Continuing the sampling period 3 more d will increase the probability of sampling the genetic contributions of later emerging males and females.

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