REARING DENSITY EFFECTS ON PREMIGRANT TRAITS OF THE FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (J. E. SMITH) (LEPIDOPTERA: NOCTUIDAE)

by

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(ABSTRACT)

Laboratory and field studies of the fall armyworm, *Spodoptera frugiperda*, were undertaken to determine if a high larval rearing density produces premigrant traits, and if these traits can be used as criteria for separating non-premigrant from premigrant groups. An increase in larval rearing density significantly decreased pupal weight and forewing width in the laboratory, but not in the field. The greatest differences in developmental time among laboratory density treatments were 0.6 d and 1.07 d for females and males, respectively, but differences were not considered biologically significant. Rearing density did not affect duration of pupal stage in the field-reared fall armyworms.

An actograph was used to measure adult flight activity and was validated with videotape recordings of moths in actograph cages. Behaviors other than flying occurred; hence, computer-recorded counts were termed activity counts (one activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage). Male moths showed a greater proportion of long-duration activity bouts (spells of activity), a significantly greater activity bout duration, and a generally greater magnitude of activity during peak periods than female moths. Generally, actograph activity

was not significantly affected by rearing density. Both mated and unmated females exposed to males showed very little actograph activity, and most of the females mated when exposed to males, regardless of rearing density.

An increase in rearing density in the laboratory significantly increased adult lipid reserves, but higher lipid reserves were not related to a higher activity potential. Although larval rearing density did not affect wing-loading values, the fall armyworm as a migrant species showed lower than theoretically expected wing-loading values.

Because some premigrant traits were produced by increasing the rearing density and other premigrant traits and behaviors were not produced, it is inconclusive that premigrants were produced by these rearing methods. Based on these results and data taken from the literature, there is reason to believe that the fall armyworm does not have a separable premigrant phase, and that components of weather are more influential than larval density in initiating its migratory behavior.

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Chapter 1

Introduction

Among migratory lepidopterous species in the southeastern U.S., the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (FAW), ranks second only to the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), in causing severe crop damage (Sparks 1986). There is a need for increased ecological and behavioral understanding of what causes FAW long-range dispersal, knowledge which would enhance the effectiveness of an integrated pest management program for FAW. Wellington (1976) was the first to stress the importance of weather/behavior interactions which cause insect dispersal. Although there are many published studies on weather conditions related to FAW migration, little is known about either its premigratory or migratory behavior. Also, studies on physiological parameters which are conducive to the onset of migratory flight need to be addressed (Stinner et al. 1983). Some moths migrate from the overwintering locales, but others remain. One of the cues for migratory flight may be high population density, probably acting in conjunction with other factors, including a decrease in food quality and food quantity (see review, Johnson 1969) (Figure 1). With an increase in population density, two or more groups of the same species, which may differ in body weight, wing size, lipid reserves, developmental time, and/or flight behavior, may be produced in a particular locale. This phenomenon occurs in a congeneric species, the African armyworm, *Spodoptera exempta* (Walker), and we proposed the same might occur in FAW. *S. exempta* has two density-dependent phases, nonmigrant (low density) and migrant (high density), which differ in the above mentioned characteristics (Faure 1943, Parker & Gatehouse 1985a and b, Simmonds & Blaney 1986, Gunn & Gatehouse 1986 and 1987). These characteristics could be used to differentiate non-premigrant from premigrant FAW. The definition of a premigrant as used here is a moth which is morphologically and physiologically capable of migratory flight and with a high propensity for migratory flight, but has not yet flown. Putative premigrant traits and behaviors include small size, high flight potential, low wing-loading (body weight/wing area), and high lipid

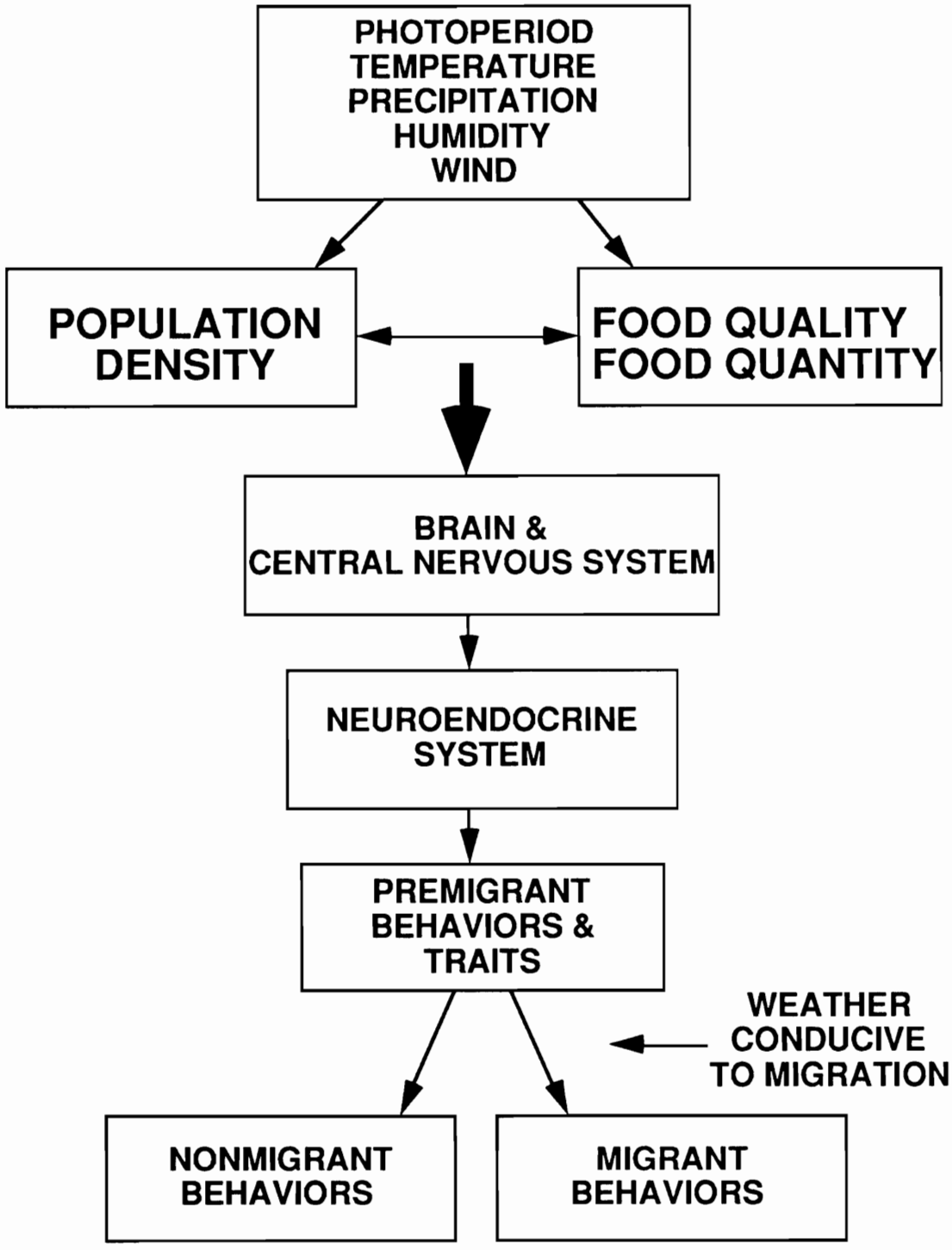

Figure 1: Schematic diagram of environmental variables which may produce premigrant traits in the fall armyworm (adapted from H. Fescemyer's seminar at V. P. I. & S. U., 1987).

reserves (see review, Angelo & Slansky 1984). If a premigrant population is identifiable in a particular overwintering site, an application of insecticide to that population could prevent further crop damage at the site as well as parts north of the site. Of course, application of pesticide to premigrants could cause collateral damage to natural enemies.

In addition to its pest status, the FAW is considered a good model for migration studies (Sparks 1986). Other lepidopteran migrant pests have similar patterns of dispersal (e.g., the velvetbean caterpillar, *Anticarsia gemmatalis* Hubner (Lepidoptera: Noctuidae) (Greene 1979)). Results from FAW migration studies may shed light on migration-related questions posed about these other lepidopterans.

The hypothesis and objectives of this dissertation are:

Overall Hypothesis: Crowding (rearing with more than one larva per cup or with multiple larvae per plant) produces premigrant traits in the adult FAW.

Specific Objectives:

1. Compare the effects of rearing FAW larvae one, two, and three to a cup on morphological, developmental, and physiological parameters believed to be associated with migration, including wing size, developmental time, and lipid reserves.
2. Compare the activity in an actograph of FAW adults from larvae reared one, two, and three to a cup.
3. Compare the effects of field-rearing FAW larvae at low and high densities on morphological, developmental, and physiological parameters believed to be associated with migration, including wing size, developmental time, and lipid reserves.
4. Compare the activity in an actograph of FAW adults from larvae field-reared at low and high densities.
5. Observe behavior of moths in an actograph with a video camera and determine a relationship between computer actograph data output and actual activity in order to validate adult activity data.

Studies in the laboratory enable us to control certain environmental factors, such as food supply and photoperiod, while manipulating the variables

of interest which may affect flight behavior and capacity. Data collected from field-reared individuals serve to complement and substantiate data from laboratory-reared FAW.

A final introductory note: It has been determined that there are two FAW "strains" (taxonomic status has not been determined) (Pashley et al. 1985, Pashley 1988). The "corn strain" and the "rice strain", differ in many ways, including their development on each other's host plant; their susceptibility to insecticides (Pashley et al. 1987a); their development on different varieties of Bermuda grass, some considered to be FAW-resistant (Pashley et al. 1987b); and their oviposition preference (Whitford et al. 1988). Because these strains differ in their preferences for host species, it is possible that their migratory behavior would differ. Also, it has been suggested that the "rice strain" FAW migrates only from tropical to subtropical zones, and the "corn strain" FAW migrates only from subtropical to temperate zones (J. N. McNeil pers. comm.). Hence, it is important to clarify that this study was conducted on FAW from a colony originally collected from volunteer corn and sorghum in Georgia (W. D. Perkins pers. comm.) and are assumed to have genetic similarity to Pashley's (1988) "corn strain".

Chapter 2

Literature Review

History of the Fall Armyworm

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (FAW) was first recognized as a pest of grains and grasses in 1797 (Smith & Abbot 1797). Since then, outbreaks occurred in the U.S. in 1845, 1854, 1864, 1868, 1870, 1872, 1878, 1884, 1889, 1892, 1896, and 1899 (Luginbill 1928). The most destructive outbreak was in 1912, when the FAW invaded nearly all the United States east of the Rockies, parts of Canada, the West Indies, British Guiana, and parts of Central America (Luginbill 1928). Outbreaks have occurred sporadically since 1912, but the most recent serious outbreak was in 1977 when agricultural losses and control costs for FAW reached nearly $300 million for the southeastern U. S. (Anon. 1979). The origin of this species is unknown, but it probably came from tropical Central or South America.

Fall Armyworm Life Cycle

Luginbill (1928) gave a thorough description of generalized FAW biology. Adults fly, feed, oviposit, and mate during the night. Females usually start emitting pheromone on the second evening after emergence (Sparks 1979). Mating activities peak prior to midnight on the second night after emergence. Females usually lay large egg masses (each having from a few to hundreds of eggs) on the lower surfaces of leaves. Oviposition takes place during the first four hours after dark, lasting until midnight for four or five days, sometimes up to 17 days (Luginbill 1928). Ali et al. (1990) determined that 12.95°C was the lower developmental threshold for all life stages, and that 38°C is the upper developmental threshold for larvae. Temperatures at or below 0°C kill FAW at all life stages and their plant hosts (Luginbill 1928). Typically, at 21 to 35°C, there are six larval instars (Ali et al. 1990). However, Ali et al. (1990) observed that at 17°C, there may be more than six instars and an increase in total developmental time. On the other hand, 26.5 and 23.9% of

larvae reared on artificial diet had five instars at 25°C and 29°C, respectively. Pupation occurs in the soil from which the adults emerge during the night. About four weeks are required for completion of the FAW life cycle in southeastern U. S. during the summer. The time from egg to adult on artificial diet ranges from 18.4 days at 35°C to 66.5 days at 18.3°C (Barfield et al. 1978).

Fall Armyworm Damage

The last three larval instars inflict the most feeding damage (Luginbill 1928). Younger instars damage by skeletonizing leaves; older larvae will strip large plants and completely devour smaller plants. Larvae have been recorded feeding on more than 60 species of plants, with a preference for graminaeous species, e.g., corn and Bermuda grass. The FAW damages corn by: feeding on leaves in the whorl stage, feeding on developing tassel until the tassel emerges, and feeding on developing ears. If the FAW damages corn during the early-tassel stage of development, the number of marketable ears can decrease significantly (Ghidiu & Drake 1989). FAW larval damage to grain sorghum is similar (Martin et al. 1980). Forage quality of Coastal Bermuda hay is greatly reduced by FAW leaf-feeding (Martin et al. 1980). Defoliation of other crops such as peanuts and rice contribute to the economic importance of the FAW (Anon. 1979).

Seasonal Abundance in the Overwintering Sites

The fall armyworm lives year-round in frost-free regions where the temperature usually remains above 10°C, which is south of ca. 26°N latitude in the Northern Hemisphere (Raulston et al. 1986). Pheromone trap data in south Florida revealed a bimodal occurrence of FAW adults with a peak in the spring and another one during the late fall/early winter (Pair et al. 1986). Pair and his collaborators surmised that the distinct wet/dry seasons of subtropical south Florida contributed to the FAW population increases/decreases in Dade and Palm Beach Counties. Pheromone trap data from the Texas-Mexico Gulf Coast area revealed a population peak in November and December and low populations during mid year (Raulston et al. 1986). In addition, peak trap

captures typically occurred at a progressively later date from southern to northern locations. Larval infestations followed a temporal developmental progression from northern Mexico (Tamaulipas) to the Lower Rio Grande Valley of Texas. Raulston et al. (1986) believed these phenomena indicate that major population movements occur from south to north in a region where FAW can survive year-round.

Fall Armyworm Larval Dispersal and Adult Flight Behavior

First instar FAW larvae may disperse great distances by ballooning on silken threads (Luginbill 1928). During the early evening, most adult FAW fly with the wind and usually remain flying less than nine meters above the canopy (Sparks 1979). After darkness falls, moths tend to fly against the wind at low altitudes. Some moths ascend above their "boundary layer," a dynamic, instantaneous layer of air above which insects cannot orient to the ground because the wind speed is greater than their flight speed (Taylor 1958). Within the boundary layer, moth flight speed is greater than wind speed, and moths can govern their behavior in relation to the ground. Migratory flight is generally assumed to be with the wind and above the boundary layer (Taylor 1974).

Fall Armyworm Migration

Annually, fall armyworm moths begin migrating northward at the beginning of spring (Snow & Copeland 1969) at an estimated rate of 480 km/generation in some years (Sparks 1986). By late June to July, moths reach Virginia and mid-California, and by August, moths reach Michigan and Minnesota (Snow & Copeland 1969). In addition to the northbound spring migration in the U. S., there is circumstantial evidence for a reverse fall migration from the northern plains of Texas to near Brownsville (overwintering habitats) (Pair et al. 1987). The existence of a return fall migration in the FAW is a controversial subject. One line of reasoning is that the FAW is a "pied piper" migrant, in that it migrates northward each year, and having no diapause mechanism, is killed back by cold weather in the autumn (Rabb & Stinner 1978). However, this "pied-piper" migration may only be apparent, not real,

because a suicidal scenario does not favor selection for migratory genes. Others hypothesize that the distinct wet and dry seasons in the tropics caused the FAW to develop and maintain a life history strategy to migrate from unfavorable dry habitats to favorable wet habitats (Johnson 1987). If the latter hypothesis is true, return FAW migration from the temperate regions is probable, but it would not be necessary to keep migratory genes in the population if migration occurs within the tropical populations between dry and wet regions.

Kennedy (1961) defined migration as an enhancement of locomotory activities with a "persistent, straightened-out movement" along with suppression of "vegetative functions" (e.g., feeding, mating, and oviposition). By this definition, pre-reproductive males and females migrate, postponing mating and oviposition until they colonize new environments. It is not known whether the FAW migrates before or after mating or does both. Interestingly, 40 to 50% of supposed migrant FAW females captured in light traps on oil platforms in the Gulf of Mexico during October 1973 were mated (females comprised 42 to 45% of the total captures) (Sparks et al. 1986). These investigators believe that most of the trapped FAW were transported on southeasterly and easterly winds. Other evidence suggests that FAW migrate during the pre-oviposition period after mating. Rose et al. (1975) investigated the remarkable transport of FAW moths from the U.S. Gulf Coast to Sault Ste. Marie, Canada, a distance of 1600 km, accomplished in 30 hours. Discovery of eggs laid on laundry left hanging overnight in Sault Ste. Marie led Rose et al. (1975) to believe that mated FAW migrated until they were reproductively mature, assisted by low-level jet and convergent surface winds.

Retrogressive atmospheric trajectories (which accounted for temperature, humidity, wind velocity, and barometric pressure) for the southeastern U. S. have pinpointed probable overwintering sites for the FAW: the Bahamas; Homestead, Fla.; Cuba; and other parts of the Caribbean basin (Westbrook & Sparks 1986). At these locales, high density premigrant fall armyworm populations can develop during the winter months if the the weather is cool and wet (Luginbill 1928). Local outbreaks may occur after a period of abundant rainfall and high humidity. Because weather conditions vary from year to year, the fall armyworm is a sporadic pest.

Generally speaking, weather fronts have been implicated in the assistance of the FAW's northward migration each spring and summer (Mitchell 1979). A front occurs when a warm air mass collides with a cold air mass; the warm air flows up and above the cool, moist, heavier air. As the warm air flows upward rapidly, insects can be lifted in these updrafts to higher altitudes and then be transported horizontally over long distances (McManus 1988). Insects, such as *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae), the African armyworm, have been documented to be carried by such weather systems (Rainey 1979). Increases in the number of moths in pheromone traps in Florida, Georgia, and Alabama during the springs of 1983, 1984, and 1985 were associated with frontal systems (Pair et al. 1986).

Besides using frontal weather systems for dispersal, which may not occur when and where insects need them, insects may use normal regional wind circulations to aid in their dispersal (Wellington 1979). Basically, the phenomena of turbulence and convection affected by surface terrain (flat or hilly) are conducive to lifting insects and transporting them short distances. A nocturnal low-level jet is a frequent wind condition in which the maximum windspeed is a few hundred meters above the ground and is capable of moving insects great distances (Drake 1985) (e.g., the transport of FAW from the U.S. Gulf Coast to Canada mentioned previously).

Certain specific weather conditions, categorized as synoptic types by Muller (1979), favor transport of FAW adults from the Gulf Coast states over the Gulf of Mexico (Johnson 1987). Such conditions include: Frontal Gulf Return, Continental High, Frontal Overrunning, Coastal Return, and Gulf High. Frontal Gulf Return happens when warm maritime tropical air meets a cold front and is associated with showers and thunderstorms, occurring during the winter, spring, and fall. Continental High is defined by fair and cool to cold weather associated with a high pressure system east of the Rockies and winds from the north. Continental Highs may occur year-round. Frontal Overrunning is a stationary polar front (interface between polar air and tropical air) during the late fall and winter, accompanied by winds from the north/northeast, low temperatures, and some precipitation. Coastal Return is characterized by surface winds going from the northeast, east, and southeast, fair and cool conditions during winter and spring, and fair but warm and muggy conditions in summer and early fall.

Gulf High weather is a summer high pressure system over the Gulf with weak winds from the southwest and maritime tropical weather.

Studies associating FAW pheromone trap captures with weather conditions have provided circumstantial evidence of FAW migration from the Caribbean to the continental U.S. and from the U.S. to Canada. Two problems with pheromone traps is that they catch both migrant and nonmigrant moths, and they catch only males. Studies which employ multiple methods of detection (such as mark/recapture, light traps, and radar), which involve genetic analyses, and which examine flight potential (such as the effect of larval density on adult behavior) can greatly contribute more conclusive evidence of FAW migration (Mitchell et al. 1991).

Larval Rearing Density Effects in Other Insects

Morphological and Developmental Parameters

The effects of larval density in the laboratory have been studied in a number of other migratory species, including the velvetbean caterpillar, *Anticarsia gemmatalis* Hubner, the cotton leafworm, *Alabama argillacea* Hubner, the cabbage looper, *Trichoplusia ni* (Hubner), the black cutworm, *Agrotis ipsilon* (Hufnagel), and the African armyworm, *S. exempta* (all Lepidoptera: Noctuidae) (Anazonwu & Johnson 1986, Fescemyer & Hammond 1986, Johnson et al. 1985, Tignor & Eaton 1986, Lewis & Keaster 1989, Sappington & Showers 1992, Parker & Gatehouse 1985a, Simmonds & Blaney 1986). In these species, rearing density affected morphological and developmental parameters which might be used to distinguish a premigrant from a non-premigrant. Pupal weight of all these species decreased when larval rearing density was increased, probably due to competition for food. Crowding in *T. ni* and *A. gemmatalis* resulted in a longer larval developmental time (Tignor & Eaton 1986, Fescemyer & Hammond 1986), but only on mature soybean leaves for the latter species. A longer developmental time for larvae could indicate that larvae had to eat longer to become reproductively competent adults (Angelo & Slansky 1984). However, duration of the larval stage in *S. exempta* was found to be shorter in crowded individuals than in singly reared

insects (Simmonds & Blaney 1986). These investigators believed that the shorter developmental time for crowded *S. exempta* larvae allowed a more rapid population increase, and their migratory capacity would enhance the spread of the high density populations to unexploited habitats. Although the FAW is an important migratory pest species similar to the ones mentioned above, it has not been the subject of comparative density studies.

Phase Variation

A. ipsilon, *A. gemmatalis*, *A. argillacea*, and *S. exempta*, among many other lepidopterous species, show color phase variation in response to increased larval density (Sappington & Showers 1992, Anazonwu & Johnson 1986, Johnson et al. 1985, Faure 1943, Long 1953). Crowded *A. ipsilon* larvae were significantly darker than uncrowded larvae (Sappington & Showers 1992). *A. gemmatalis* larvae were predominantly green when reared at low densities, but mostly black when reared at higher densities (Anazonwu & Johnson 1986). *A. argillacea* larvae exhibited increased melanization with increased crowding (Johnson et al. 1985). *S. exempta* has two distinct behavioral, physiological, and colored phases. There is a migrant high density dark-colored phase which conspicuously inflicts crop damage contrasted with a nonmigrant low-density light-colored phase which establishes a covert reservoir population during suboptimal weather conditions (Faure 1943). Faure (1943) termed the high density phase *gregaria* and the low density phase *solitaria*. There is no published evidence that the FAW has density-induced melanization. It is still possible that this species has distinct behavioral or physiological phases related to its migration.

Lipid Reserves

The effect of larval rearing density on the amount of lipid reserves has been studied extensively in only one noctuid species, *S. exempta*, the African armyworm (Gunn & Gatehouse 1986 and 1987). Gunn & Gatehouse (1987) found that the abdominal glyceride contents (including tri-, di-, and mono-) were 2.5 to 6.1 times greater in *gregaria* adults than in *solitaria* adults. Generally

speaking, for species in Lepidoptera, Coleoptera, and Diptera, the presumed migrant phase individuals were found to have higher lipid reserves than nonmigrant members of a species (see review, Angelo & Slansky 1984).

Long-range migratory flight depends on large lipid reserves, which are not depleted as rapidly as glycogen, the primary fuel for short-range flying (Van Handel & Nayar 1972a and b). Lipids are important because: (1) they are an economical energy source because 1 mg of lipid is equal to 8 mg of stored glycogen in energy, releasing many more calories per gram than carbohydrates (Weis-Fogh 1968); (2) they can be stored more economically because less space is taken up by water in lipid storage than in carbohydrate storage (Beenakkers et al. 1985); and (3) lipids release more metabolic water/mol oxidized than carbohydrates (Beenakkers et al. 1985). This helps in maintaining hemolymph volume and osmotic status quo while flying (Teo et al. 1987).

Nayar & Van Handel (1971) observed that in most FAW moths flown from four to seven hours, the lipid content did not decrease. However, whole body trehalose and glycogen decreased more than 50% after six hours of flight (Van Handel & Nayar 1972b). Whole body lipid in the FAW was reduced only after 16 to 30 hours of tethered flight (Van Handel 1974).

In addition to providing fuel for flight, lipids are a vital resource for egg production, which becomes important for a colonizing migrant moth when it reaches its destination. Gunn et al. (1988) found that there was a trade-off for use of lipid reserves between flight and reproduction in *S. exempta*. Female moths which flew on tethers for the two nights after emergence had significantly less fecundity if mated after the flight test and allowed only distilled water when compared to unflown mated moths allowed distilled water. Flight-tested females subsequently mated and given sucrose solution showed a smaller but significant decrease in fecundity as compared to unflown mated sucrose-fed females. *S. exempta* moths normally feed on field nectar sources and also fed on molasses sprayed on plants (Rose et al. 1985). The results of Gunn et al. (1988) indicate that carbohydrate sources are important in restoring lipid reserves used during flight.

Flight Behavior and Wing-Loading

In one group of tethered flight experiments with *A. ipsilon* , as density was increased from three larvae to five larvae per 30 ml cup, flight activity in subsequent adults, as measured in mean seconds of flight per hour, increased significantly, but none of the flights they observed were ≥ 1 hour (Lewis & Keaster 1989). More recent tethered flight studies with *A. ipsilon* found no effect of larval density on subsequent adult long-duration (presumed migratory) flight (≥ 1 hour) (Sappington & Showers 1992). In both studies, flight behavior of *A. ipsilon* varied considerably, regardless of density treatment. Tethered flight studies of *S. exempta* revealed that larvae reared at higher densities were more likely to produce adults that were "long-fliers" (Parker & Gatehouse 1985a). The authors suggested that larval population density was the only significant environmental factor influencing migration in the African armyworm; food availability and quantity were not as important. Further research with *S. exempta* indicated that flight capacity may have a greater genetic basis than realized before. Flight capacity could be increased or decreased through directional selection of either "long-fliers" or "short-fliers" (Parker & Gatehouse 1985b, Woodrow et al. 1987).

Also, in *S. exempta*, there was a tendency for wing area to decrease with increasing rearing density. However, there were no significant differences in wing-loading (whole body weight/total wing area) among density treatments or between long-fliers and short-fliers, when all density treatment individuals were pooled (Parker & Gatehouse 1985a). Lower wing-loading values allow the moths to be more efficient flyers and fly longer and further. Studies of other lepidopterans revealed lower wing-loading values in the presumed migrant phases (see review, Angelo & Slansky 1984). There are no data on the effects of rearing density on wing-loading and flight behavior in the fall armyworm.

Measurement of Flight Behavior in the Laboratory

Flight behavior of Lepidoptera has been studied in the laboratory using many different measurement techniques. These include: the static tether (Dingle 1965), the vibration-sensitive actograph (Leppla et al. 1979), the flight

mill (Kishaba et al. 1967), and the "pivot" flight balance (Gatehouse & Hackett 1980, Wales et al. 1985). Two design problems of these measurement techniques, the use of multiple insects in one cage and the use of tethers, need to be addressed. The use of multiple insects in a cage in some designs such as the vibration-sensitive actograph (Leppla et al. 1979), has several drawbacks: individual flight capacity and population variability cannot be quantified, the "domino effect" caused by one moth initiating flight and others following suit cannot be measured, and moth behavior is altered as moths bump into other moths and into the walls of a container. Tethered flight techniques, which allow the study of one insect, have been evaluated by Gatehouse & Woodrow (1987) who argued that: (1) tethers alter flight behavior and oviposition, if measured simultaneously, (2) there are problems when inactive periods of time and temporal patterns are not considered in data analysis which uses flight duration as an indicator of migratory capability, and (3) they should be substantiated by field free-flight studies to make valid conclusions about migratory potential. In the laboratory, there is a lack of usual field stimuli such as varying temperatures and wind currents. In addition to these problems, flight duration data distributions from tethered flight studies are almost always right skewed, i.e., most insects are short flyers (Davis 1980). This phenomenon is found also in non-tethered flight experiments. Davis (1980) speculated that only a few of the tested animals in a given experiment are physiologically equipped for long flight (i.e., are premigrants), and that short flights (for foraging, mating, and oviposition) are adaptive most of the time. Longer flight, being costly (see review, Rankin & Burchsted 1992), is undertaken only when there are adverse habitat conditions (e.g., lack of food, mates, hiding places, and/or oviposition sites).

With the increase in availability of microcomputers, old-design actographs were modernized with computer interfaces (Woodrow et al. 1987, Lewis & Keaster 1989, Barfield et al. 1988, Resurreccion et al. 1988, Eaton 1985). The only computerized actograph which exists today that does not entail tethering moths is one that was developed at V. P. I. & S. U. (Eaton 1985). This system can collect data from 32 moths simultaneously, each moth isolated in a screen-top cage with an opening on the side near the top to allow an infrared beam to pass across the top of the cage. Moths are allowed to fly freely within

the confines of the 13 cm dia by 16.5 cm high cages. One count is recorded each time the beam is interrupted. Eaton made observations during the first trials of this actograph that cabbage looper moths do not fly into the cage walls or screen covers, and usually they fly in the top third of the cage. Flight behavior data from this actograph have been published (Eaton 1985, Sprint & Eaton 1987, Judge et al. 1991), but validation of this paradigm for studying flight behavior has not been accomplished until now (Chapter 3).

Chapter 3

Comparison of Computer-Interfaced Actograph Output with Visual Observations via Videotape for the Study of Flight Behavior in the Laboratory

Introduction

Questions about specific variables influencing flight behavior of migratory moths bring about the necessity of laboratory flight measurement devices. In the laboratory, certain environmental variables can be controlled and parameters such as sex, age, reproductive status, and rearing density can be studied to determine their effects on adult activity. Flight and other movement can be measured with actographs which have evolved from a simple running wheel type (Lipton & Sutherland 1970) to the present-day computer-interfaced instruments (e.g., Eaton 1985).

Early actographs using electronic transducers include the vibration-sensitive type (Leppla et al. 1979), the capacitance discharge device (Chabora & Shukis 1979), thermistors (Macauley 1972), and infrared photocells (Brown & Unwin 1961). These early methods had design flaws which affected the analysis of activity. The use of multiple insects in one cage with vibration and capacitance discharge transducers has several drawbacks: individual flight capacity and population variability cannot be quantified, the "domino effect" caused by one moth initiating flight and others following suit cannot be measured, and moths become battered as they bump into other moths repeatedly. Thermistors generate heat which may affect moth behavior. Red and infrared photocells work as long as the insect does not react to the radiation. Eaton (1980b) tested the compound eye and ocellus of *Trichoplusia ni* (Hubner) for a response to infrared from the infrared emitting diode used in his actograph and found no electroretinogram response. Unlike the vibration-sensitive actograph and the capacitance discharge device, both thermistor and photocell actographs permit the study of a single insect. Various tethers have been designed to isolate individual moths and are being used presently even though the tether interferes with normal moth activity (Dingle 1965, Kishaba et al. 1967, Gatehouse & Hackett 1980, Barfield et al. 1988, Lewis & Keaster 1989, Resurreccion et al. 1988, Mason et al. 1989).

With an increase in availability of computers, old-design actographs were modernized with computer interfaces. There are at least five computer-interfaced actographs in use at the present time. Woodrow et al. (1987) and Lewis & Keaster (1989) modified Gatehouse & Hackett's (1980) tethered flight

balance and replaced the event recorder with a microcomputer. Barfield et al. (1988) created a photocell-based tethered actograph interfaced with a microcomputer, fashioned to monitor flight and oviposition simultaneously. Resurreccion et al. (1988) made a computer interfaced, photocell-based (infrared light-emitting diode (LED)) tethered flight mill, designed especially for large moths such as the black cutworm. The fifth computerized actograph, the only one of the five which does not tether moths, is the one that was used for this dissertation.

The prototype of the current V. P. I. & S. U. actograph was a 20-channel infrared LED based electronic actograph which was connected to an event recorder (Eaton 1980b). Artificial sunrise and sunset were created with a cam-operated light control (Eaton 1980a). Eventually, a larger, more sophisticated, infrared LED based actograph (Eaton 1985) was produced for the purpose of studying noctuid moth migratory flight behavior (J. L. Eaton pers. comm.). The system included 32 channels, a microcomputer interface, a radiometer, temperature and humidity detectors, and electronically simulated sunrise and sunset by Byers & Unkrich (1983). It was housed inside a walk-in environmental chamber. A single insect was placed in a screen top cage (13 cm in dia by 16.5 cm high) with an opening on the side near the top to allow a 940-nm infrared beam to pass across the inside of the cage to the phototransistor detector opposite the beam emitter. One activity count was recorded each time the beam was interrupted. The actograph was interfaced with a Digital Equipment Minc II Microcomputer which collected and stored data. Data were transferred to the IBM 3084/3090 main frame for analysis using SAS (SAS Institute 1985). During its seven years of operation, cabbage loopers, corn earworms, fall armyworms, American cockroaches, European earwigs, and house flies have flown or walked about in its cages.

The fall armyworm (FAW) project was the only one which attempted to index migratory flight potential with the actograph. A single method of studying flight activity in the laboratory may lead to misleading or incomplete conclusions about behavior. The objective of this study was to substantiate and validate the activity data recorded electronically by the V. P. I. & S. U. actograph with visual observations via video camera of moth activity in actograph cages. Three assumptions that were made during the design of this actograph were

examined: (1) moths fly in the cages, with minimal walking, so that counts recorded are in direct relationship to amount of time spent flying; (2) moths fly near the top of the cage, hence the LED window is near the top; and (3) migratory potential can be indexed using data produced with this actograph.

Materials and Methods

Fall armyworm pupae were obtained from the Insect Biology and Population Management Research Laboratory, USDA, ARS, Tifton, Ga. Moths were placed in holding cages at 26° C on the first night after emergence and fed 10 % sucrose solution *ad libitum*. On the second night after emergence, four moths were placed individually in actograph cages and supplied with 10% sucrose solution. The second night was chosen because preliminary research showed that activity is usually greatest on that night. The light regime was 1 h of simulated sunset starting at 1600 h, 9 h of darkness, 1 h of simulated sunrise, followed by 13 h of light. Moth activity was recorded from 1600 h to 2200 h with a VHS video cassette recorder connected to a video camera positioned to view inside four cages. To provide enough light for the camera in the darkness while still allowing the moths to fly, two goose-neck lamps with red light bulbs were placed inside the actograph above the four cages. Simultaneously, the computer recorded activity counts for every 15-minute period. Three nights of recording with three different sets of four unmated males were conducted, followed by three nights with different sets of four unmated females.

Unfortunately, one of the channels did not function during video camera recordings. Also, some of the males did not initiate their activity until after 2200 h or were inactive. Thus, data from only four male moths and nine female moths were analyzed.

The general behavior of moths inside the cages was observed and noted to determine if certain behavioral activities were universal, individual, or gender-specific. Activity bouts (spells of activity) were timed with a chronographic watch to the nearest 0.01 s; the end of a bout was called after five s of settlement. The five s of settlement was subtracted from each bout time. Mean activity bout time was compared between males and females with the

TTEST procedure of SAS (SAS Institute 1985) ($\alpha = 0.05$). Percent of long-duration activity bouts (≥ 61 s) was compared between the sexes using χ^2 analysis ($\alpha = 0.05$). To determine the validity of the electronically recorded behavior data, the number of activity counts recorded was compared to the sum of all activity bout times within each 15-min period, both accumulated over 6 h, using a SAS two-way analysis of covariance ($\alpha = 0.05$) with time as the covariate and sex and measurement method as the classification variables. The mean number of activity counts and the mean number of timed min per moth for each 15-min period were graphed together to see if peaks of activity from 1600 to 2200 h matched up between methods.

Results and Discussion

Five generalized behavioral activities, going from feeding to very rapid flying, were identified and occurred in both unmated males and females but not in all moths (Table 1). Feeding and walking were observed for most of the male and female moths. Walk-flying and flitting (slow flying) were found more often in the females, while very rapid flying was more prevalent in the males. All of these behaviors, except feeding, could break the infrared beam and register an activity count. The predominant activity was flying (walk-flying, flitting, and very rapid flying).

Flying, walking, and feeding took place in the upper third of the cage. Occasionally, a moth would venture down to the bottom of the cage, but usually for only a few seconds. Flying would naturally lift the moth upward, hence the design of the cage with the LED window near the top.

The mean bout time was significantly more for males than for females (males: 51.46 ± 4.34 s, n = 179, females: 36.45 ± 1.81 s, n = 499, $P = 0.0016$). The shortest (0.6 s) and the longest bout (7.3 min) was found in males. Although the majority of bouts were 1 min or less in duration, the proportion of long bouts (≥ 61 s) was significantly greater in males (26.82%) than in females (15.03%) ($P > 0.001$). It is not surprising to find the data skewed toward short flights, for right skewness is often found with insect flight data distributions (Davis 1980). Davis (1980) suggested that most of the time, short flights (to find

Table 1. Generalized behavioral activity in the fall armyworm observed in actograph cages via videotape.

	Activity[a]				
	Feeding	Walking	Walk-flying[b]	Flitting[c]	Very Rapid Flying
Female Replicate					
1	+	+	+	+	-
2	+	+	-	+	-
3	+	+	-	+	+
4	-	+	+	+	-
5	-	+	+	+	+
6	+	+	-	-	+
7	+	+	-	-	+
8	+	+	+	-	+
9	+	+	-	-	+
% Exhibiting Activity	77.8	100.0	44.4	55.6	66.7
Male Replicate					
1	+	+	-	-	+
2	+	+	+	-	+
3	+	+	-	-	+
4	+	+	-	+	-
% Exhibiting Activity	100.0	100.0	25.0	25.0	75.0

[a] (+) indicates that moth exhibited activity and (-) indicates that moth did not exhibit activity.
[b]Wings flapping with tarsal contact with surface.
[c]Slow, erratic flying; fluttering.

food, mates, hiding places, and/or oviposition sites) are more adaptive than long flights in the field. If there is a lack of available food, mates, hiding places, and/or oviposition sites, the few individuals with a predisposition to migrate would disperse to a better habitat despite the danger and energetic costs.

Analysis of covariance revealed that the two methods of quantifying activity were not significantly different ($P = 0.8934$). Therefore, the number of activity counts correlates nicely with the amount of timed activity during a 15-min period for both males and females (Figures 2 and 3). A significant gender effect was found ($P = 0.0001$), which contributed to the growing evidence in this study that male activity was different from female activity in the actograph.

The amount of activity was dependent on the time of night, indicated by a significant 15-min period effect ($P = 0.0001$). The pattern for mean timed activity (min) per 15-min period closely followed the pattern for mean number of computer-recorded counts (Figures 4 and 5). Female activity began at sunset, but male activity started later at about 1830 h. A peak of female activity occurred at sunset, followed by a drastic drop. Radar and airplane observations revealed FAW and corn earworm moths taking off on migratory flight just after sunset from the Rio Grande Valley in 1989 (C. E. Rogers pers. comm.). A similar sunset peak was recorded for female *Spodoptera exempta* (Walker), the African armyworm (Parker & Gatehouse 1985a). After the sunset peak, several peaks of activity for FAW females were noted. Female evening activity could represent migratory flights, foraging, or movement to a calling position. Male activity consisted of several peaks from 1830 h to 2100 h, followed by the beginning of a potentially great peak. Computerized activity count data, which was logged until 0700 h the next morning, showed that this peak declined two hours before the end of the scotophase. Male activity, occurring much later and at a greater magnitude than female activity, could represent migratory flights, but there is evidence from radar and visual observations that FAW and other noctuids commence migratory flight shortly after dusk (Riley et al. 1983, Drake 1984, C. E. Rogers pers. comm.). Activity in male FAW moths which occurs in the middle of the scotophase on the second night after emergence is usually associated with mating (Mitchell et al. 1974, Sparks 1979). It is possible that male FAW moths with the propensity for migratory flight migrate only on the first night after emergence and mate on the second night after emergence.

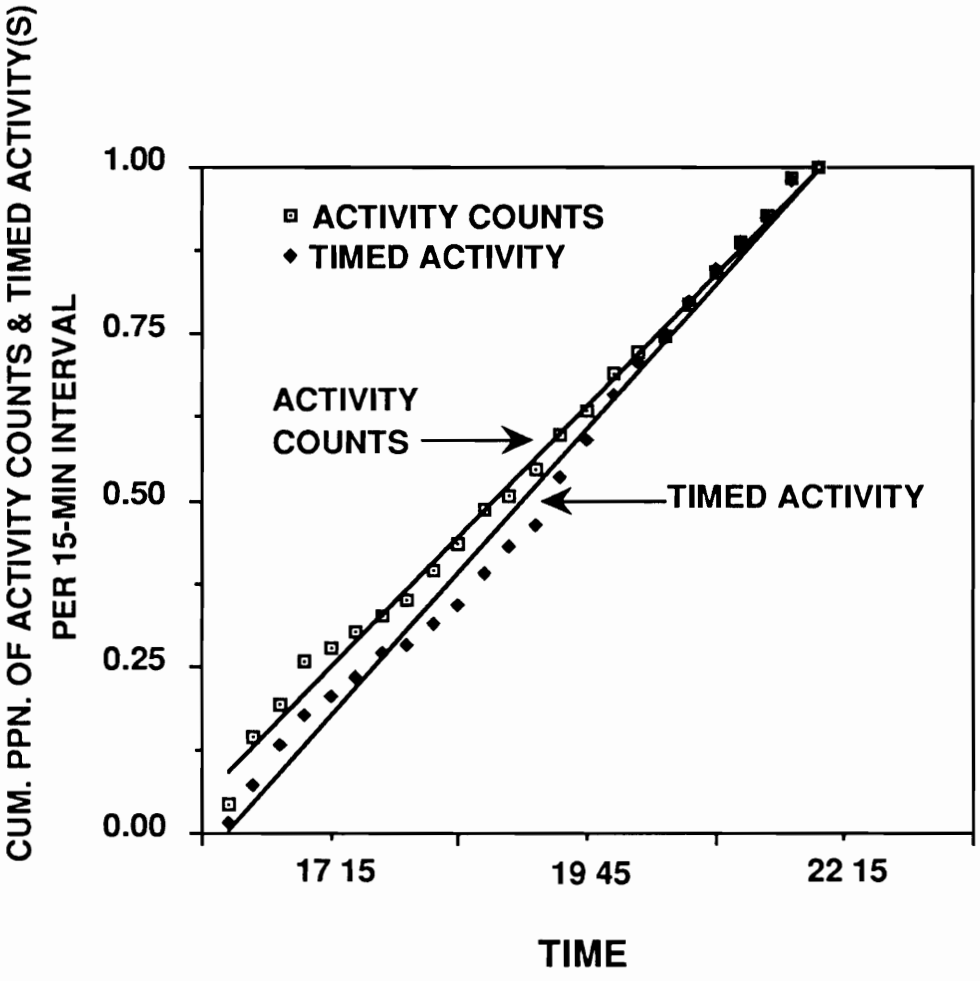

Figure 2: Comparison of computer-recorded activity counts and timed activity (timed from videotape) during a six-hour night period in female fall armyworm moths (n = 9). One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Activity counts: $y = 0.05 + 0.04x$, $r^2 = 0.995$; timed activity: $y = -0.04 + 0.04x$, $r^2 = 0.99$.

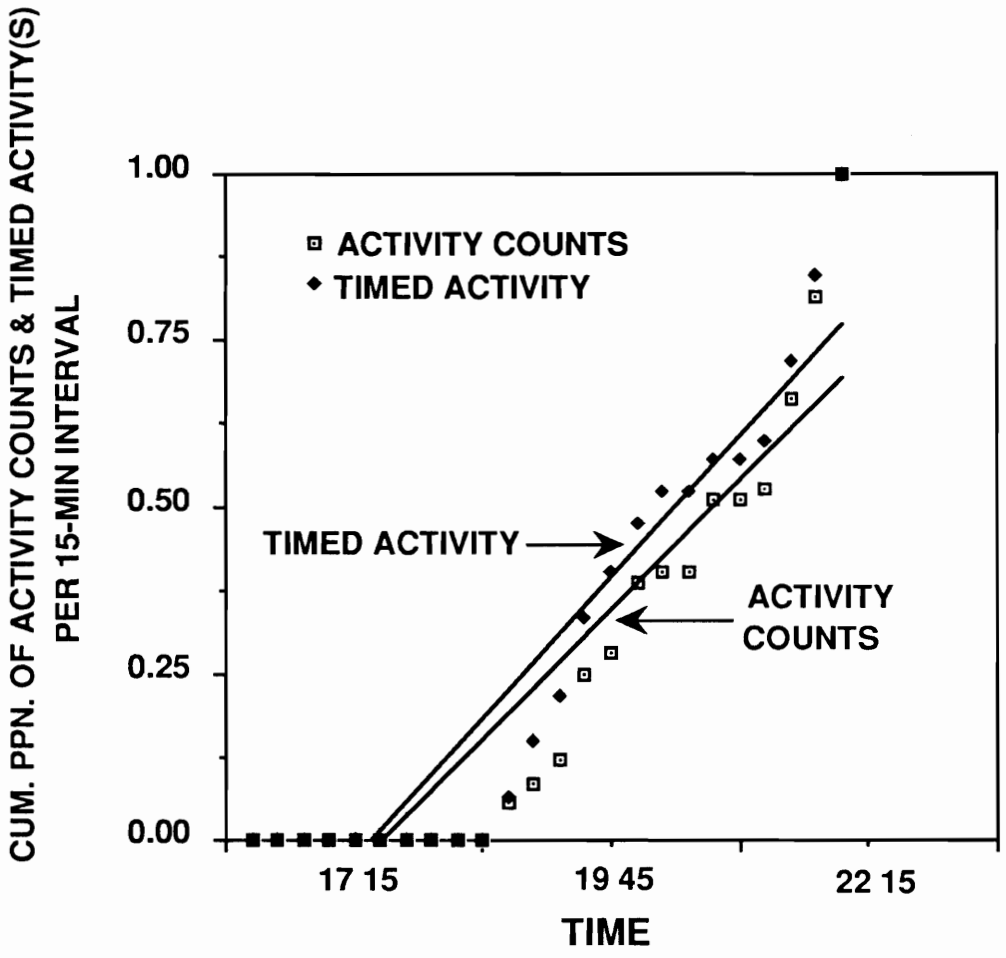

Figure 3: Comparison of computer-recorded activity counts and timed activity (timed from videotape) during a six-hour night period in male fall armyworm moths (n = 4). One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Activity counts: $y = -0.23 + 0.04x$, $r^2 = 0.84$; timed activity: $y = -0.24 + 0.04x$, $r^2 = 0.88$.

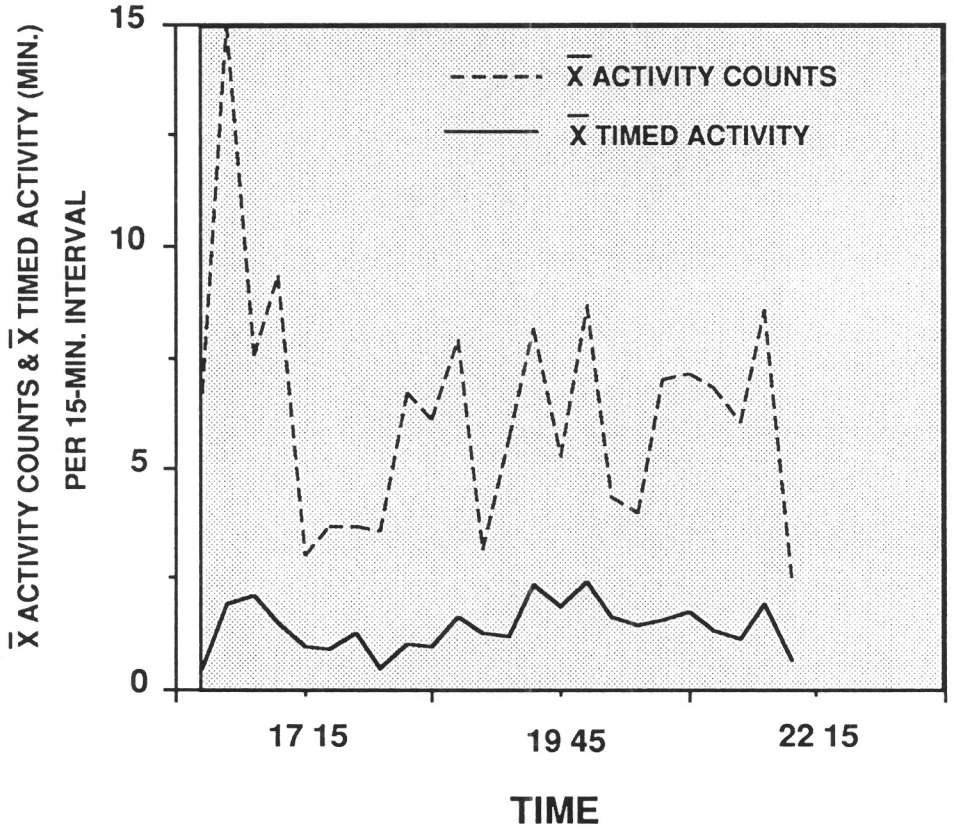

Figure 4: Activity pattern for female fall armyworm moths during a six-hour night period as recorded by computer (activity counts) and timed from videotape (timed activity) (n = 9). One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. The shaded area represents the scotophase.

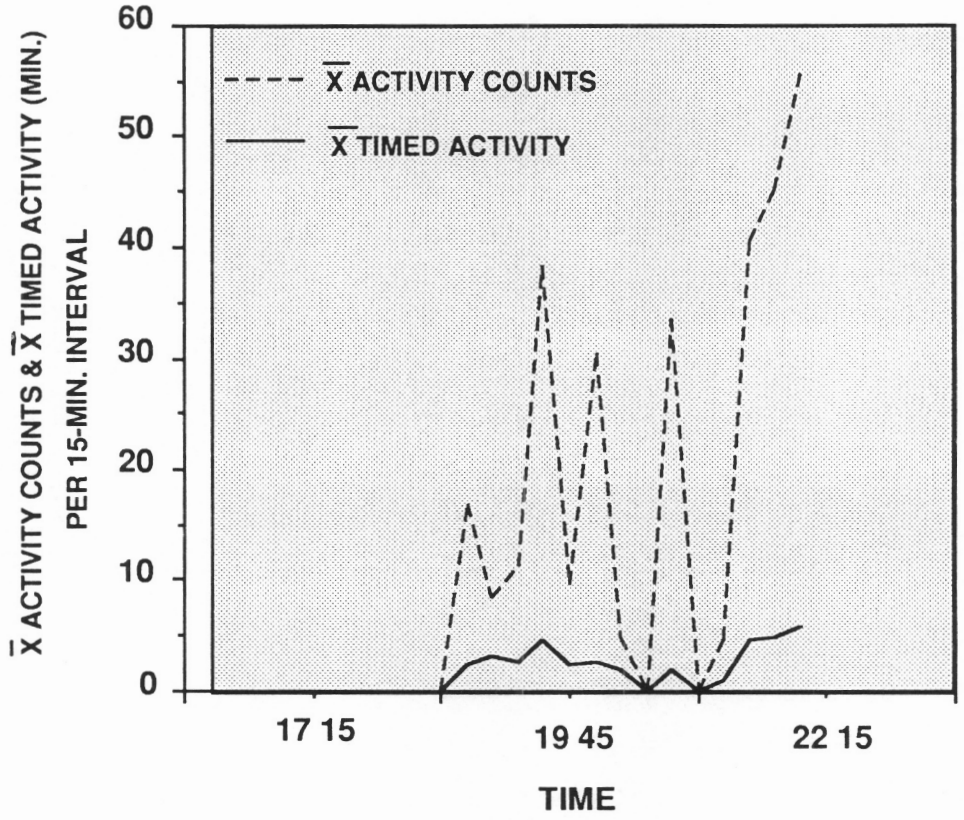

Figure 5: Activity pattern for male fall armyworm moths during a six-hour night period as recorded by computer (activity counts) and timed from videotape (timed activity) (n = 4). One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. The shaded area represents the scotophase.

Consistently, males showed greater activity than females by having a significantly greater mean activity bout time, a greater proportion of long-duration activity bouts, and a generally greater magnitude of activity during peak periods. Greater activity in males occurs not only in fall armyworm, but also in other noctuid moths including cabbage looper (Sprint & Eaton 1987), African armyworm (Woodrow et al. 1987), and corn earworm (Judge et al. 1991). While males may exhibit intense activity in search of a mate and perhaps food, females may be less active to conserve their energy reserves for reproduction. Also, females would not be flying while calling or ovipositing, while male flight activity increases in response to pheromone release.

The original premises on which the design of the V. P. I. & S. U. actograph was based must be modified in light of the preceding evidence. The first assumption should be altered to include all activity, not just flying, such that computer-recorded activity counts are in direct relationship to the amount of time a moth was active. The second assumption that most of the flying took place in the upper portion of the cage is correct, but other activities such as walking, which may register activity counts, occur at the top of a cage too. The third assumption that actograph data may be used as an index of migratory potential is questionable. From the results of this study, actograph data have the potential for validly indexing adult activity potential but may or may not be a valid index for migratory potential. The evening peak of activity for female FAW recorded with the actograph matches the massive take-off of FAW migrants just after sunset observed in the field (C. E. Rogers pers. comm.). However, no evening peak of activity was recorded for male FAW with the actograph. Additional free flight studies of male and female FAW in the field to compare to actograph data are needed to make valid conclusions about male and female FAW migratory capacity.

Chapter 4

Larval Rearing Density Effects on Pupal Weight, Larval and Pupal Development, and Adult Activity of the Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

Introduction

Among migratory lepidopterous species in the southeastern U.S., the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (FAW), ranks second only to the corn earworm, *Helicoverpa zea* (Boddie), in causing severe crop damage (Sparks 1986). There is a need for increased understanding of the factors resulting in FAW dispersal before an effective integrated pest management program can be devised. Of particular importance in the study of FAW flight movement is the "identification of physiological and behavioral mechanisms conducive to initiation . . . of flight" (Stinner et al. 1983). One of the cues for migratory flight from the overwintering locales may be high population density, probably acting in conjunction with other factors, including a decrease in food quality and food quantity (Johnson 1969). With an increase in population density, two or more groups of the same species may emerge from a particular locale. These supposed groups may differ in body weight, wing size, developmental time, and adult activity. This phenomenon occurs in a congeneric species, the African armyworm, *Spodoptera exempta* (Walker), which has two density-dependent phases (Faure 1943). The phases of *S. exempta*, migrant (high density) and nonmigrant (low density), differ in the above mentioned characteristics (Parker & Gatehouse 1985a and b, Simmonds & Blaney 1986), and we proposed that the same might occur in the FAW. The hypothesis of this study is that premigrant FAW (defined as moths which are morphologically and physiologically capable of migratory flight and with a high propensity for flight but have not yet flown) are separable from non-premigrant individuals based on differences in weight, wing size, developmental time, and adult behavior.

Research on factors other than weather in the overwintering locales which are associated with migratory flight in FAW is limited. In studies on *S. exempta* (Walker), Parker & Gatehouse (1985a) showed that adults from larvae reared at a higher density were more likely to fly longer than adults from larvae reared at a lower density. They suggested that larval population density was an important factor influencing migration in the African armyworm. The objective of this study was to determine the effects of larval rearing density in the laboratory

and in the field on developmental time, subsequent size, and adult activity of the FAW.

Materials and Methods

Fall armyworm eggs for the laboratory studies and first-instar larvae for the field studies were obtained from the USDA, ARS, Insect Biology and Population Management Research Laboratory, Tifton, Ga. This colony was originally collected from volunteer corn and mature sorghum in the fall of 1986. Field-collected larvae have been added to the laboratory colony regularly to reduce laboratory adaptation.

Laboratory Studies

Size and Developmental Time. Newly emerged first-instar larvae were placed in 30 ml plastic cups with approximately 15 ml diet (an excess) at an initial rearing density of one, two, or three larvae per cup. For each replicate, 100 to 150 cups were set up for each density. There were nine replicates. Larvae were reared on a modified artificial diet from Guy et al. (1985) (brewer's yeast was used instead of torula yeast). Cups were kept in an environmental chamber, with a 14:10 (L:D) photoperiod (to simulate summer conditions) at 26^{o}C. The cups were checked every 2 d initially and daily after detecting the first prepupa to record mortality, days to pupation, and days to emergence. When pupation occurred, pupae were placed individually in clean 30 ml cups. Pupae were weighed and sexed 3 d after pupation, and adults were allowed to emerge in the 30 ml cups.

Newly emerged moths were frozen for wing measurement. The right forewing and right hindwing of each moth were taped to white paper. The widths of both forewings and hindwings were measured to the nearest 0.01 mm with a hand-held micrometer. The width of a wing was defined as the widest point of the wing perpendicular to its long axis (the length). The width was chosen over length as an indicator of wing size because part of the length of the wing was often torn during removal from the body.

Data on pupal weight, wing width, days to pupation, and days to emergence, were pooled from nine replicates, because larvae were subjected to identical temperature, photoperiod, and diet conditions. Sample sizes were unequal due to mortality and because of the omission of the density treatment of three larvae per cup in some of the earlier replicates. To test for a density effect on pupal weight, wing width, days to pupation, and days to emergence, the GLM analysis of variance procedure of SAS was used (SAS Institute 1985) (α = 0.05). Means were separated using Tukey's studentized range test (α = 0.05).

Measurement of Adult Activity. On the day of emergence, unmated female moths were placed in an actograph, a 32-channel computerized system which can monitor activity of 32 moths simultaneously (Eaton 1985). The actograph was housed in the same environmental chamber in which the rearing occurred. Inside the actograph, there is a sunset/sunrise light control to simulate dusk and dawn conditions (Byers & Unkrich 1983). The light regime was 1 h of simulated sunset starting at 1600 h, 9 h of darkness, 1 h of simulated sunrise, followed by 13 h of light. A cup containing an emerged moth was placed inside an actograph cage and the lid removed without disturbing the moth. Moths were placed inside the actograph cages a few hours before simulated dusk. An actograph cage consisted of a screen-top paper carton (13 cm in dia by 16.5 cm high) with an opening (2.5 cm wide by 1.5 cm high) on the side 1.5 cm from the top to allow a 940-nm infrared beam to pass through. Activity was detected and logged as an activity count when a moth broke the beam. Validation studies using this actograph have shown that the number of activity counts is directly related to the amount of actual time a moth was active. The actograph was interfaced with a Digital Equipment Minc II Microcomputer which collected and stored data. Data were transferred to the IBM 3084/3090 main frame for analysis using SAS. Our actograph system had a significant advantage over pivot-stick actographs (as per Gatehouse & Hackett 1980) in that the moths were never handled before or during the collection of activity data. Moths were left in the actograph for up to 10 d and fed 10% sucrose solution *ad libitum*. Activity counts were continuously recorded for 15 h each night starting 2 h before sunset. Preliminary data showed that there is an evening peak of activity in females beginning soon after sunset. Also, radar and

airplane observations revealed FAW moths taking off for migration shortly after sunset (C. E. Rogers pers. comm., Pair & Sparks 1986).

One actograph replicate consisted of a six-day actograph recording of up to 30 moths. There were five actograph replicates. The first three actograph replicates had moths which came from larvae reared at one and two larvae per cup. The fourth replicate had moths which came from larvae reared at one and three per cup. The fifth replicate included moths which came from larvae reared at one, two, and three larvae per cup. Sample sizes within a single replicate were equivalent among or between density treatments unless a moth died or an actograph channel failed during the activity observations. The sample sizes for the first replicate were: 9 and 10, for the second replicate: 15 and 14, and for the third replicate: 14 and 14, per respective density treatment of one and two larvae per cup. Sample sizes for the fourth replicate were 14 for each of the two density treatments. Sample sizes for the fifth replicate were 10 for each of the three treatments. Usually, there were not enough moths which emerged on one day to obtain equal sample sizes for the treatment groups. Therefore, moths were initially placed in the actograph over two or three days, and the data were grouped later to compare equally-aged moths.

Nonparametric statistical analyses were used on the activity data because these data did not come from normal-like distributions, and nonparametric tests do not assume normality. For each of five 6-day recordings, data were analyzed, each night separately, by comparing the number of activity counts accumulated by 0.5 h after activity onset, 2 h after onset, and by the end of the night between or among density treatments using the nonparametric Wilcoxon Rank Sum for two treatments or the Kruskal-Wallis one-way analysis of variance for three treatments ($\alpha = 0.05$) (NPAR1WAY procedure, SAS Institute 1985). In addition, data from the fifth replicate were analyzed using the Jonckheere test for ordered alternatives ($\alpha = 0.05$) (Hollander & Wolfe 1973). The ordered alternative hypothesis was that activity counts of moths from the density treatment of one larva per cup $\leq$ the activity counts of moths from the treatment of two larvae per cup $\leq$ the activity counts of moths from the treatment of three larvae per cup. The Jonckheere test is more powerful than Kruskal-Wallis because it increases the probability that a difference will be detected in the direction of the ordered alternative. A more

powerful test decreases the probability of a Type II error which occurs when a false null hypothesis is not rejected (Zar 1984).

Parker & Gatehouse (1985a and b) analyzed flight activity of tethered *S. exempta* adults by dividing the moths into proportions of long-flyers and short-flyers based on duration of individual flights. To examine the FAW activity count data in like manner, the proportions of infrequent (≤ 200 activity counts) and frequent (≥ 201 activity counts) "flyers"[1] of different density treatments on the second night in the actograph were analyzed with χ^2 ($\alpha = 0.05$). The Yates correction for continuity when df = 1 was applied when appropriate (Zar 1984). Activity counts accumulated by 2 h after activity onset and accumulated by the end of the night were examined for this analysis.

Field Studies

The effects of larval crowding in whorl-stage field corn on developmental time, size, and adult activity of FAW were studied in 1989. Two 0.1 ha plots of 'Pioneer 3320-F11' corn were planted at Tifton, Ga., and artificially infested with first instar larvae from the Tifton laboratory colony, one plot for low density and one for high density (Table 2). Infestation was accomplished using the mechanical dispenser method of Wiseman et al. (1980). Corn was replanted before each infestation. Plants were at the eight-leaf stage (middle whorl) during the first infestation, and at the six-leaf stage (early whorl) during the last two infestations.

To recover larvae, plants were cut at their base and taken to the edge of the field for examination. Larvae were removed from the plants and placed individually in 30 ml cups containing diet. Larvae were either fifth or sixth instar at the time of collection. The following number of larvae were recovered from Field Trials 1, 2, and 3, respectively: 90 low density (L), 15 high density (H); 177 L, 289 H; 300 L, 325 H. Field-collected larvae were shipped via overnight mail to V. P. I. & S. U., Blacksburg, Virginia.

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[1]Moths sometimes *walked* around the actograph cage and registered activity counts.

Table 2. Dates for planting corn field plots, infestation and recovery of fall armyworm larvae and number of larvae infested per plant on each infestation date, Tifton, Georgia.

Trial	Date of Planting	Date of Infestation	Date of Recovery	$\bar{x}$ No. larvae/plant	
				Low	High
1	5/5/89	6/14/89	6/27/89	1	20
2	6/28/89	7/26/89	8/7-9/89	4	25
3	8/28/89	9/20/89	10/2/89	5	25

Size and Developmental Time. Larvae were allowed to complete development under the same environmental conditions as the laboratory-reared FAW. Pupae were weighed 3 d after pupation. Total times to pupation and emergence could not be determined, but duration of pupal stage was compared between the low and high density treatments . Forewing and hindwing widths were measured on newly emerged moths as described above. Most larvae pupated within a week after being placed on artificial diet. However, because larvae may undergo ca. 25-35% of their development during the last 7-9 days of the larval stage, the effects of feeding on artificial diet on pupal weight, wing width, and duration of pupal stage were examined.

Sample sizes for analyses were reduced from collection sample sizes because of natural mortality. Data on pupal weights, duration of pupal stage, and wing widths from each infestation were analyzed separately because of the differences in initial density treatments among the field trials. Shortly after beginning Field Trial 1, a heavy rain washed many of the first instar larvae from the plants. Thus, there were not enough data collected on the parameters of interest from Field Trial 1 to statistically analyze; they are presented in this chapter for completeness. For Field Trials 2 and 3, the effects of larval density and days on artificial diet on pupal weight, wing width, and duration of pupal stage were analyzed using the GLM analysis of variance procedure (SAS Institute 1985) ($\alpha = 0.05$). Means were separated using Tukey's studentized range test ($\alpha = 0.05$).

Measurement of Adult Activity. Unmated female adults from field-reared larvae were placed in the actograph for collection of activity data. Sample sizes were unequal for females from Field Trials 1 (25 and 5 for low and high density, respectively) because of the low number of high density larvae collected in Field Trial 1. From Field Trial 2, not enough female moths from the low density treatment emerged in time to achieve equal sample sizes for each treatment in the actograph study. Hence, sample sizes for female moths from Field Trial 2 were 10 and 20 for low and high density treatments, respectively. Fifteen female moths from each density treatment in Field Trial 3 were observed for only three d in the actograph at which time they were removed, and twelve unmated male moths from the same field trial (six from

each treatment) were placed in the actograph to compare their activity with that of the field-reared females.

Activity data from Field Trial 1 were not analyzed because of the low sample size for the high density treatment, but they are presented in this chapter for completeness. Activity data from Field Trials 2 and 3 were analyzed separately but otherwise treated similarly to laboratory-reared moth activity data. The effect of feeding on artificial diet instead of field corn on adult activity was assumed to be minimal and uniform. Moths used for actograph studies were the first emerged moths from each field trial collection, and as larvae, they had spent the least amount of time on artificial diet before pupation.

Results and Discussion

Laboratory Studies

Size and Developmental Time. In the female FAW, pupae from larvae reared two and three per cup weighed less than pupae reared one per cup ($P = 0.0001$) (Table 3). In the males, pupae from larvae reared three per cup weighed less than pupae reared one per cup ($P = 0.0035$), but pupae reared two per cup had comparable weights to pupae reared one and three per cup.

Wing width, an indicator of wing size, is assumed to be directly proportional to adult size, based on Lepidopteran studies which showed that wing measure was a good index of adult size (Miller 1977). The mean forewing width in female FAW was significantly greater in the individuals from larvae reared one per cup ($P = 0.0001$) (Table 3). However, results were more variable in the males in which the mean forewing width was significantly greater in moths from larvae reared one and three per cup than in moths from larvae reared two per cup ($P = 0.0029$). For both females and males, means of hindwing width among the groups were not significantly different ($P > 0.05$).

Presumed migratory individuals of several Lepidopteran species were shown to be smaller as pupae and/or adults (see review, Angelo & Slansky 1984; Lewis & Keaster 1989, Tignor & Eaton 1986, Parker & Gatehouse 1985a).

Table 3. Pupal weight, wing width, time to pupation and emergence of fall armyworms from larvae reared at three densities in the laboratory.

No. Larvae/ 30 ml Cup	Pupal Weight (mg) $\bar{x} \pm$ SE (n)	Forewing Width (mm) $\bar{x} \pm$ SE (n)	Hindwing Width (mm) $\bar{x} \pm$ SE (n)	Days To Pupation $\bar{x} \pm$ SE (n)	Days to Emergence $\bar{x} \pm$ SE (n)
FEMALE					
1	268.19 ± 1.65(326)a	6.93 ± 0.02(237)a	9.48 ± 0.04(187)a	19.56 ± 0.13(336)ab	30.85 ± 0.15(327)b
2	254.76 ± 2.49(258)b	6.76 ± 0.03(257)b	9.37 ± 0.05(148)a	19.99 ± 0.15(260)a	31.38 ± 0.16(257)a
3	250.72 ± 2.92(183)b	6.77 ± 0.03(188)b	9.36 ± 0.05(107)a	19.49 ± 0.12(188)b	30.78 ± 0.12(188)b
MALE					
1	264.12 ± 1.65(337)a	6.72 ± 0.02(327)a	9.34 ± 0.03(269)a	19.22 ± 0.12(350)b	32.17 ± 0.13(334)b
2	260.52 ± 2.19(247)ab	6.64 ± 0.02(236)b	9.24 ± 0.04(184)a	20.26 ± 0.17(249)a	33.24 ± 0.18(245)a
3	254.94 ± 2.44(188)b	6.75 ± 0.02(181)a	9.34 ± 0.04(132)a	19.71 ± 0.13(190)b	32.76 ± 0.14(190)a

Means followed by the same letter are not significantly different, $\alpha = 0.05$, Tukey's studentized range test.

Decreased body size would make the insect a more efficient flyer over long distances and times. These FAW data are consistent with the hypothesis that crowding produces individuals which are morphologically equipped for migratory flight. However, in studies with nonmigratory species, larvae reared at a higher density were smaller as pupae than larvae reared at a lower density (see review, Peters & Barbosa 1977). As crowding increases, there is more competition for food. Thus, the effects of rearing density on pupal weight and wing size may not be indicative of a separable premigrant phase in the FAW, but rather a typical response to crowded conditions. Additionally, in non-Lepidopteran migratory species such as *Oncopeltus fasciatus* (Dallas), there was no correlation between long duration (presumed migratory) flight and body size (Rankin & Burchsted 1992).

Both females and males reared at one larva per cup and three larvae per cup required fewer days to pupation than those reared at two per cup (Table 3) (males: $P = 0.0001$, females: $P = 0.0273$). Females reared at one and three larvae per cup took significantly less time to emerge than those reared at two larvae per cup ($P = 0.0126$), while the mean days to emergence was greatest in males reared at one larva per cup ($P = 0.0001$). The greatest differences in pupation and emergence times for females was 0.6 d and for males, 1.07 d which may not be biologically significant, but within the normal range of variation for FAW developmental time. Crowding in other migratory species has resulted in both longer (Tignor & Eaton 1986, Fescemyer & Hammond 1986) and shorter developmental times (Simmonds & Blaney 1986) with differences among treatments much greater than 1 day. Thus, developmental time is not a good factor to use in differentiating between non-premigrant and premigrant FAW.

There was a tendency for male FAW to emerge later than cohort female FAW (Table 3, 1.32 to 1.98 days later for males). Presumably, the females emerge first to allow time for ovary maturation before mating on the second night after emergence (Luginbill 1928). Females emerging before males could indicate that females migrate before mating without males. However, overlaps in developmental time between males and females would allow reproductively immature adults of both sexes to migrate simultaneously and mate upon arrival of the new habitat. Also, immature female and male moths of different ages

could migrate from more than one source and convene at the same location to mate.

Adult Activity. Typically, the pattern of activity for laboratory-reared females during the night consisted of a pronounced peak at dusk and one or two peaks following dawn (Figure 6). FAW moths have been observed via radar migrating from Tifton, Ga., shortly after dusk (C, E, Rogers pers. comm., Pair & Sparks 1986). These results also resemble the findings for *S. exempta* (Parker & Gatehouse 1985a). The FAW dusk activity may be associated with migratory and foraging flight, while the post-dawn activity may be attributed to foraging or searching for a hiding place (Dreisig 1986). The lull in activity between peaks may be related to the time when females engage in calling behavior. A pattern of frequent activity during the first and second nights after emergence was followed by a progressive decrease in activity as the moths aged. High activity during the first night may be due in part to the moths' adjustment to the actograph cages. Therefore, the second night was assumed to have had the greatest activity.

Activity counts were graphed as mean rank (rank sum/sample size) to alleviate magnitude differences in graphing mean number of activity counts among the FAW groups tested. There were no significant differences in activity counts among moths from larvae reared at different density treatments for each night ($P > 0.05$) (Figures 7-11). The more powerful Jonckheere test on the fifth replicate (Figure 11) did not show any significant effects due to rearing density ($P > 0.05$).

The proportion of infrequent "flyers" versus frequent "flyers" was compared among density treatments for the second night (Tables 4 and 5). It is quite evident that most moths were infrequent flyers 2 h after onset and by the end of the night. There were no significant differences due to rearing density between the proportions of infrequent versus frequent flyers ($P > 0.05$).

These findings for FAW are in contrast to those obtained for *S. exempta* (Parker & Gatehouse 1985a). In tethered flight studies with *S. exempta*, an increase in larval density from 20 to 40 larvae per 450 ml jar significantly increased the proportion of subsequent moths which were long-flyers (>120 min during one night). Perhaps the density treatment of three FAW larvae per cup

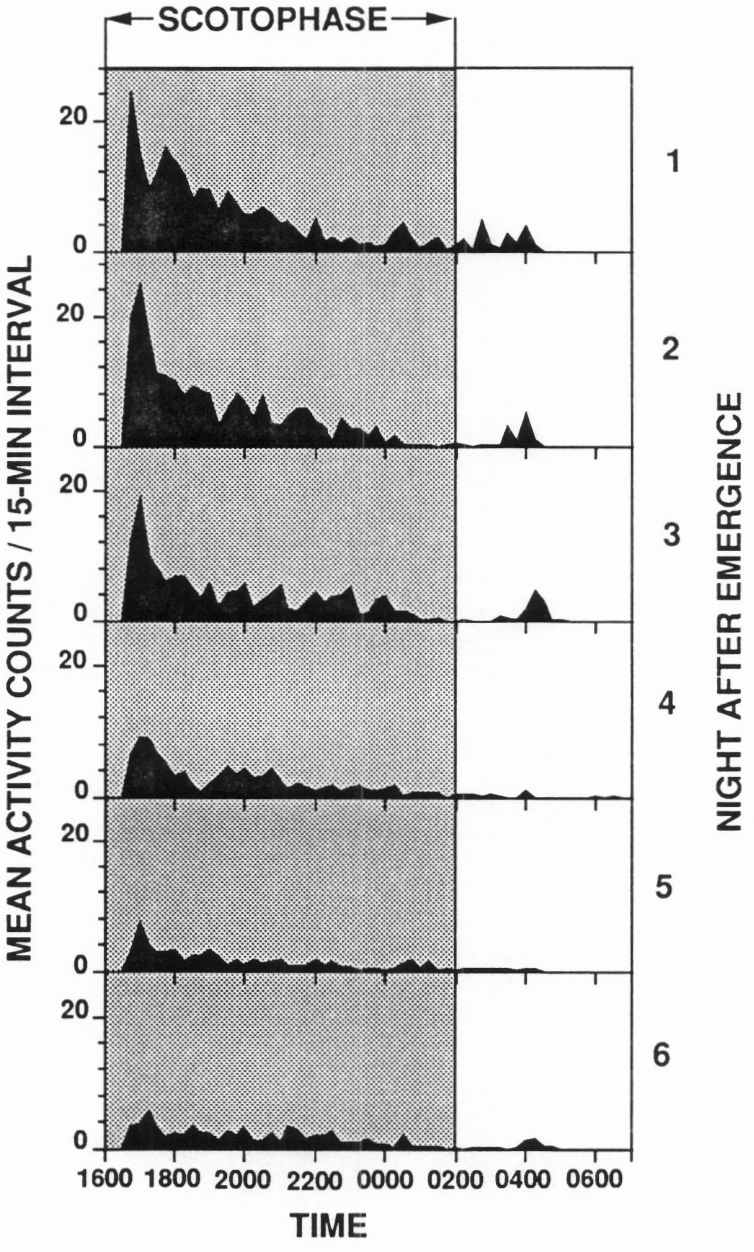

Figure 6: Activity patterns for female fall armyworm moths from laboratory-reared larvae recorded in the actograph over a six-day period (n = 30). One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage.

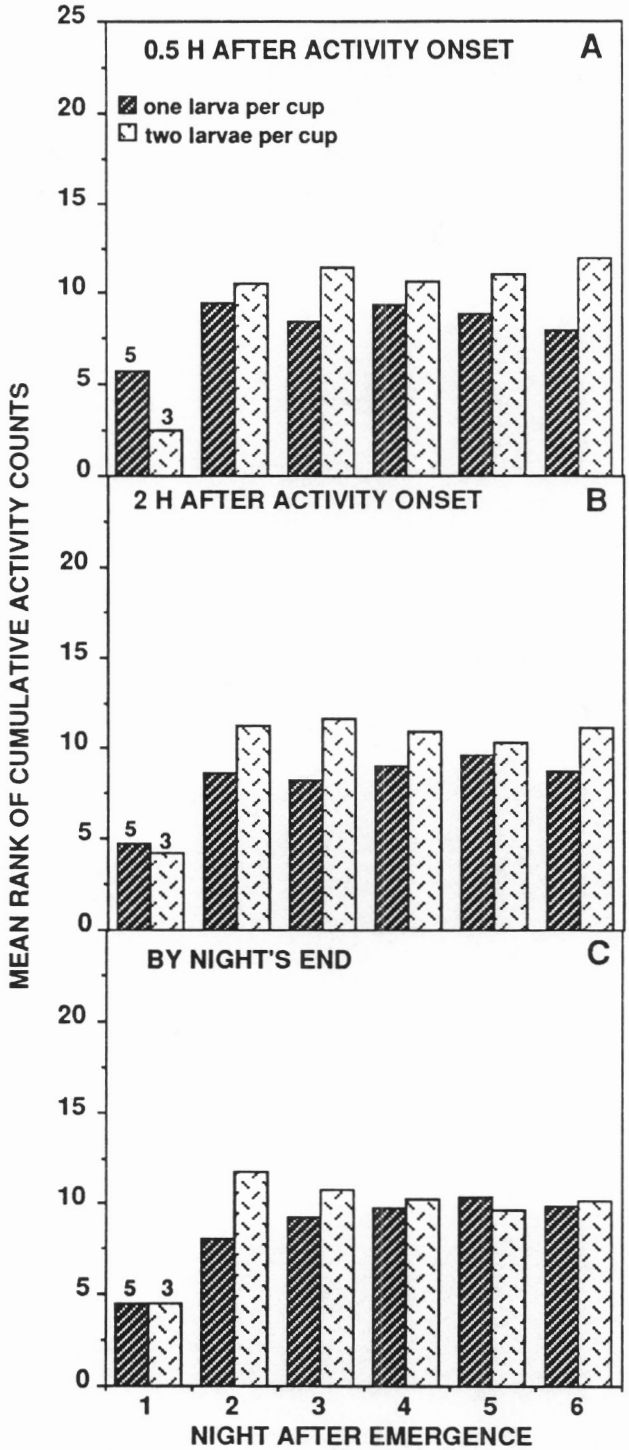

Figure 7: Mean ranks (rank sums / sample sizes) of cumulative activity for female fall armyworm moths from the first laboratory replicate. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample sizes are 9 (one larva per cup) and 10 (two larvae per cup) unless noted above the bar.

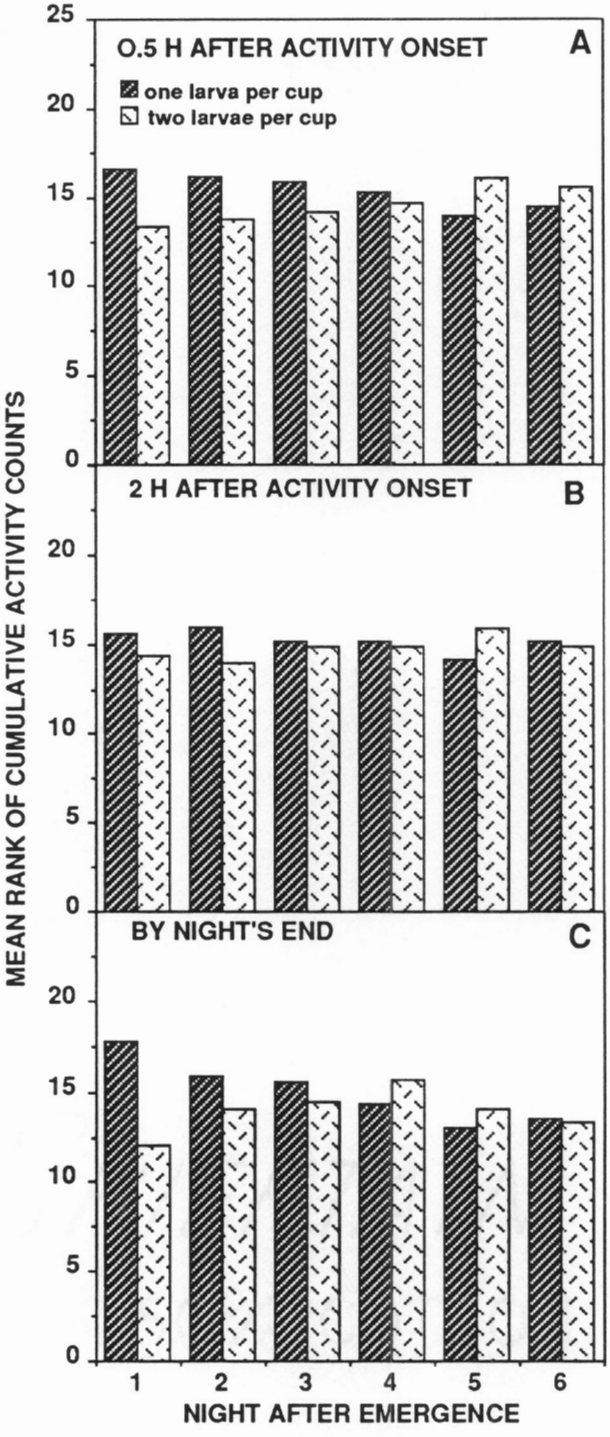

Figure 8: Mean ranks (rank sums / sample sizes) of cumulative activity for female fall armyworm moths from the second laboratory replicate. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample sizes are 15 (one larva per cup) and 14 (two larvae per cup).

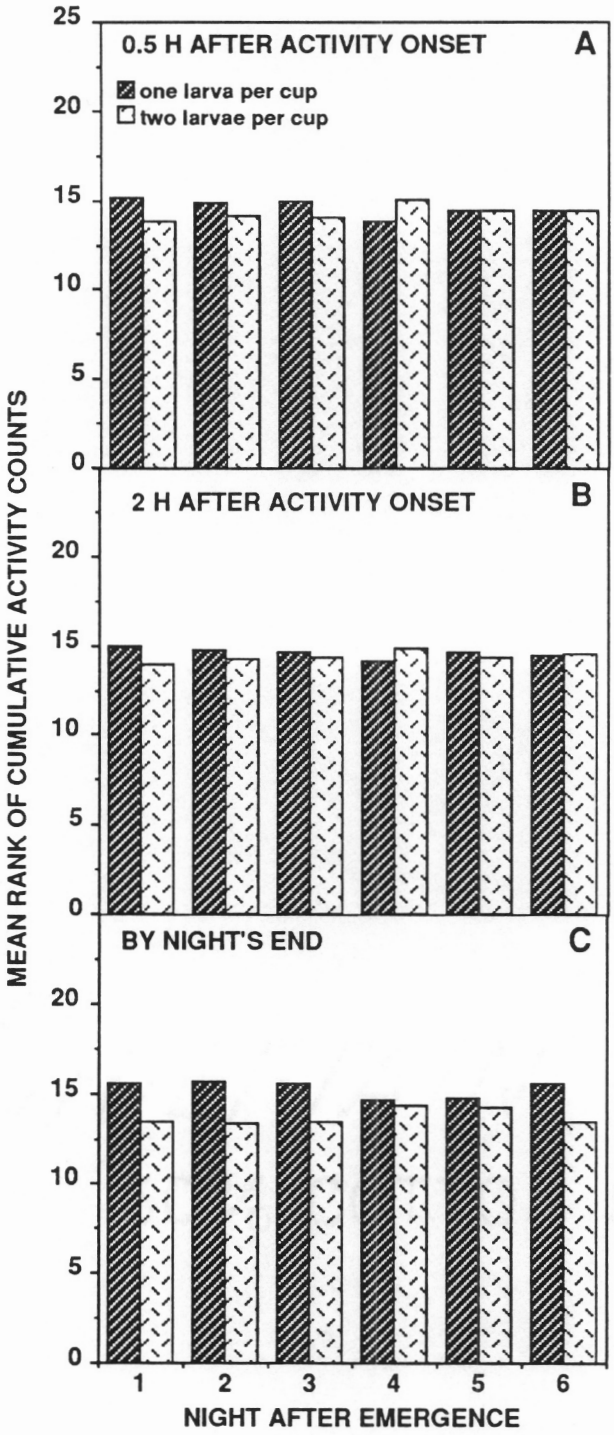

Figure 9: Mean ranks (rank sums / sample sizes) of cumulative activity for female fall armyworm moths from the third laboratory replicate. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample size is 14 for each treatment.

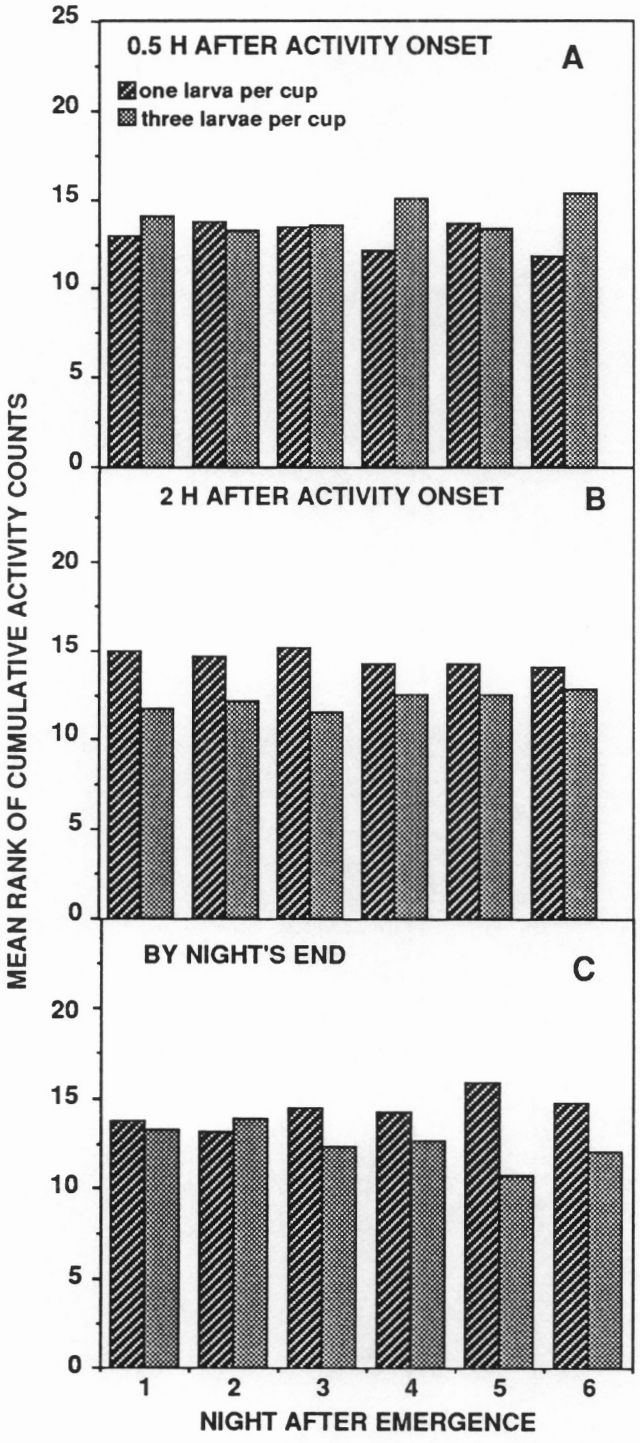

Figure 10: Mean ranks (rank sums / sample sizes) of cumulative activity for female fall armyworm moths from the fourth laboratory replicate. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample sizes are 14 (one larva per cup) and 12 (three larvae per cup).

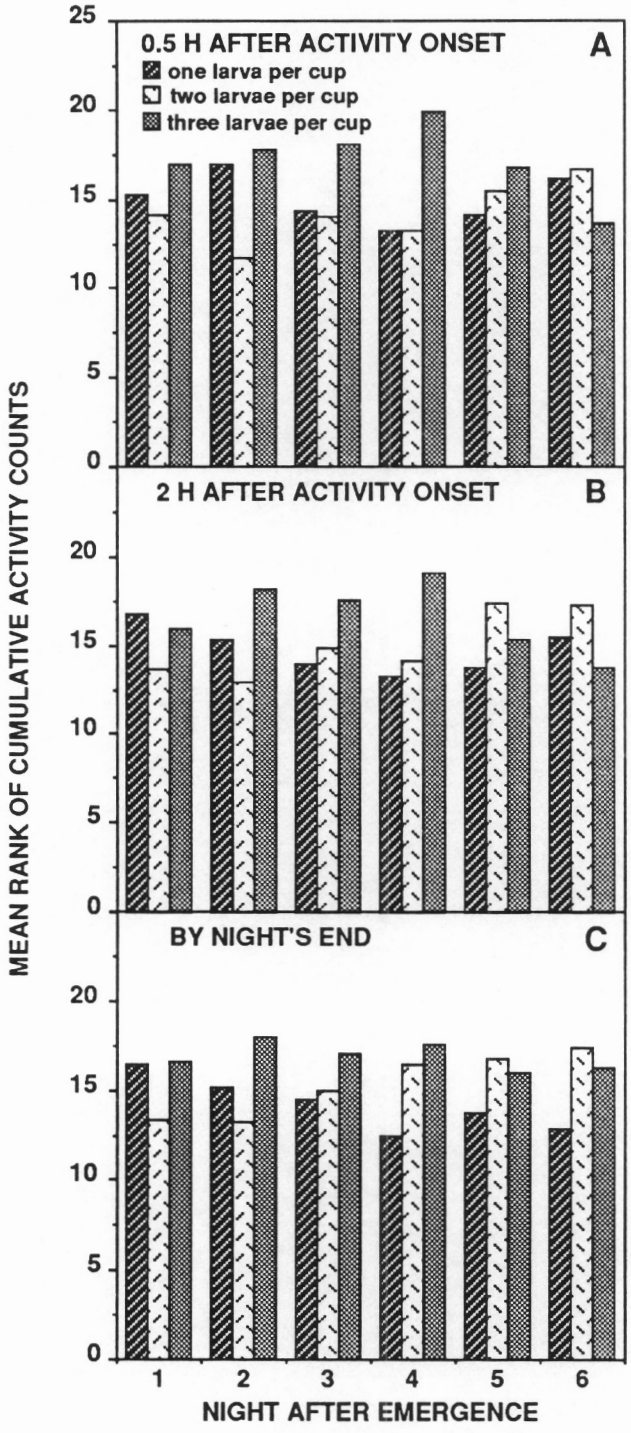

Figure 11: **Mean ranks (rank sums / sample sizes) of cumulative activity for female fall armyworm moths from the fifth laboratory replicate. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample size is 10 for each treatment.**

Table 4. Proportions of infrequent and frequent "flyers"[a] in female fall armyworm adults from laboratory-reared larvae in different density treatments, using data collected by two hours after activity onset on the second night after emergence. Analyzing replicates separately, no significant differences were found, χ^2 analysis, $P > 0.05$. Sample sizes are in parentheses.

Replicate	No. Larvae Per 30 ml Cup	Proportion of Infrequent Flyers	Proportion of Frequent Flyers
1	1	.78(9)	.22(9)
	2	.60(10)	.40(10)
2	1	.93(15)	.07(15)
	2	.93(14)	.07(14)
3	1	.93(14)	.07(14)
	2	.93(14)	.07(14)
4	1	1.00(14)	.00(14)
	3	1.00(12)	.00(12)
5	1	.70(10)	.30(10)
	2	.90(10)	.10(10)
	3	.80(10)	.20(10)

[a]Infrequent refers to ≤ 200 accumulated activity counts, and frequent refers to ≥ 201 accumulated activity counts. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Moths sometimes *walked* across the beam window and registered activity counts.

Table 5. Proportions of infrequent and frequent "flyers"[a] in female fall armyworm adults from laboratory-reared larvae in different density treatments, using data collected by the end of the second night after emergence. Analyzing replicates separately, no significant differences were found, χ^2 analysis, $P > 0.05$. Sample sizes are in parentheses.

Replicate	No. Larvae Per 30 ml Cup	Proportion of Infrequent Flyers	Proportion of Frequent Flyers
1	1	.67(9)	.33(9)
	2	.30(10)	.70(10)
2	1	.73(15)	.27(15)
	2	.79(14)	.21(14)
3	1	.71(14)	.29(14)
	2	.71(14)	.29(14)
4	1	.79(14)	.21(14)
	3	.67(12)	.33(12)
5	1	.70(10)	.30(10)
	2	.80(10)	.20(10)
	3	.60(10)	.40(10)

[a]Infrequent refers to ≤ 200 accumulated activity counts, and frequent refers to ≥ 201 accumulated activity counts. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Moths sometimes *walked* across the beam window and registered activity counts.

was not sufficiently crowded to affect subsequent adult behavior. In tethered flight studies, *Agrotis ipsilon* (Hufnagel) moths from larvae reared five per 30 ml cup were significantly more active than moths from larvae reared three per cup (Lewis & Keaster 1989). However, none of the *A. ipsilon* flights that Lewis & Keaster (1989) examined were of long-duration (≥ 1 hour). In more recent *A. ipsilon* tethered flight studies which involved higher levels of larval crowding, there were no significant larval density effects on amount of presumed migratory long-duration flight (≥ 1 hour) (Sappington & Showers 1992).

Field Studies

Size and Developmental Time. The second field trial was conducted during late July to early August, 1989. Some of the larvae collected may have come from natural populations. Generally, pupae were lighter, wings were smaller, and duration of pupal stage was shorter for this group than from the first field trial (Table 6). However, there were no significant differences in the parameters examined for FAW from low and high density treatments ($P > 0.05$).

The third field trial took place during late September to early October, 1989. Again, as in the second trial, some of the larvae collected may have come from natural populations. The trends toward lighter pupae and shorter developmental times were continued for this trial (Table 6). However, no significant differences were found in the parameters examined for FAW collected from low and high density plots ($P > 0.05$).

Although field rearing density did not affect pupal weight, wing width, and duration of pupal stage, the length of time spent on artificial diet significantly affected these parameters. For instance, from the second field trial, female pupal weight of larvae which fed on artificial diet for 4-5 d < pupal weight of larvae on diet for 6-7 d < pupal weight of larvae on diet for 8-10 d (means ± SE (n): 205.41 ± 3.27 (68), 246.07 ± 3.43 (74), 269.40 ± 3.73 (57), respectively) ($P = 0.0001$). In the same group of females, duration of pupal stage of larvae which fed on artificial diet for 8-10 d < duration of pupal stage of larvae on diet for 6-7 d < duration of pupal stage of larvae on diet for 4-5 d (means ± SE (n): 9.33 ± 0.08 (57), 9.62 ± 0.07 (74), 10.10 ± 0.06 (68) respectively) ($P = 0.0001$).

Table 6. Pupal weight, wing width, and duration of pupal stage in fall armyworms reared at two density levels in corn field plots, Tifton, Georgia, 1989. Analyzing field trials separately, no significant differences were found between density treatments within sex, *t*-test, $P > 0.05$.

Trial	Sex	$\bar{x}$ No. Larvae/Plant	Pupal Weight (mg) $\bar{x} \pm$ SE (n)	Forewing Width(mm) $\bar{x} \pm$ SE (n)	Hindwing Width(mm) $\bar{x} \pm$ SE (n)	Duration of Pupal Stage(days) $\bar{x} \pm$ SE (n)
1[a]	Female	1	267.83 ± 6.30(35)	7.29 ± 0.13(8)	9.62 ± 0.22(5)	12.06 ± 0.10(35)
	Female	20	261.40 ± 6.70(5)	n.d.[b]	n.d.	12.00 ± 0.00(5)
	Male	1	271.90 ± 5.76(39)	6.73 ± 0.05(38)	9.38 ± 0.08(30)	13.69 ± 0.10(39)
	Male	20	261.50 ± 10.37(6)	6.73 ± 0.10(6)	9.40 ± 0.07(5)	13.67 ± 0.21(6)
2	Female	4	234.90 ± 4.51(73)	6.66 ± 0.04(55)	9.47 ± 0.07(29)	9.71 ± 0.07(73)
	Female	25	241.15 ± 3.39(126)	6.62 ± 0.03(99)	9.36 ± 0.07(42)	9.70 ± 0.06(126)
	Male	4	242.59 ± 3.99(75)	6.48 ± 0.04(72)	9.27 ± 0.06(38)	10.92 ± 0.15(75)
	Male	25	248.54 ± 3.03(117)	6.51 ± 0.03(113)	9.40 ± 0.06(54)	10.76 ± 0.06(117)
3	Female	5	213.25 ± 3.19(105)	6.69 ± 0.04(47)	8.98 ± 0.07(44)	9.08 ± 0.04(105)
	Female	25	212.30 ± 3.08(91)	6.75 ± 0.04(36)	9.11 ± 0.07(33)	9.12 ± 0.07(91)
	Male	5	223.82 ± 3.24(83)	6.49 ± 0.04(71)	8.81 ± 0.06(66)	10.14 ± 0.06(83)
	Male	25	222.71 ± 3.24(104)	6.49 ± 0.03(87)	8.74 ± 0.06(80)	10.23 ± 0.06(104)

[a] Data from Trial 1 were not analyzed because there were too few fall armyworms collected from the high density plot.

[b] n.d. = no data collected.

There were no significant interactions of the effect of rearing density with the effect of days on artificial diet for pupal weight, wing width, and duration of pupal stage ($P > 0.05$).

It is apparent that despite efforts to create artificially crowded conditions in the field, many larvae died, were washed from the plants, or dispersed from the treatment plots. Thus, at the time of collection, typically only one larva was found on each plant examined. What happened in the field is comparable to what occurred in the laboratory. Frequently, high density cups (with more than one larva initially) eventually became cups with only one larva. Certainly, mortality due to cannibalism occurred in both the crowded laboratory (observed) and field situations (Luginbill 1928). However, the laboratory crowding produced differences in weight, developmental time, and wing size, while field crowding did not. High dispersal and mortality of early instar larvae (Morrill & Greene 1973a and b, 1974) along with the confounding effect of the larvae feeding on artificial diet instead of field corn until pupation were the primary causes of the lack of a crowding effect on size and developmental time in the field-reared FAW.

Adult Activity. The pattern of activity for field-reared females was similar to the activity pattern of laboratory-reared females, except the dusk peak was broader, lasting for several hours (Figure 12). The dawn peaks, which might be associated with foraging or searching for a hiding place (Dreisig 1986), were quite pronounced.

Activity data for adults from Field Trial 1, which were not analyzed, are presented in Figure 13. In Field Trial 2, females from the high larval density plot generally exhibited more activity on Nights 1 through 3 (Figure 14). Yet only on the third night, 2 h after activity onset, was there significantly more activity in the high density females ($P = 0.0055$). In Field Trial 3, moths from larvae reared at a higher density had generally more activity counts on the second and third nights than moths from larvae reared at a lower density (Figure 15). However, no significant differences were found ($P > 0.05$). There was a tendency for more activity in the males from high density field-reared larvae by the end of the first, second, fourth, fifth, and sixth nights, but no significant differences were found

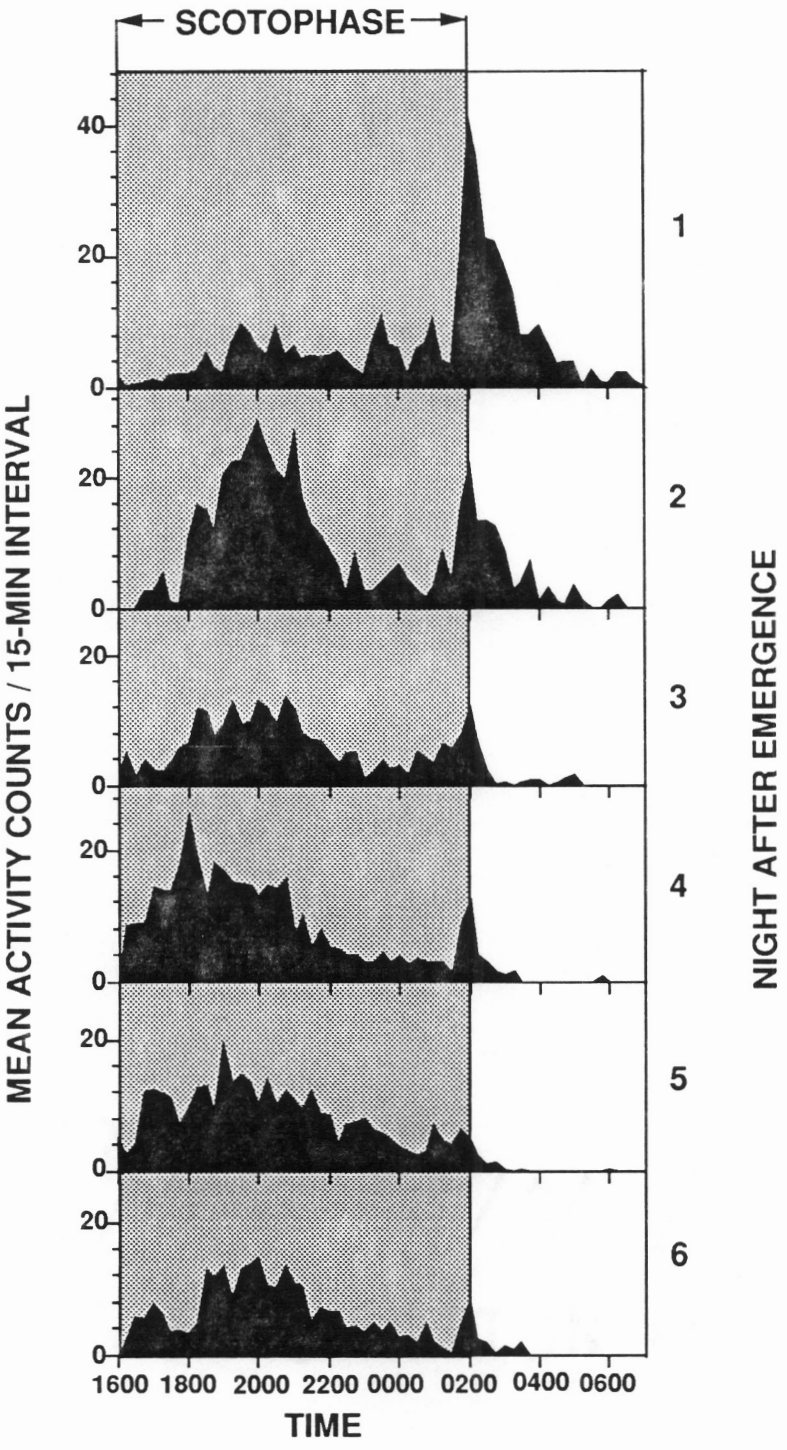

Figure 12: Activity patterns for female fall armyworm moths from field-reared larvae recorded in the actograph over a six-day period (n = 30). One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage.

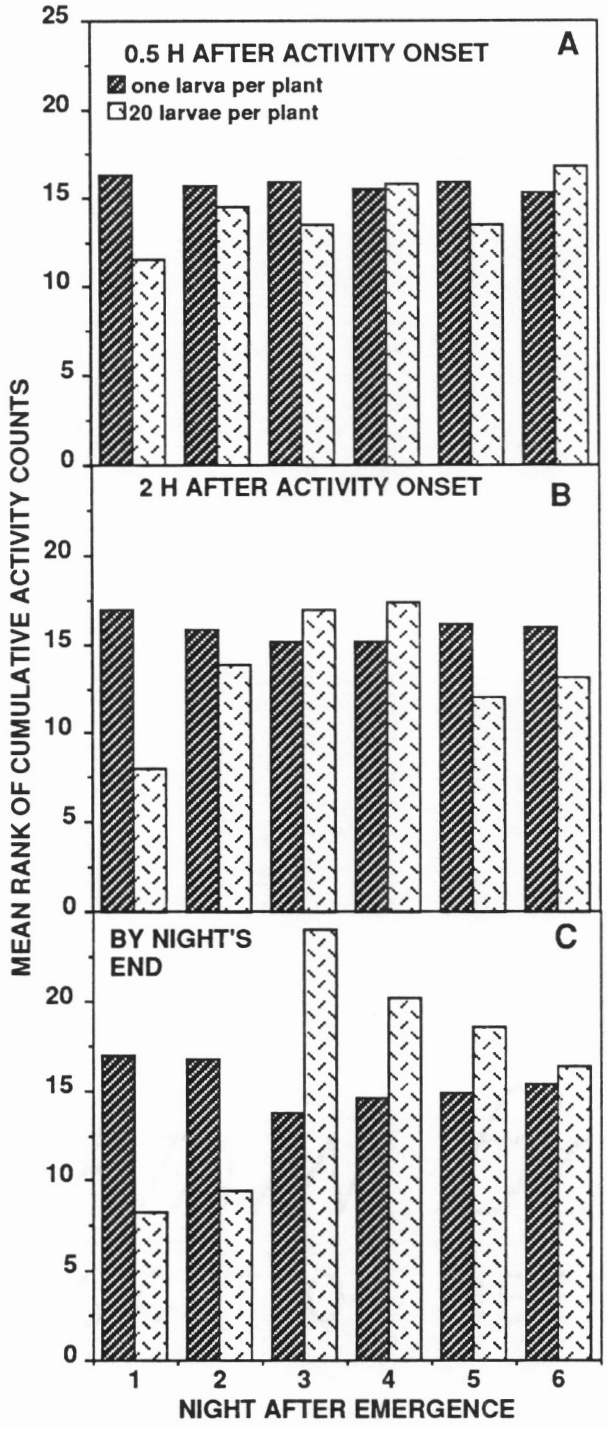

Figure 13: Mean ranks (rank sums / sample sizes) of cumulative activity for female fall armyworm moths from Field Trial 1. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample sizes are 25 (one larva per plant) and 5 (20 larvae per plant).

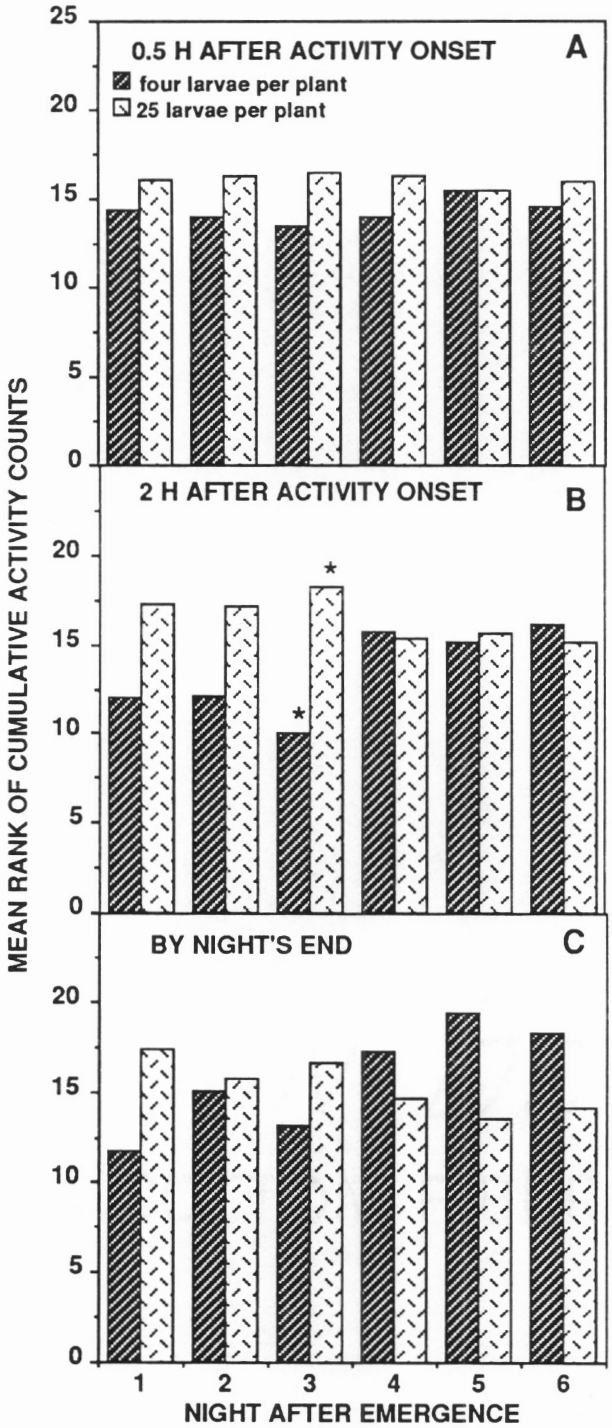

Figure 14: Mean ranks (rank sums / sample sizes) of cumulative activity for female fall armyworm moths from Field Trial 2. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample sizes are 10 (four larvae per plant) and 20 (25 larvae per plant). Asterisks denote significant differences between treatments ($P < 0.05$).

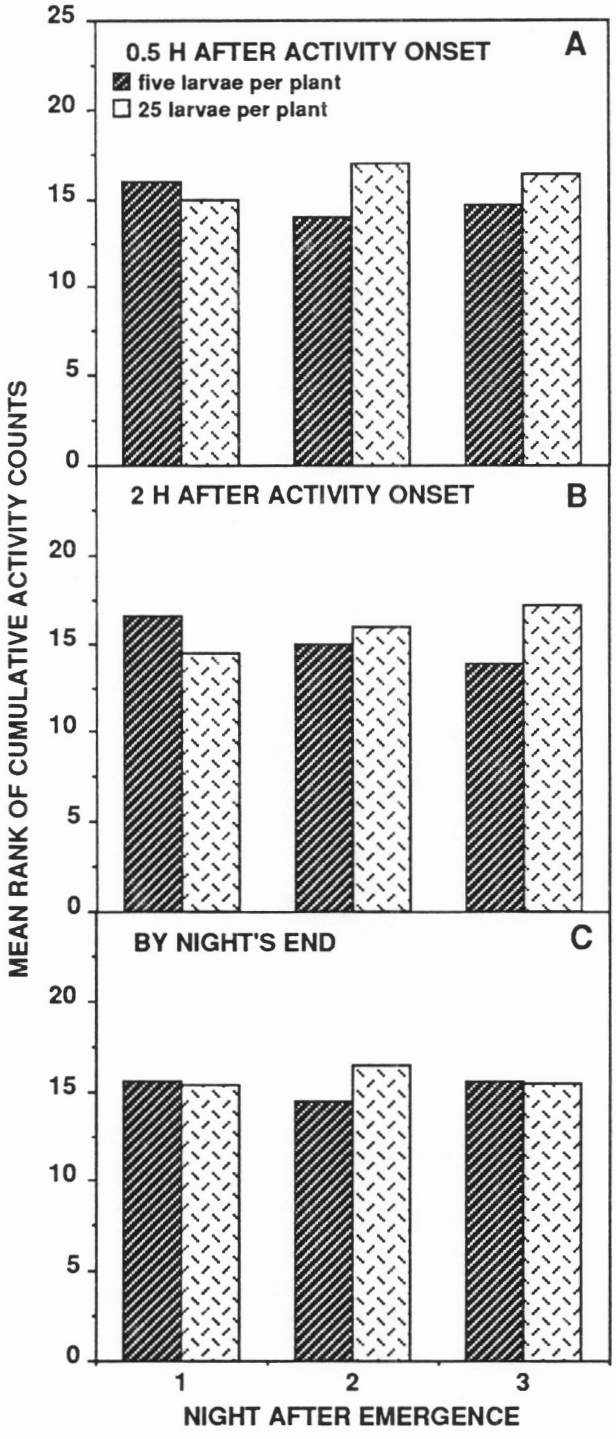

Figure 15: **Mean ranks (rank sums / sample sizes) of cumulative activity for female fall armyworm moths from Field Trial 3. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample size is 15 for each treatment.**

(Figure 16) ($P > 0.05$). The adult male activity may have been affected by residual pheromone emitted by the previous female actograph occupants.

In Field Trial 2 and 3, field density did not significantly affect the proportions of frequent "flyers" or infrequent "flyers" at either 2 h after activity onset or by the end of the second night after emergence (Tables 7 and 8) ($P > 0.05$). Because moths from field-reared larvae had nearly twice as much activity overall than moths from laboratory-reared larvae, more moths from both density treatments had accumulated ≥ 201 activity counts by the end of the second night in the actograph. Because field-reared male moths may have been affected by residual pheromone from females tested earlier in the actograph, the effect of field density on the proportions of frequent and infrequent flyers in males was not examined.

Actograph data for moths from field-reared larvae demonstrated that activity is enhanced by living conditions in the corn field. Even though the eggs came from the same laboratory colony, the amount of actograph activity in moths from field-reared larvae probably more closely approximates the amount of actual field activity than the activity of moths from laboratory-reared larvae.

Generally, differences in larval rearing density in the laboratory and in the field did not significantly affect subsequent adult behavior as measured in our actograph. Individual behavioral variability (ranging from 0 to thousands of activity counts) and a greater proportion of the laboratory-reared moths registering ≤ 200 activity counts each night made significant differences unobtainable. Transformations of activity data sets did not help to normalize their distributions. The Jonckheere test used on the fifth laboratory replicate, a more powerful nonparametric test than Kruskal-Wallis, detected no significant treatment effects even though activity was higher in moths from higher density treatments on 16 occasions. Also, analyses of covariance (SAS Institute 1985) were performed on linearized curves representing activity counts accumulated as proportions of the six-night total from the first night to the sixth night. These analyses did not affect our results significantly either. Variability in behavioral data regardless of density treatment has been shown for other moths, including *S. exempta* (Parker & Gatehouse 1985) and *A. ipsilon*, the black cutworm (Lewis & Keaster 1989). Parker and Gatehouse (1985a) believe that the variability in *S. exempta* flight behavior can be attributed to genetic variation or

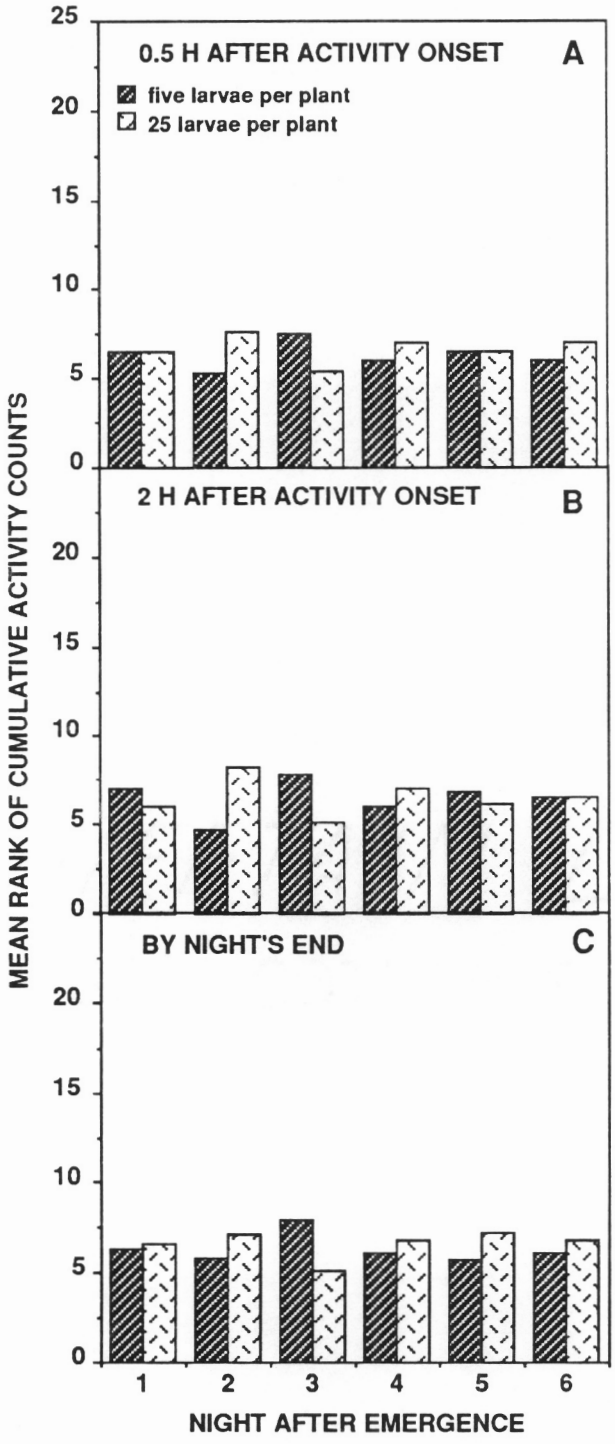

Figure 16: Mean ranks (rank sums / sample sizes) of cumulative activity for male fall armyworm moths from Field Trial 3. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample size is 6 for each treatment.

Table 7. Proportions of infrequent and frequent "flyers"[a] in female fall armyworm adults from larvae field-reared at different densities in corn plots at Tifton, Ga., 1989, using data collected by two hours after activity onset on the second night after emergence. Analyzing field trials separately, no significant differences were found, χ^2 analysis, $P > 0.05$. Sample sizes are in parentheses.

Field Trial	$\bar{x}$ No. Larvae Per Corn Plant	Proportion of	
		Infrequent Flyers	Frequent Flyers
2	4	1.00(10)	.00(10)
	25	.85(20)	.15(20)
3	5	.93(15)	.07(15)
	25	.93(15)	.07(15)

[a]Infrequent refers to ≤ 200 accumulated activity counts, and frequent refers to ≥ 201 accumulated activity counts. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Moths sometimes *walked* across the beam window and registered activity counts.

Table 8. Proportions of infrequent and frequent "flyers"[a] in female fall armyworm adults from larvae field-reared at different densities in corn plots at Tifton, Ga., 1989, using data collected by the end of the second night after emergence. Analyzing field trials separately, no significant differences were found, χ^2 analysis, $P > 0.05$. Sample sizes are in parentheses.

Field Trial	$\bar{x}$ No. Larvae Per Corn Plant	Proportion of	
		Infrequent Flyers	Frequent Flyers
2	4	.40(10)	.60(10)
	25	.35(20)	.65(20)
3	5	.33(15)	.67(15)
	25	.20(15)	.80(15)

[a]Infrequent refers to ≤ 200 accumulated activity counts, and frequent refers to ≥ 201 accumulated activity counts. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Moths sometimes *walked* across the beam window and registered activity counts.

other unidentified environmental factors. Lewis & Keaster (1989) suggested that in the black cutworm, difference in flight capacity among the density treatments may manifest itself in other parameters such as fecundity.

To summarize, changes in laboratory rearing density did produce significant differences in pupal weight and wing size, but smaller individuals produced by an increase in rearing density are not necessarily premigrants. Similar results were obtained in nonmigratory species (see review, Peters & Barbosa 1977). The differences in developmental time due to laboratory rearing density were not large enough to be biologically important. Because of dispersal and low survival in the field, the desired low and high levels of crowding were not achieved. In addition, supplying field-collected larvae with artificial diet until they pupated affected size and developmental time. There was a general lack of a density-dependent phase variation in the activity of female adults from larvae reared in the laboratory and in the field. The results of these studies put the existence of a density-dependent premigrant phase in FAW in question. Perhaps a density level of more than three larvae per cup may be needed to get separation of premigrant and non-premigrant individuals. Another possibility is that there may be significant genetic influences on adult flight potential which has been shown for *S. exempta* (Parker & Gatehouse 1985b, Woodrow et al. 1987). Finally, components of weather (wind circulations, temperature, photoperiod, etc.) may be more important than larval density in initiating FAW migrant behaviors (Pair et al. 1986, Westbrook & Sparks 1986, Johnson 1987).

Chapter 5

Larval Rearing Density Effects on Mating and on Activity of Adult Males and Mated Females in the Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Introduction

Among migratory lepidopterous species in the southeastern U.S., the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (FAW), ranks second only to the corn earworm, *Helicoverpa zea* (Boddie), in causing severe crop damage (Sparks 1986). There is a need for increased understanding of the factors resulting in FAW migration before an effective integrated pest management program can be devised. Of particular importance in the study of FAW dispersal is the "identification of physiological and behavioral mechanisms conducive to initiation . . . of flight" (Stinner et al. 1983). One of the cues for migratory flight from the overwintering locales may be high population density, probably acting in conjunction with other factors, including a decrease in food quality and quantity (Johnson 1969). An increase in population density may produce premigrants, those moths which are morphologically and physiologically capable of migratory flight or with a high propensity for migratory flight but have not yet flown. *Spodoptera exempta* (Walker), the African armyworm, has two density-dependent phases, migrant (high density) and nonmigrant (low density) (Faure 1943). *S. exempta* phases differ in size, developmental time, and flight behavior (Parker & Gatehouse 1985a and b, Simmonds & Blaney 1986). Earlier studies demonstrated that a decrease in FAW pupal weight was produced with an increase in larval rearing density. However, rearing density did not appreciably affect wing width, larval and pupal developmental time, nor did density affect adult activity of unmated female FAW. The effects of rearing density on adult activity of FAW males and mated females needs to be examined.

Migration, as defined by Kennedy (1961), involves an enhancement of locomotory activities with a "persistent, straightened-out movement" along with suppression of "vegetative functions" (e.g., feeding, reproduction). Presumably, prereproductive males and females migrate from unfavorable habitats, postponing mating and oviposition until after colonizing new environments. It is not known whether the FAW migrates before or after mating or does both. Interestingly, 40 to 50% of the supposed migrant FAW females captured in light traps on oil platforms in the Gulf of Mexico during October 1973 were mated (females comprised 42 to 45% of the total numbers captured) (Sparks et al.

1986). Other evidence suggests that FAW migrate during the pre-oviposition period after mating. Rose et al. (1975) investigated the remarkable transport of FAW moths from the U.S. Gulf Coast to Sault Ste. Marie, Canada, a distance of 1600 km, accomplished in 30 hours. Discovery of eggs laid on laundry left hanging overnight in Sault Ste. Marie led them to believe that mated FAW migrated until they were reproductively mature, assisted by low-level jet and convergent surface winds. These two pieces of circumstantial evidence from the field are inconsistent with the traditional Kennedy (1961) definition of migration because in both cases, "vegetative functions" were not suppressed.

The following experiments were undertaken to determine if an increase in rearing density affects adult male activity, and if density affects the incidence of mating and mated female activity in an actograph. If males migrate with females, I would expect that they would have evening activity similar to the females which would be greater in the higher density treatments if premigrants are produced. If females postponed mating until after they migrated, assuming that crowding produces premigrants, then an increase in rearing density would lower the incidence of mating. Also, unmated females exposed to males should exhibit more activity than mated females. This study seeks to provide evidence in the laboratory that both sexes of FAW migrate together and that FAW moths migrate before they mate which would be indicated by reduced levels of activity in mated females.

Materials and Methods

Rearing Methods. Fall armyworm eggs were obtained from a colony in the USDA, ARS, Insect Biology and Population Management Research Laboratory, Tifton, Ga. This colony was originally collected from volunteer corn and mature sorghum in the fall of 1986. Field-collected larvae have been added to the laboratory colony regularly to reduce laboratory adaptation.

First-instar larvae were placed in 30 ml plastic cups with approximately 15 ml diet (an excess) at an initial density of one, two, or three larvae per cup. One hundred cups were set up for each density treatment for a total of 300 cups for each replicate. There were four replicates accomplished for FAW adult male

activity studies, and three replicates for mated female activity studies. Larvae were reared on a modified artificial diet from Guy et al. (1985) (brewer's yeast was used instead of torula yeast). Cups were kept at 26°C in an environmental chamber, with a 14:10 (L:D) photoperiod (simulating summer conditions). When pupation occurred, pupae were placed individually in clean 30 ml plastic cups. Adults were allowed to emerge in the plastic cups.

Mating Procedure. Newly emerged males and females of the same age were paired from each larval density treatment, 10 pairs per treatment. Each pair was placed in a screen-top paper carton (8.75 cm in dia by 8.5 cm high) and provided with 10% sucrose solution *ad libitum*. Pairs were kept together for two nights; subsequently females were removed to measure their nightly activity. To confirm that mating had occurred, the females were dissected after activity analysis to look for spermatophores.

Measurement of Activity. On the day of emergence, virgin male moths were placed in the V. P. I. & S. U. actograph, with 10 moths per density treatment. Mated females, initially 10 moths per treatment, were placed in the actograph on the day before the third night after emergence. There were usually less than 10 male or female moths from each treatment which emerged on the same day. Therefore, the 30 moths in a particular replicate were initially placed in an actograph over two or three days. Activity data were grouped later so that equally-aged moths could be compared.

The actograph is a 32-channel computerized system which monitors moth activity (Eaton 1985). It was housed in the same environmental chamber in which the rearing occurred. Inside the actograph, there was a sunset/sunrise light control to simulate dusk and dawn conditions (Byers & Unkrich 1983). An actograph cage consisted of a screen-top paper carton (13 cm in dia by 16.5 cm high) with an opening (2.5 cm wide by 1.5 cm high) on the side 1.5 cm from the top to allow a 940-nm infrared beam to pass through. Activity was detected and logged as an activity count when a moth broke the beam. The actograph was interfaced with a Digital Equipment Minc II Microcomputer which collected and stored data on a hard disk. Data were transferred to the IBM 3084/3090 main frame for analysis using SAS. Validation studies using this actograph have

shown that the number of activity counts is directly related to the amount of actual time a moth was active.

Moths were left in the actograph for up to 10 d and fed 10% sucrose solution *ad libitum*. Activity counts were continuously recorded for 15 hours each night starting 1 h before sunset. In these experiments, a simulated 1-h dusk began at 1600 h which was followed by 9 h of darkness with lights coming on at 0200 h. Our preliminary data have shown that there is an evening peak of activity in females beginning after sunset. Also, radar and airplane observations revealed FAW moths taking off for migration shortly after sunset (C. E. Rogers pers. comm.). Therefore, it was important to record before sunset to ensure that the initial activity data were collected.

Data Analyses. Activity data consisted of four replicates of 6-day recordings from males and three replicates of 4-day recordings from mated and unmated females exposed to males. For each replicate, data were analyzed, each night separately, by comparing the number of activity counts accumulated at 0.5 h after activity onset, 2 h after onset, and by the end of the night among density treatments using the nonparametric Kruskal-Wallis one-way analysis of variance (NPAR1WAY procedure, SAS Institute 1985) ($\alpha = 0.05$). Nonparametric statistical analyses were used on the activity data because these data did not come from normal-like distributions, and nonparametric tests do not assume normality. If a significant density effect was found, treatments were separated using the Wilcoxon Rank Sum Test on two treatments at a time (Hollander & Wolfe 1973) ($\alpha = 0.05$). In addition, data from the first male replicate were subjected to the nonparametric Jonckheere test for ordered alternatives (Hollander & Wolfe 1973) ($\alpha = 0.05$). The ordered alternative hypothesis was that activity counts of moths from the density treatment of one larva per cup $\leq$ the activity counts of moths from the treatment of two larvae per cup $\leq$ the activity counts of moths from the treatment of three larvae per cup. The Jonckheere test is more powerful than Kruskal-Wallis because it increases the probability that a difference will be detected in the direction of the ordered alternative. A more powerful test decreases the probability of a Type II error, which occurs when a false null hypothesis is not rejected (Zar 1984). Sample sizes for males were 9, 10, and 10, for the first replicate, and 10, 10, and 10, for

the second through fourth replicates for density treatments of one, two, and three larvae per cup, respectively. A lightning storm during the third male replicate caused the loss of some data, so total accumulated activity counts were available for only 3, 5, and 6 moths on the first night and 7, 5, and 4 moths on the second night per respective density treatment. Sample sizes for mated females were reduced because some of the females that were exposed to males did not mate. They were: first replicate, 10, 8, and 8; second replicate, 9, 7, and 8; and third replicate, 8, 9, and 10 for density treatments of one, two and three larvae per cup, respectively.

Parker & Gatehouse (1985a and b) analyzed flight activity of tethered *S. exempta* adults by dividing the moths into proportions of long-flyers and short-flyers based on duration of individual flights. To examine the male FAW activity count data in like manner, the proportions of infrequent ($\leq$ 200 activity counts) and frequent ($\geq$ 201 activity counts) "flyers"[1] from different density treatments on the second night in the actograph were analyzed with χ^2 ($\alpha = 0.05$). Activity counts accumulated 2 h after activity onset and accumulated by the end of the night were examined for this analysis. Activity counts for females exposed to males were too low (the greatest number of activity counts was below 200) for the infrequent/frequent "flyer" analysis employed with the unmated males.

The effect of rearing density on the incidence of mating was examined using χ^2 ($\alpha = 0.05$). Replicates were analyzed separately.

Results and Discussion

Male and Mated Female Activity Patterns. For the first two nights after emergence, male FAW moths showed a broad peak of activity beginning in the evening and peaking 5 to 7 h later (Figure 17). Similarly, Mitchell et al. (1974) noted maximum pheromone trap captures of males 4 to 7 h after sunset. A large peak of activity occurred after the lights came back on (at 0200 h) at ca. 0315 h. During the next four nights, the broad evening peak shifted forward in time, such that activity did not drop until near the end of the scotophase. The post-dawn peak was present but smaller on the third through the sixth nights.

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[1]Moths sometimes *walked* around the actograph cage and registered activity counts.

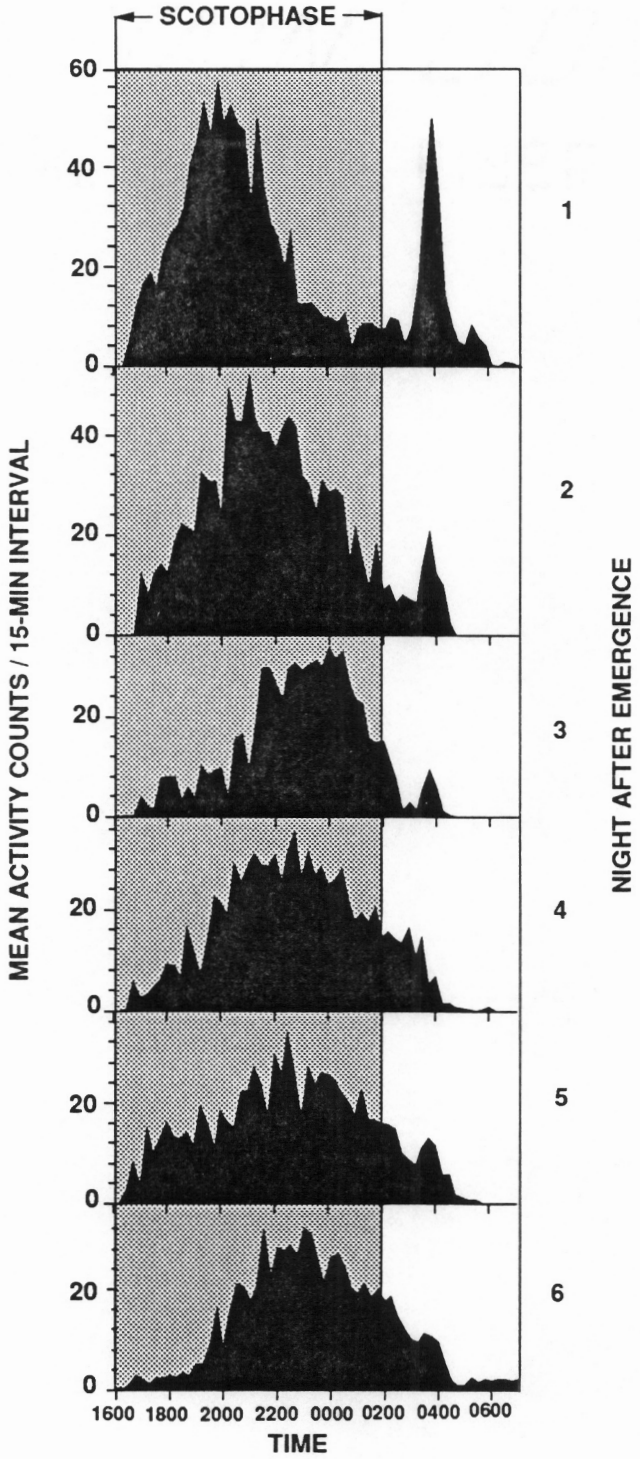

Figure 17: Activity patterns for male fall armyworm moths from laboratory-reared larvae recorded in the actograph over a six-day period (n = 30). One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage.

Leppla et al. (1979) reported similar FAW male behavior patterns, but their use of multiple moths per actograph cage undoubtedly produced a "domino effect" when one moth flying caused the rest of the moths to fly at once. In the present study, independent observations could be made of individual moths. Male activity in the evening during the first night could represent migratory flight or foraging because mating usually does not occur until at least the second night after emergence (Sparks 1979). Previous studies have shown that unmated females have a sharp evening peak which could be associated with migration too. Later scotophase activity in the males might represent foraging and mating flights (Mitchell et al. 1974). This later activity remained at a generally high level through the 6-day period with some waning with time. Unmated males probably remain active until they have mated at least once. During the peak male activity period, unmated females in previous studies showed little actograph activity because they were probably engaging in calling behavior. The post-dawn peak in males, present in unmated females too (previous study), could be associated with foraging or searching for a hiding place (Dreisig 1986).

Compared to unmated females in earlier studies , reduced levels of activity were recorded for mated females, particularly in the evening when migratory activity is presumed to occur in the field (Figure 18). Less than ten mean activity counts were recorded per 15-min period for mated females. Similar results were obtained by Leppla et al. (1979), but multiple pairs per actograph cage were used in their study. Most mated females laid viable eggs on the sides and tops of the actograph cages. Thus, activity detected by the actograph was mostly oviposition-related.

Male Activity. Activity counts were graphed as mean rank (rank sum/sample size) to alleviate differences in magnitude among the FAW groups tested, e.g., the highest mean number of activity counts for males was 1594.70, for mated females, 161.38. At the three times examined over 6 days (0.5 h after activity onset, 2 h after onset, and total activity for the night) (Figures 19 - 22), only one significant rearing density effect was found in males using the Kruskal-Wallis analysis of variance (Figure 21, $P = 0.0058$, third male replicate, fifth night, by the end of the night). In this instance, activity was significantly greater in the male moths from larvae reared one per cup than in moths from larvae reared

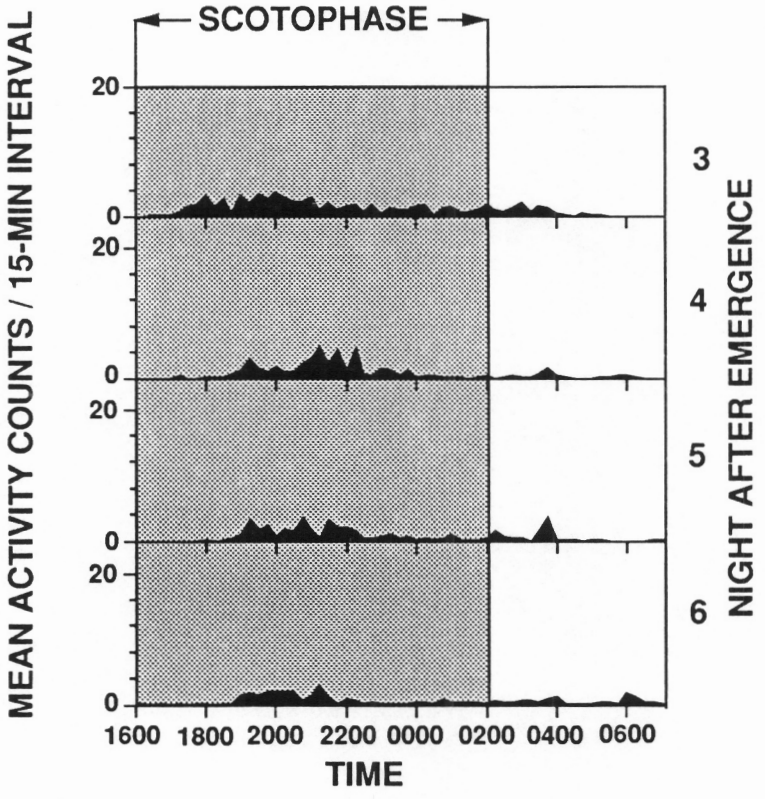

Figure 18: Activity patterns for mated female fall armyworm moths from laboratory-reared larvae recorded in the actograph over a four-day period (n = 26). Females were exposed to male fall armyworm moths during the first two nights after emergence. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage.

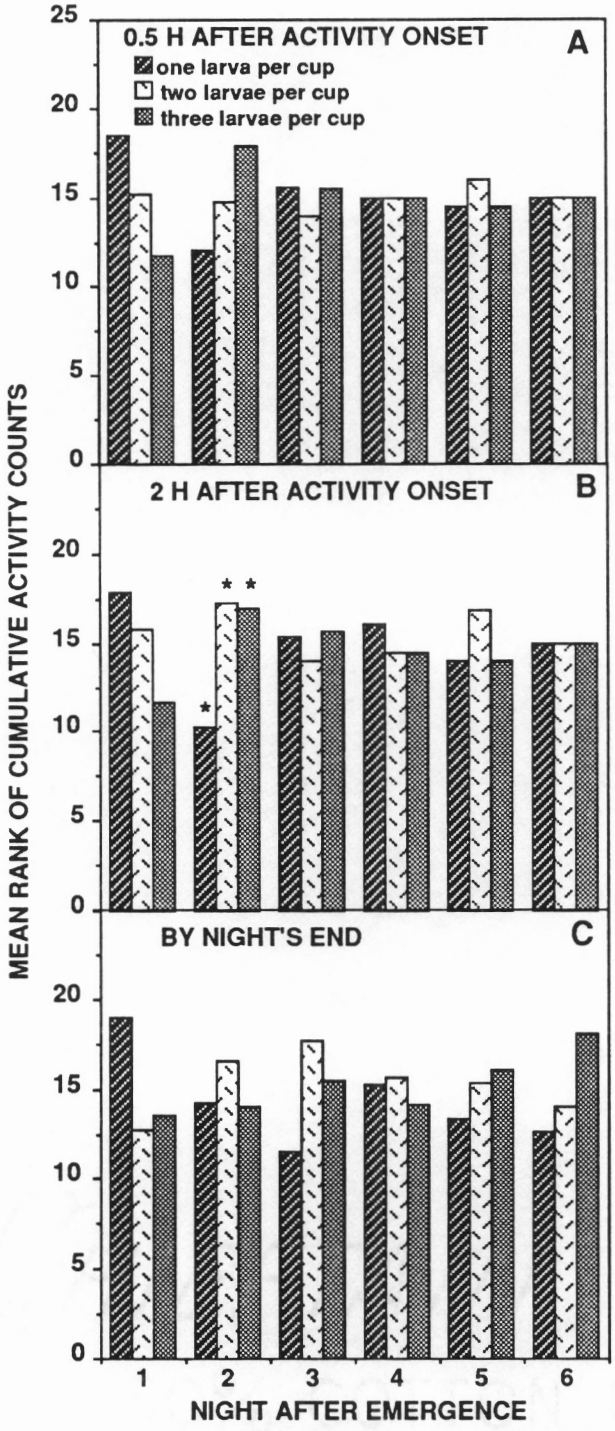

Figure 19: Mean ranks (rank sums / sample sizes) of cumulative activity for male fall armyworm moths from the first laboratory replicate. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample sizes are 9 (one larva), 10 (two larvae), and 10 (three larvae per cup). Asterisks denote significant differences between treatments ($P < 0.05$).

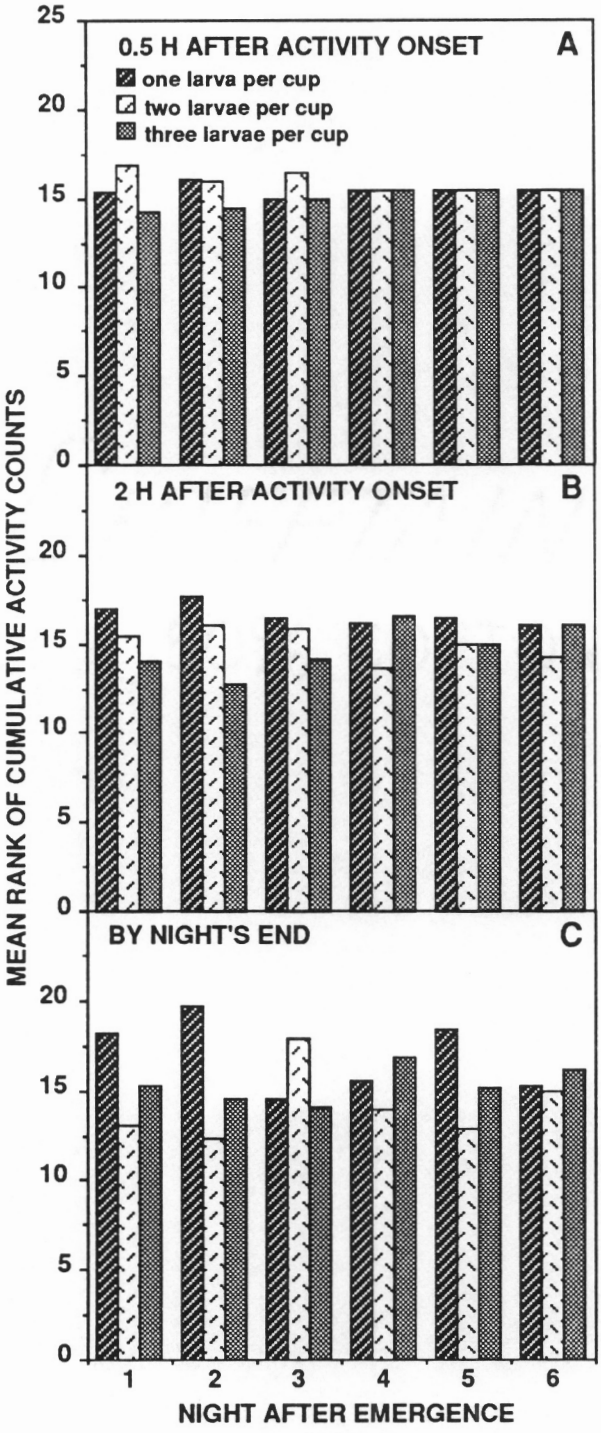

Figure 20: Mean ranks (rank sums / sample sizes) of cumulative activity for male fall armyworm moths from the second laboratory replicate. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample size is 10 for each treatment.

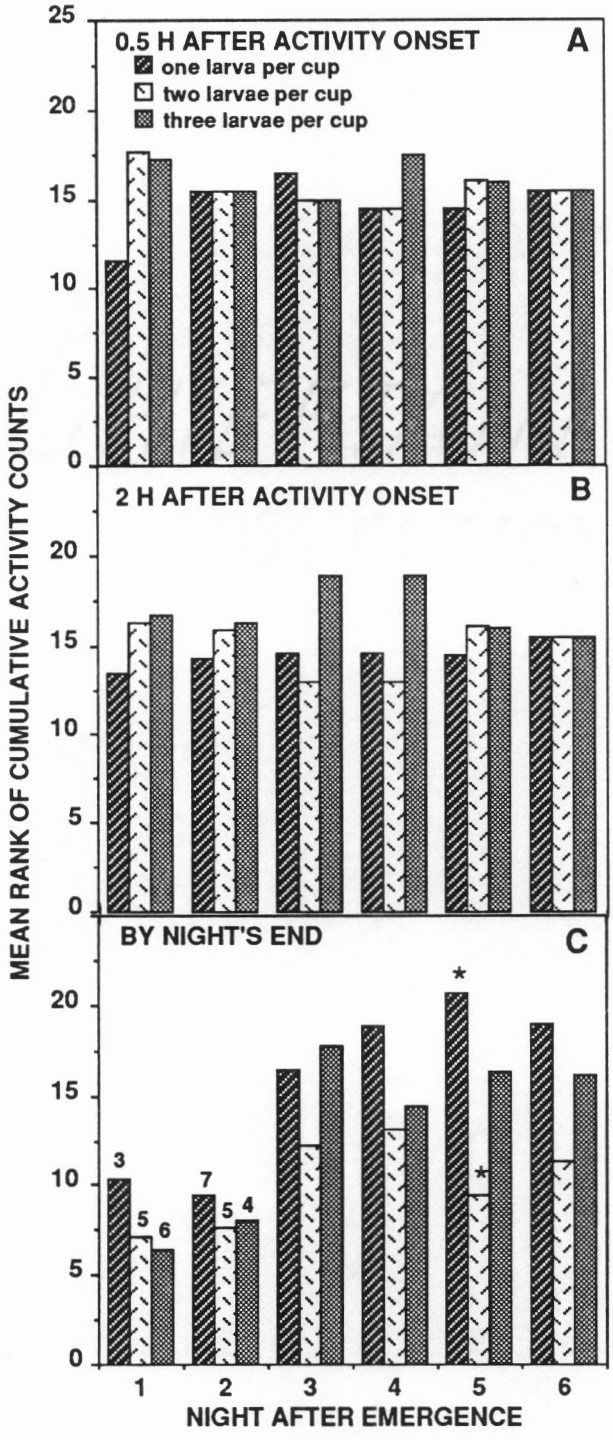

Figure 21: Mean ranks (rank sums / sample sizes) of cumulative activity for male fall armyworm moths from the third laboratory replicate. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample size is 10 for each treatment unless noted above the bar. Asterisks denote significant differences between treatments ($P < 0.05$).

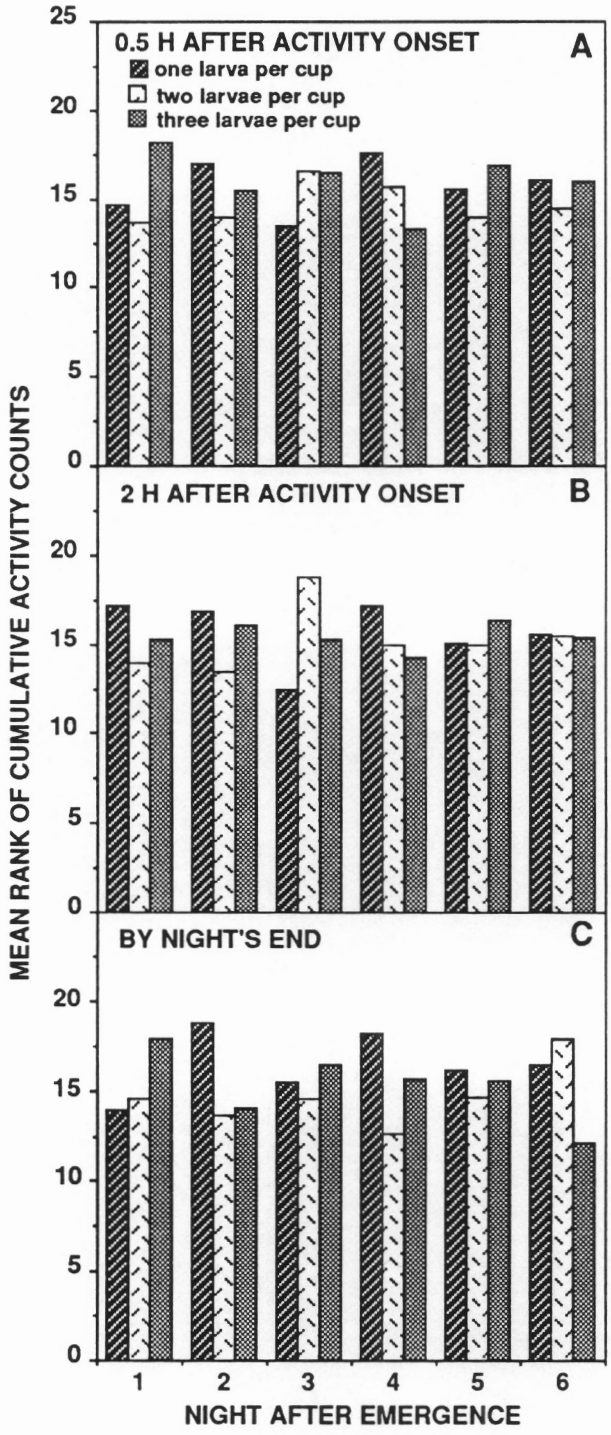

Figure 22: **Mean ranks (rank sums / sample sizes) of cumulative activity for male fall armyworm moths from the fourth laboratory replicate. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample size is 10 for each treatment.**

two per cup. This significant effect may be inconsequential to migratory potential, considering that moths were five days old when it occurred. Using the Jonckheere test on the first male replicate revealed that activity significantly increased with increased rearing density only on one occasion: two hours after activity onset on the second night ($P < 0.05$, Figure 19B).

The proportions of infrequent versus frequent male "flyers" were compared among density treatments on the second night after emergence (Tables 9 and 10). No significant differences were found between the proportions among density treatments ($P > 0.05$). Because males registered more activity counts overall, most male moths were frequent "flyers" (80%) by the end of the night, while most of the unmated females were infrequent "flyers" (68.9%) by the end of the night (previous study).

Most of the male activity occurred following 2 h after onset (about 1800 h) (Figure 17). Hence, the majority of the male activity, regardless of rearing density, probably was related to mating flight potential, rather than their migratory potential. Results similar to the present study were obtained with male *S. exempta* (Woodrow et al. 1987). No significant effects due to density phase were found on tethered flight performance in male *S. exempta*, and males had markedly greater activity levels than female *S. exempta*. Woodrow et al. (1987) suggested that too much "noise" (read: variability) in flight activity interfered with assessing the migratory potential of male *S. exempta*. These researchers theorized that male flight activity was mostly associated with "non-migratory activity". Individual variability (ranging from 0 to thousands of activity counts) made it difficult to show significant density effects. The Jonckheere test used on the fifth laboratory replicate, a more powerful nonparametric test than Kruskal-Wallis, detected only one significant treatment effect even though activity was higher in male moths from higher density treatments on ten occasions. It appears that both tethered (Woodrow et al. 1987) and caged flight methods (i.e., the V. P. I. & S. U. actograph) are not very useful for predicting the migratory capability of males. Field studies utilizing mark-recapture and pheromone traps might be better choices for determining the migrating abilities of male FAW and *S. exempta*.

Table 9. Proportions of infrequent and frequent "flyers"[a] in male fall armyworm adults from laboratory-reared larvae in different density treatments, using data collected by two hours after activity onset on the second night after emergence. Analyzing replicates separately, no significant differences were found, χ^2 analysis, $P > 0.05$. Sample sizes are in parentheses.

Replicate	No. Larvae Per 30 ml Cup	Proportion of Infrequent Flyers	Proportion of Frequent Flyers
1	1	.89(9)	.11(9)
	2	.80(10)	.20(10)
	3	.80(10)	.20(10)
2	1	.80(10)	.20(10)
	2	.80(10)	.20(10)
	3	1.00(10)	.00(10)
3	1	1.00(10)	.00(10)
	2	.90(10)	.10(10)
	3	.80(10)	.20(10)
4	1	.70(10)	.30(10)
	2	.90(10)	.10(10)
	3	.70(10)	.30(10)

[a]Infrequent refers to ≤ 200 accumulated activity counts, and frequent refers to ≥ 201 accumulated activity counts. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Moths sometimes *walked* across the beam window and registered activity counts.

Table 10. Proportions of infrequent and frequent "flyers"[a] in male fall armyworm adults from laboratory-reared larvae in different density treatments, using data collected by the end of the second night after emergence. Analyzing replicates separately, no significant differences were found, χ^2 analysis, $P > 0.05$. Sample sizes are in parentheses.

Replicate	No. Larvae Per 30 ml Cup	Proportion of Infrequent Flyers	Proportion of Frequent Flyers
1	1	.11(9)	.99(9)
	2	.10(10)	.90(10)
	3	.30(10)	.70(10)
2	1	.10(10)	.90(10)
	2	.40(10)	.60(10)
	3	.30(10)	.70(10)
3	1	.14(7)	.86(7)
	2	.20(5)	.80(5)
	3	.25(4)	.75(4)
4	1	.00(10)	1.00(10)
	2	.30(10)	.70(10)
	3	.20(10)	.80(10)

[a]Infrequent refers to ≤ 200 accumulated activity counts, and frequent refers to ≥ 201 accumulated activity counts. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Moths sometimes *walked* across the beam window and registered activity counts.

Incidence of Mating and Unmated Female Activity. Larval rearing density had no significant effect on the incidence of mating in any of the three replicates ($P > 0.05$). Thirteen out of 90 female moths exposed to male moths did not mate, three out of 30, six out of 30, and four out of 30 for female moths from larvae reared at one, two, and three per cup, respectively. However, the unmated females exposed to males (< 10 mean activity counts per 15-min interval, Figure 23) had comparable activity levels to mated females (< 10 mean activity counts per 15-min interval, Figure 18), levels which were markedly reduced from the activity levels of females which had not been exposed to males (up to 25 activity counts per 15-min interval, previous study). Clearly, the unmated female moths in the present study were not behaving as if they were postponing the "vegetative function" of mating in order to engage in migratory flight. Pooling the data from three replicates, the unmated females exposed to males from the lower density treatments generally had more activity accumulated by the end of the night than those from the higher density treatments (Figure 24C), but data were not analyzed statistically because of the low number of unmated moths.

Unfortunately, the design of the experiment prevented the collection of activity data during the first two nights after emergence. It is possible that the unmated moths may have spent more time flying and less time calling than the moths which readily mated. It is also conceivable that the unmated females had called and attempted to couple with the males but were unsuccessful.

Mated Female Activity. At the three times examined over 4 days (0.5 h after activity onset, 2 h after onset, and at the end of the night), significant differences were found among rearing density treatments for mated females on three occasions (Figures 25 - 27). In the second replicate, at both two hours after activity onset and by the end of the third night, mated females from the lowest density treatment showed significantly greater activity than mated females from the highest density treatment ($P = 0.0305$ and $P = 0.0093$, respectively; Figure 26B and C). In the third replicate, by the end of the third night, mated females from the density treatment of three larvae per cup showed significantly more activity than the mated females from the treatment of two larvae per cup

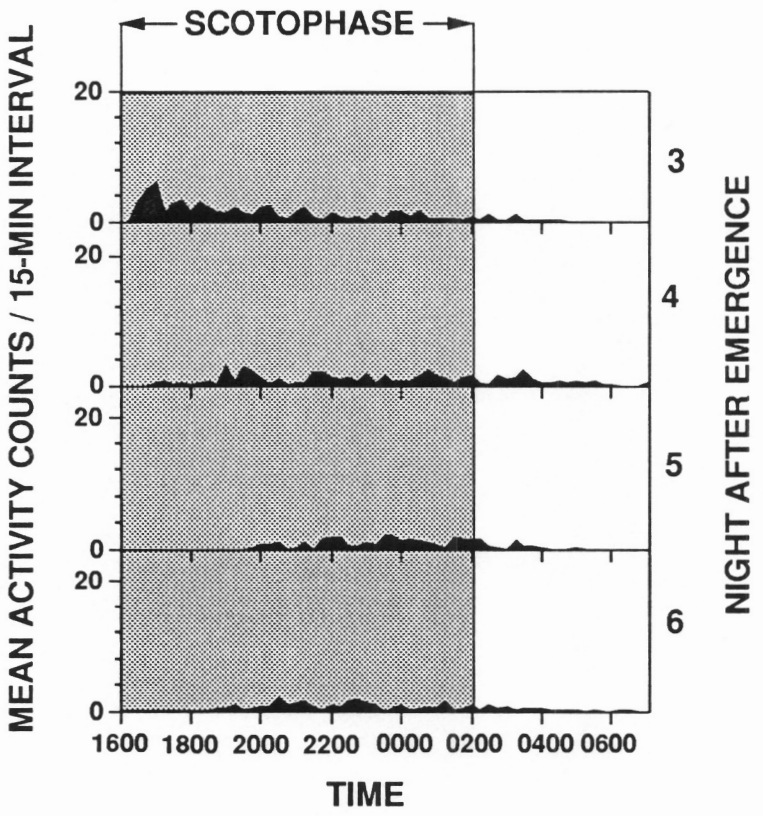

Figure 23: Activity patterns for unmated female fall armyworm moths from laboratory-reared larvae recorded in the actograph over a four-day period (n = 13). Females were exposed to male fall armyworm moths during the first two nights after emergence. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage.

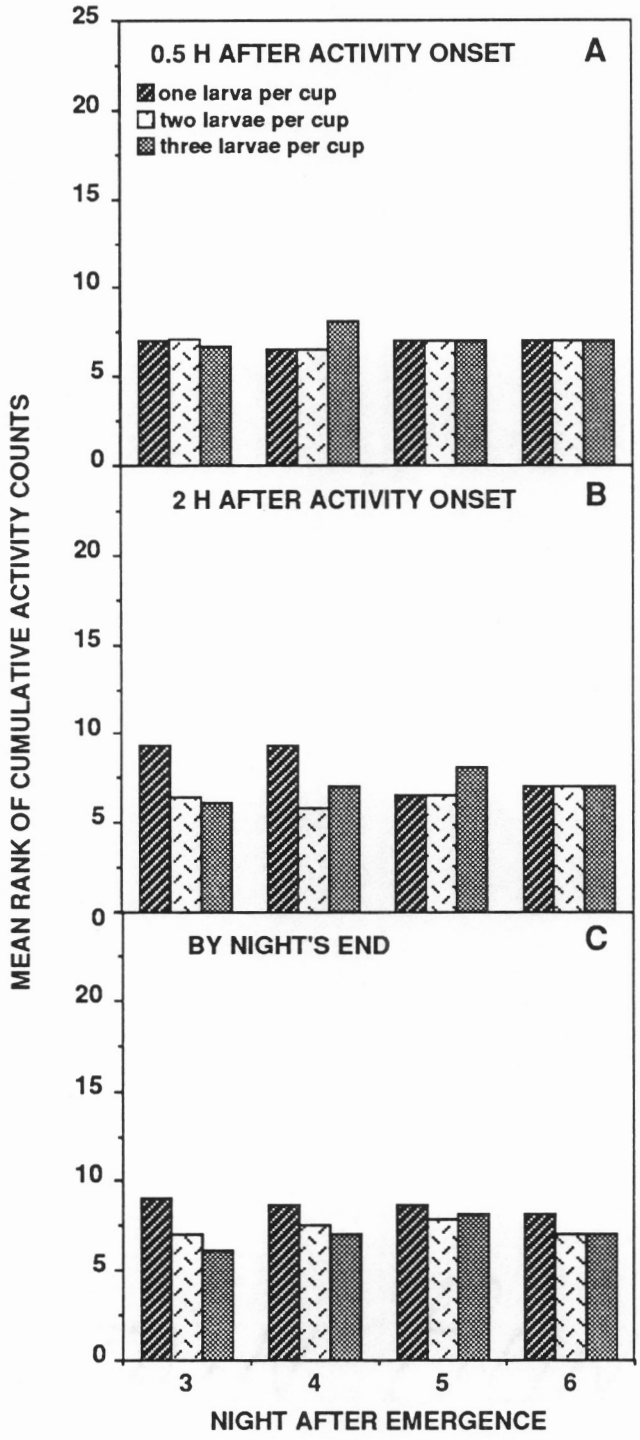

Figure 24: Mean ranks (rank sums / sample sizes) of cumulative activity for unmated female fall armyworm moths exposed to males the first two nights after emergence. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample sizes are 3 (one larva), 6 (two larvae), and 4 (three larvae per cup).

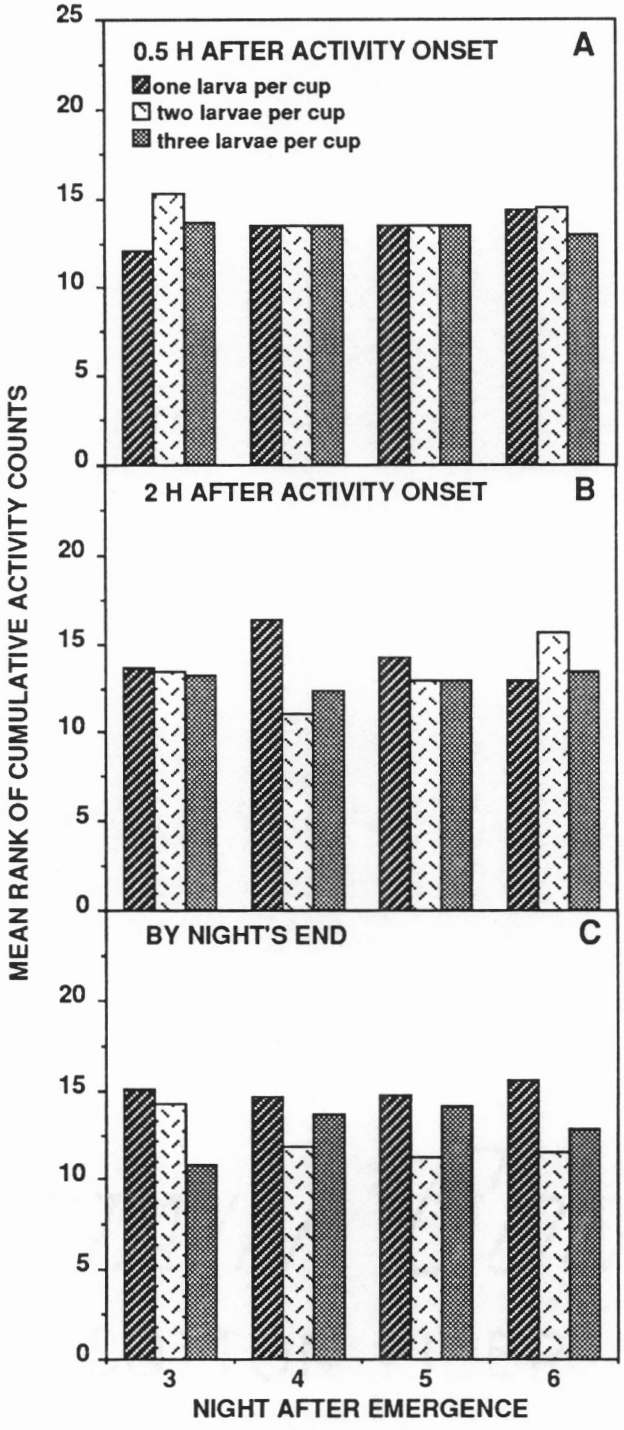

Figure 25: Mean ranks (rank sums / sample sizes) of cumulative activity for mated female fall armyworm moths from the first replicate. Females were exposed to males the first two nights after emergence. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample sizes are 10 (one larva), 8 (two larvae), and 8 (three larvae per cup).

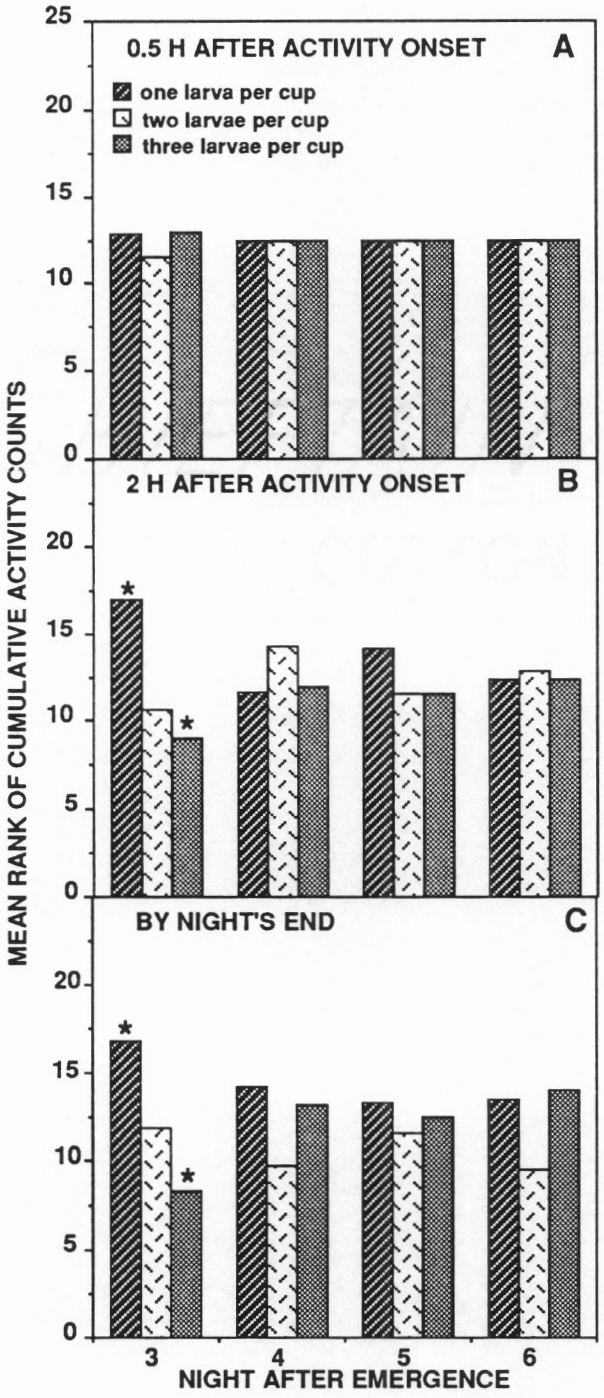

Figure 26: Mean ranks (rank sums / sample sizes) of cumulative activity for mated female fall armyworm moths from the second replicate. Females were exposed to males the first two nights after emergence. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample sizes are 9 (one larva), 7 (two larvae), and 8 (three larvae per cup). Asterisks denote significant differences between treatments ($P < 0.05$).

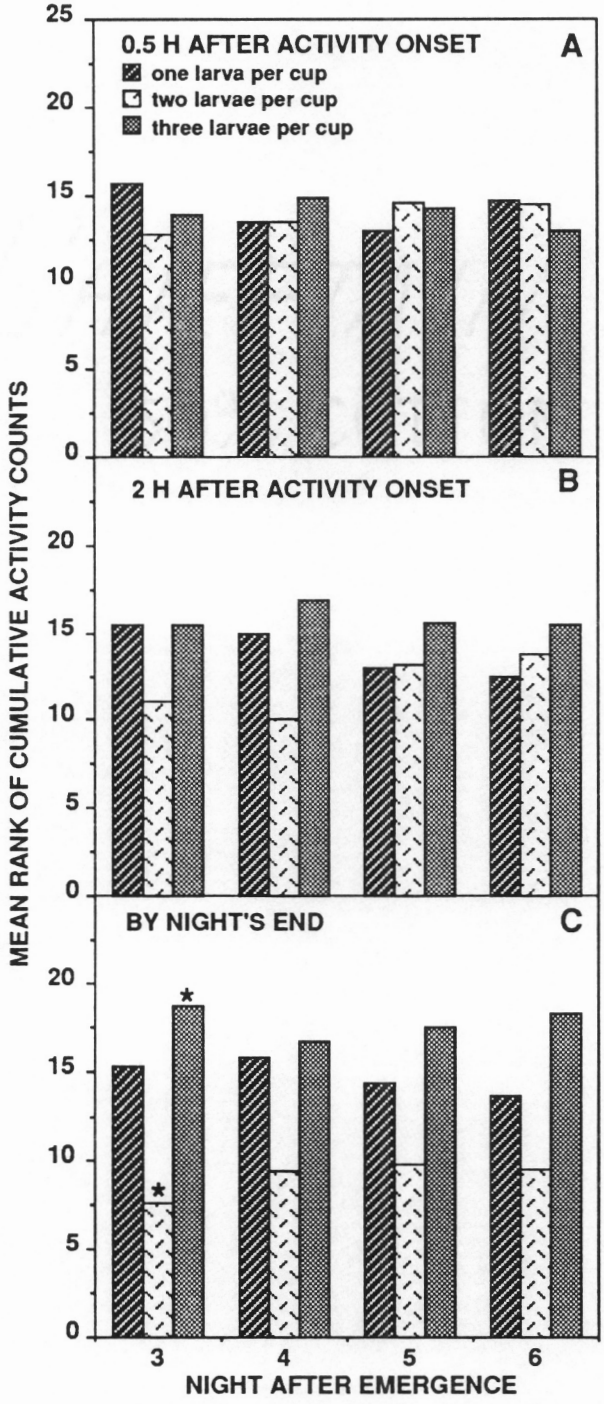

Figure 27: Mean ranks (rank sums / sample sizes) of cumulative activity for mated female fall armyworm moths from the third replicate. Females were exposed to males the first two nights after emergence. One activity count was recorded each time a moth broke an infrared beam directed through a window in an actograph cage. Sample sizes are 8 (one larva), 9 (two larvae), and 10 (three larvae per cup). Asterisks denote significant differences between treatments ($P < 0.05$).

($P = 0.0017$, Figure 27C). Aside from these three instances, larval rearing density had no effect on mated FAW female activity.

An interesting direction to take from this experiment would be to examine the effect of larval rearing density on fecundity. Both *Pieris brassicae* L. (Pieridae) (Zaher & Long 1959) and *Epiphyas postvittana* (Walker) (Tortricidae) (Danthanarayana et al. 1982) had reduced fecundity in moths from larvae reared in crowded conditions. *Plusia gamma* L. (Noctuidae) moths from larvae reared at high densities produced more eggs than moths from larvae reared at lower densities (Zaher & Long 1959). Research is needed in the FAW to determine the relationship between rearing density and fecundity.

The actograph data show that male FAW moths could migrate on the first or second night after emergence during the early evening concurrent with the females. Indeed, these data are supported by the fact that both female and male FAW (presumed migrants) were caught in light traps in the Gulf of Mexico (Sparks et al. 1986). Generally, larval rearing density did not affect actograph activity of unmated males and mated females. An increase in larval rearing density did not affect the incidence of mating in females. The majority of the females mated when given the opportunity. The actograph data neither support nor refute the hypothesis proposed by Kennedy (1961) that FAW and other insects migrate before they mate, mature during the migratory flight, and mate upon arrival at the new habitat. Mated females showed very little actograph activity compared to unmated females not exposed to males in earlier studies, supporting Kennedy's (1961) hypothesis. However, the few unmated female moths which had been exposed to males showed similar suppressed actograph activity, refuting Kennedy's hypothesis. These unmated moths did not behave as if they were migrants which postpone mating to undergo migratory flight. Additionally, published evidence indicates that both mated and unmated moths migrate. Forty to 50% of presumed migrant FAW females caught in light traps in the Gulf of Mexico were mated (Sparks et al. 1986), and mated FAW females traveled wind-borne from the U.S. Gulf Coast to Canada (Rose et al. 1975).

There is evidence to support the existence of a premigrant phase in the FAW on the basis of morphological differences (previous study). However, there were few significant differences in adult activity as a result of a change in

rearing density in unmated males, mated females, and unmated females (this chapter and previous study). Also, the majority of the females mated when given the opportunity, regardless of rearing density. Thus, there does not appear to be density-dependent behavioral migrant and nonmigrant phases in the FAW. Components of weather (wind circulations, temperature, photoperiod, etc.) may be more important than larval density in initiating FAW migrant behavior (Pair et al. 1986, Westbrook & Sparks 1986, Johnson 1987).

Chapter 6

Larval Rearing Density Effects on Lipid Reserves and Wing-Loading in Adult Fall Armyworms, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

Introduction

Among migratory lepidopterous species in the southeastern U.S., the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (FAW), ranks second only to the corn earworm, *Helicoverpa zea* (Boddie), in causing severe crop damage (Sparks 1986). There is a need for increased understanding of the factors resulting in FAW dispersal before an effective integrated pest management program can be devised. Of particular importance in the study of FAW dispersal is the "identification of physiological and behavioral mechanisms conducive to initiation . . . of flight" (Stinner et al. 1983). One of the cues for migratory flight from the overwintering locales may be high population density, probably acting in conjunction with other factors, including a decrease in food quality and quantity (Johnson 1969). An increase in population density may produce premigrants, moths which are morphologically and physiologically capable of migratory flight and have a high propensity for migratory flight but have not yet flown. Putative premigrant traits include small size, high flight potential, low wing-loading (body weight/wing area), and high lipid reserves (see review, Angelo & Slansky 1984). Previous research has lent evidence to support the existence of density-dependent non-premigrant and premigrant phases of the FAW on the basis of certain morphological, but not developmental or behavioral differences. There are no studies on the effect of rearing density on lipid reserves and wing-loading in the FAW.

Lipids are a more economical energy source than carbohydrates for long-range flying because: (1) they yield more energy calories per gram than carbohydrates (Weis-Fogh 1968), (2) they release more metabolic water per mole oxidized than carbohydrates, and (3) they take up less storage with water than carbohydrates (Beenakkers et al. 1985). In addition, lipids are a vital resource for egg production, which becomes important for a colonizing migrant moth when it reaches its destination.

Wing-loading is the ratio of whole body weight to total wing area (derived from the aeronautical term of the gross weight of an airplane or glider divided by its gross wing area (American Heritage Dictionary 1969). A moth with a lower wing-loading means that it would carry less weight per unit wing area over long

distances and times and burn up less fuel than one with a relatively higher wing-loading.

The objective of this study was to determine the effects of rearing density on lipid reserves and wing-loading. If we produce premigrants of the fall armyworm in the laboratory and in the field by increasing the larval rearing density, then I would expect those moths to have higher lipid reserves and lower wing-loadings.

Materials and Methods

Rearing Methods. Fall armyworm eggs were obtained from the Insect Biology and Population Management Research Laboratory, USDA, ARS, Tifton, Ga. This colony was originally collected from volunteer corn and mature sorghum in the fall of 1986. Field-collected larvae are added to the laboratory colony regularly to reduce laboratory adaptation.

For the laboratory studies, first-instar larvae were placed in 30 ml plastic cups with approximately 15 ml diet (an excess) at an initial density of one, two, or three larvae per a cup. One hundred to 150 cups were set up for each density, replicated three times. Larvae were reared on a modified artificial diet from Guy et al. (1985) (brewer's yeast was used instead of torula yeast). Cups were kept in an environmental growth chamber, with a 14:10 (L:D) photoperiod (to simulate summer conditions) at 26°C. Newly formed pupae were placed individually into clean 30 ml cups. Adults were allowed to emerge in the cups.

For the field studies, two 0.1 ha plots of 'Pioneer 3320-F11' corn were planted at Tifton, Ga., and artificially infested with first instar larvae from the Tifton laboratory colony, one plot for low density and one for high density (Table 11). Infestation was accomplished using the mechanical dispenser method of Wiseman et al. (1980). Corn was replanted before each of three infestations. Plants were at the eight-leaf stage (middle whorl) during the first infestation, and at the six-leaf stage (early whorl) during the last two infestations. To recover larvae, plants were cut at their base and taken to the edge of the field for examination. Larvae were removed from the plants and placed individually in 30 ml cups containing diet. Larvae were either fifth or sixth instar at the time of

Table 11. Dates for planting corn field plots, infestation and recovery of fall armyworm larvae and number of larvae infested per plant on each infestation date, Tifton, Georgia.

Trial	Date of Planting	Date of Infestation	Date of Recovery	$\bar{x}$ No. larvae/plant	
				Low	High
1	5/5/89	6/14/89	6/27/89	1	20
2	6/28/89	7/26/89	8/7-9/89	4	25
3	8/28/89	9/20/89	10/2/89	5	25

collection. The following number of larvae were recovered from the first, second, and third field infestation trials, respectively: 90 low density (L), 15 high density (H); 177 L, 289 H; 300 L, 325 H. Field-collected larvae were shipped via overnight mail to V. P. I. & S. U., Blacksburg, Va. Larvae were allowed to complete development to adult under the same environmental conditions as the laboratory-reared FAW. Most larvae pupated within a week after being placed on artificial diet. However, because larvae may undergo ca. 25-35% of their development during the last 7-9 days of the larval stage, the effect of feeding on artificial diet on adult dry body weight and % whole body lipid were examined.

Lipid Studies. Moths were frozen on the day of emergence for lipid analyses. Whole body lipid content was determined using the following protocol:

1. Individual moths without wings were placed in 5 ml (1 dram) vials (Bioquip®), capped with aluminum foil punctured once. Vials were placed in freeze-drier for at least three days.
2. Vials were weighed with moths; moths were removed and placed individually in preweighed 1.5 ml microcentrifuge tubes. Vials were weighed without moths for calculation of dry body weights.
3. One steel ball (3 mm in dia) was placed into each tube. Two tubes were fastened to a Wig-L-Bug® amalgamator (Model # 3110-3A, Crescent Dental Manufacturing Co.). Moths were ground for at least one minute or until moth was crushed to a fine powder.
4. The steel balls were removed from the tubes containing moths and 400 ul 2:1 chloroform:methanol solution were added to each tube. Tubes were vortexed to mix contents thoroughly and then were centrifuged for three minutes.
5. Supernatants containing the lipids were pulled off and placed in a second set of corresponding preweighed 1.5 ml microcentrifuge tubes.
6. Another 400 ul chloroform:methanol were added to each of the tubes in the first set. Tubes were vortexed and then centrifuged for three minutes. Supernatants were pulled off and added to initial corresponding supernatants.

7. Open tubes were placed in a sand bath at 40°C, and the solvent was allowed to evaporate overnight under the hood.
8. Both sets of tubes were weighed. The proportion of whole body lipid content was determined by dividing the weight of the solvent-free supernatant by the initial dry body weight.

Verification analyses using spikeovers with Mazola® corn oil revealed a mean recovery of 98.8% using this method.

Fall armyworm males of unknown age were caught in pheromone traps in the Blacksburg, Virginia area during August and September of 1988 (n = 63). These moths were assayed to compare their lipid content to the lipid content of newly emerged males. Also, 29 females collected from the first field infestation trial (24 from the low density plot and 5 from the high density plot), which had spent a 6-day period in an actograph (Eaton 1985) were tested for lipid content to determine if there was a relationship between activity and amount of lipid reserves. The actograph was a 32-cage computerized system which monitors moth activity. Each moth was isolated in a screen-top cage and supplied with 10% sucrose solution *ad libitum*. Each cage had an opening on the side near the top to allow an infrared beam to pass across the top of the cage. One activity count was recorded each time the beam was interrupted. Validation studies using this actograph have shown that the number of activity counts is directly related to the amount of actual time spent in activity.

Analyses to determine the kinds of neutral lipids (those without a reactive sugar or phosphate group attached) found in the FAW were accomplished using the high-performance thin-layer chromatography (HPTLC) methods developed by Judge (1988) with one modification. The second developing solution was 70:30 hexane:ether to enhance the separation of cholesterol ester and triglyceride (D. N. Judge pers. comm.).

Wing-loading Studies. The right forewing and hindwing of each moth were taped to white paper. The length and width of these wings were measured to the nearest 0.01 mm with a hand-held micrometer. The length was measured at its maximum from the tegula to the end of the fringe. The width was measured at the widest point of the wing perpendicular to its long axis (the length).

Several methods to estimate wing area were evaluated, including passing photographic negatives of enlarged wings through a leaf area meter, tracing outlines of wings on 20 squares-per-inch graph paper, and using the triangle area formula, but the following method proved to be the most reliable and time-efficient. Outlines of wings were traced on weighing paper, cut out, weighed, and compared to a known area's weight to get an estimate of wing area. Forewing area was regressed on forewing width, and hindwing area was regressed on hindwing width to evaluate the wing area estimates. The width was chosen over length because part of the length of the wing was often torn during removal from the body. Total wing area was obtained by adding the forewing area to the hindwing area and multiplying by two.

Statistical Methods. Laboratory data on adult dry body weight and percent whole body lipid (transformed to arcsine of the square root of the proportion) were analyzed with the GLM analysis of variance procedure of SAS (SAS Institute 1985) ($\alpha = 0.05$). Means were separated using Tukey's studentized range test ($\alpha = 0.05$). Data on dry body weight and % whole body lipid from each field infestation trial were analyzed separately because of differences in initial density treatments between field experiments. The GLM analysis of variance procedure of SAS was used for field data, including the effect of feeding on artificial diet in the model ($\alpha = 0.05$). Means were separated using Tukey's studentized range test ($\alpha = 0.05$). No statistical analyses were performed on the pheromone-trapped male data or the actograph-tested field-reared female data because of uncontrolled conditions. Regressions to predict forewing area and hindwing area, and regressions to determine wing-loading were accomplished with the REG procedure of SAS ($\alpha = 0.05$). Total wing area and dry body weight data were log-transformed (base 10) before wing-loading analysis. Regression lines among or between different density treatments or between sexes were compared using the GLM test for heterogeneity of slopes and analysis of covariance procedures of SAS ($\alpha = 0.05$).

Results and Discussion

Lipid Analyses. Percent lipid in laboratory-reared fall armyworms ranged from 20.1 to 51.2% and 19.1 to 56.5% in females and males, respectively (Table 12). In field-reared females, lipid content ranged from 16.6 to 45.6%, while lipid content of field-reared males extended from 17.5 to 48.1% (Table 13).

Although the dry body weight of females did not differ among laboratory density treatments, mean % whole body lipid in females was significantly greater in the higher density treatments of two and three larvae per cup ($P < 0.05$, Table 12). In laboratory-reared males, both mean dry body weight and percent whole body lipid were significantly greater in the two higher density treatments ($P < 0.05$). It is not clear why mean dry body weight of adults from the lowest density group was significantly lower. In previous companion studies, pupal weight increased with decreasing larval rearing density. In the field-rearing experiments, adult dry body weight was similar between density treatments, but males reared in the high larval density plot during the third field infestation trial showed significantly higher lipid content ($P < 0.05$, Table 13).

Dry body weight was significantly affected by the length of time the field-reared larvae fed on artificial diet before pupating ($P < 0.05$), except in the females from the third field trial. For example, male FAW adults from the third field trial which fed on artificial diet for 3-4 days weighed significantly less than those which fed on diet for 5-6 days ($P = 0.0069$) (mean dry body weight $\pm$ SE (n): 3-4 days group, 27.75 $\pm$ 0.57 mg (59); 5-6 days group, 30.85 $\pm$ 1.09 mg (37)). Percent whole body lipid was significantly less in adults from field-reared larvae which spent fewer days on artificial diet before pupating ($P < 0.05$), except in the only comparison when there was a significant density effect on lipid content (males, third field trial). For instance, female FAW adults from the third field trial which fed on artificial diet for 3-4 days had significantly less whole body lipid content than those which fed on diet for 5-6 days ($P = 0.0004$) (mean % lipid $\pm$ SE (n): 3-4 days group, 20.3 $\pm$ 0.7 (9); 5-6 days group, 26.3 $\pm$ 0.8 (34)). There were no significant interactions of the effect of rearing density with the effect of days on artificial diet for adult dry body weight and whole body lipid content ($P > 0.05$). Because only one replicate showed a significant difference in lipid reserves as a result of a change in rearing density, and because there

Table 12. Dry body weight and percent whole body lipid in adults from laboratory-reared fall armyworm larvae.

No. Larvae/ 30 ml cup	Dry Body Weight (mg)[a]		% Whole Body Lipid[a]	
	$\bar{x} \pm$ SE(n)	Range	$\bar{x} \pm$ SE(n)	Range
FEMALE				
1	54.29 ± 1.79 (61) a	23.70 - 79.70	36.1 ± 0.8 (61) b	20.1 - 44.8
2	58.71 ± 1.55 (46) a	37.90 - 89.70	38.8 ± 0.7 (46) a	25.9 - 51.1
3	57.54 ± 1.55 (78) a	22.00 - 82.50	40.1 ± 0.6 (78) a	27.3 - 51.2
MALE				
1	47.14 ± 1.31 (62) b	18.90 - 71.40	39.0 ± 1.0 (62) b	19.1 - 51.7
2	53.51 ± 1.16 (35) a	39.90 - 64.90	44.9 ± 0.7 (35) a	35.0 - 56.6
3	52.61 ± 1.16 (67) a	34.10 - 80.50	43.5 ± 0.7 (67) a	27.6 - 54.1

[a] Means within the same column and sex followed by the same letter are not significantly different, Tukey's Studentized Range Test, $\alpha = 0.05$.

Table 13. Dry body weight and percent whole body lipid in adults from fall armyworm larvae reared at two density levels in corn field plots, Tifton, Georgia, 1989.

Field Trial	Sex	$\bar{x}$ No. Larvae/Plant[b]	Dry Body Weight (mg)[a] $\bar{x} \pm$ SE(n)	Dry Body Weight (mg)[a] Range	% Whole Body Lipid[a] $\bar{x} \pm$ SE(n)	% Whole Body Lipid[a] Range
2	Female	4	51.23 ± 2.54 (16)a	34.50 - 71.80	31.7 ± 1.3 (16)a	24.6 - 45.6
	Female	25	50.19 ± 2.00 (27)a	25.90 - 68.10	29.9 ± 1.0 (27)a	16.6 - 37.1
	Male	4	39.14 ± 2.55 (17)a	20.00 - 66.40	28.8 ± 1.5 (17)a	19.0 - 45.6
	Male	25	41.43 ± 1.45 (35)a	24.40 - 55.60	32.3 ± 1.2 (35)a	18.8 - 48.1
3	Female	5	37.39 ± 1.87 (25)a	23.80 - 62.10	23.3± 1.0 (25)a	16.7 - 33.5
	Female	25	41.54 ± 1.94 (18)a	24.20 - 56.50	27.4 ± 1.9 (18)a	20.9 - 33.3
	Male	5	29.81 ± 0.89 (41)a	19.40 - 43.30	23.9 ± 0.7 (41)b	17.5 - 38.8
	Male	25	28.30 ± 0.72 (55)a	19.50 - 44.30	25.9 ± 0.7 (55)a	17.8 - 44.9

[a] Analyzing field trials separately, means within the same column and sex followed by the same letter are not significantly different, Student's *t*-test, $\alpha = 0.05$.

[b] Initial density at the time of artificial infestation of first instar larvae.

was a confounding effect of artificial diet on dry body weight and lipid content, the evidence is inconclusive that rearing density in the field affects adult dry body weight and lipid reserves.

The differences in whole body lipid content in FAW adults due to a change in laboratory rearing density were not nearly as great as those found for *Spodoptera exempta* (Walker) (Gunn & Gatehouse 1987). The greatest increases in whole body lipid content due to increased density were 11.1 and 15.1% in female and male FAW moths from laboratory-reared larvae, respectively. Increased rearing density in the field caused a significant increase in whole body lipid content of 8.4% from low to high density treatment in males from the third field trial. In contrast, *S. exempta* adults of the high density migrant phase contained 250 to 610% more abdominal lipids (> 92.5% of whole body glycerides) than adults of the low density nonmigrant phase (Gunn & Gatehouse 1987). Gunn & Gatehouse (1987) contended that the increase in lipid reserves in the moths reared under crowded conditions was related to their dispersal potential. If larvae are reared at unfavorable density levels, they would stockpile lipid reserves in the larval stage for use in prolonged flight as adults. Their hypothesis was substantiated by data which demonstrated that larvae reared at higher densities (*gregaria* phase) were more likely to produce adults which were "long fliers" (Woodrow et al. 1987). Because of the relatively small increases in FAW whole body lipid content as a result of increasing laboratory rearing density in the present study, results are inconclusive that higher larval rearing density in the laboratory appreciably increases lipid reserves. Moreover, increased lipid reserves are not backed up by evidence that crowded FAW larvae emerge as adults with higher activity (previous studies).

If males are compared with females within each laboratory larval density treatment, males weighed significantly less but had a significantly greater % lipid content than females in all three laboratory density treatments ($P < 0.05$). Males from field-reared larvae weighed significantly less than females from field-reared larvae ($P < 0.05$), but % whole body lipid was comparable between the sexes. The greatest significant difference between male and female whole body lipid content in FAW adults was 13.6% which may not be biologically significant. This difference between the sexes is relatively small compared to

those detected in *S. exempta* (Gunn & Gatehouse 1986). Laboratory- and field-reared female *S. exempta* contained 55.9 and 25.6% more abdominal lipids, respectively, than their male counterparts. These scientists speculated that male larvae did not accumulate as much lipid as female larvae which need lipid for flight fuel and reproduction as adults.

A nonstatistical comparison of the whole body lipid content between FAW moths from field- and laboratory-reared larvae revealed that in both sexes, mean dry body weight and % whole body lipid were higher in laboratory-reared groups. Gunn & Gatehouse (1986) obtained similar results with female *S. exempta*, but not with males. Not only could the difference in larval diet quality and availability in the field affect adult lipid content as suggested by Gunn & Gatehouse (1986), but perhaps the field-reared larvae were more active than their laboratory-reared counterparts which would affect the amount of lipid accumulated in the larval stage. Field-collected larvae initially must have been quite lean, considering the weight and lipid they apparently gained by feeding on artificial diet before they pupated.

Differences in the results of lipid analyses between *S. exempta* and *S. frugiperda* could be a species difference, but they may be the result of dissimilar experimental protocols between Gunn & Gatehouse's studies (1986 and 1987) and my studies. Gunn & Gatehouse's 1987 data are not comparable to my data because their method used to determine glyceride content extracted only triglycerides and glycerol (Eggstein & Kuhlmann 1974), whereas I retrieved tri-, di-, and mono-glycerides, cholesterol, cholesterol ester, and glycerol. In Gunn & Gatehouse's 1986 paper, chloroform/methanol lipid extraction was used as in the present study, but aliquots of each sample were assayed for amount of lipid and glycerol (Goldsworthy et al. 1972, Candy et al. 1976), and I assayed for whole body lipid by weighing the total amount of extracted lipid. More importantly, both of Gunn & Gatehouse's studies (1986 and 1987) used spectrophotometric methods for quantifying lipids. D. N. Judge (pers. comm.) determined that lipid values obtained from the spectrophotometer were often underestimates or overestimates of actual lipid present in sample. Gunn & Gatehouse (1986) expressed abdominal and thoracic lipid as mg/100 mg of pharate adult weight. I expressed lipid as a percentage of newly emerged adult freeze-dried body weight. If their high density laboratory-reared female data are

recalculated to obtain % whole dry body lipid, the mean % is 14.6, a value considerably lower than 40.07 %, the mean lipid content calculated for high density laboratory-reared female FAW in the present study (low density females were not tested in Gunn & Gatehouse's 1986 study). Additionally, in another study using a lipid extraction protocol similar to that reported here, the mean % lipid was 37.0 in 8-day-old female moths of *Anticarsia gemmatalis* (Hubner), a migratory noctuid very similar to the FAW (Teo et al. 1987). Therefore, Gunn & Gatehouse's results (1986 and 1987) showing differences in lipid content between male and female *S. exempta* and between the different phases bear reconsideration.

An analysis of the lipid content of four groups (corresponding to four collection dates in August and September,1988) of pheromone-trapped FAW males demonstrated a wide range of dry body weight (13.3 - 59.9 mg) and % whole body lipid (12.2 - 71.4%). The extensive ranges are to be expected in data from a mixed-age, mixed-level-of-activity, and mixed-food-source group of FAW. The mean dry body weights for the four separate groups in chronological order were, in mg: 24.93, 30.01, 25.51, and 23.5. The mean percentages of whole body lipid were: 25.5, 26.9, 32.4, 30.0, respectively. The range of % whole body lipid content for free-flying pheromone-trapped FAW males presumed to be migrants (12.2 - 71.4%) is wider than the range of % lipid content for newly emerged field-reared FAW males (17.5 - 48.1%) (Table 13). This could indicate that moths utilized very little of their lipid reserves for flight, relying mostly on wind assistance. Additionally, moths may feed extensively on nectar sources to augment lipid reserves prior to migratory flight. FAW moths and other migrant noctuids are known to feed on nectar soon after emergence (Sparks 1979, Rose & Dewhurst 1979, Rose et al. 1985).

Evidence of lipid synthesis from carbohydrate sources was found in field-reared female FAW which were tested in the actograph. These moths had spent six days in the actograph with 10% sucrose solution provided *ad libitum*. Subsequently, their mean dry body weights were 23.3% and 58.2% higher than the weights of newly emerged females reared at low and high densities from the second field infestation trial, respectively (low density 6-day-old females: 63.13 mg, high density 6-day-old females: 79.42 mg). Also, mean % whole body lipid in these actograph-tested females were 34.1% and 70.6% greater than the %

lipid content from the same newly emerged females (low density 6-day-old females: 42.5%, high density 6-day-old females: 51.0%). (The highest mean dry body weight and % lipid content of newly emerged moths from field-reared larvae were found in the second field trial.) Similarly, Gunn & Gatehouse (1986) reported that fed, mated females gained lipids during the first 48 h after emergence, but as mentioned previously, their lipid data are questionable because of the low values for lipid content they obtained spectrophotometrically. Although it is interesting that 6-day-old female moths from the high density treatment gained more weight and more lipid than 6-day-old female moths from the low density treatment, no conclusion can be drawn about the relationship between rearing density and the increase in lipid reserves after feeding because sucrose solution intake by moths was not controlled and could not be measured in this study.

HPTLC analyses showed the presence of fatty acid, cholesterol ester, cholesterol, triglycerides, diglycerides, and traces of monoglycerides. The predominant lipid class was triglyceride in all 14 samples, ranging from 26.5 to 47.7% of whole body lipid. Less than 10% diglycerides per sample was detected. An abundance of triglycerides was expected in these moths, because these lipids are the primary fat in the whole insect, stored in the fat body (Beenakkers et al. 1985). Triglycerides are converted to diglycerides in the fat body and transported through the hemolymph to the flight muscles and the developing ovaries.

Wing Area Estimation and Wing-loading Analyses. Analysis of covariance revealed that rearing density did not significantly affect the regression of forewing area on forewing width or the regression of hindwing area on hindwing width in females from laboratory- and field-reared larvae. In fact, there was no significant difference between the regressions of forewing area on forewing width of females from laboratory-reared and field-reared larvae (Figure 28) ($P > 0.05$, combined regression: $y = -45.01 + 18.83x$, $r^2 = 0.69$, $n = 118$). Similarly, laboratory-reared and field-reared female hindwing area regressions on hindwing width could be combined (Figure 29) ($P > 0.05$, $y = -55.68 + 15.61x$, $r^2 = 0.68$, $n = 114$). Male wing measurement data were slightly more variable (Figures 30 - 32). No rearing density effects were found

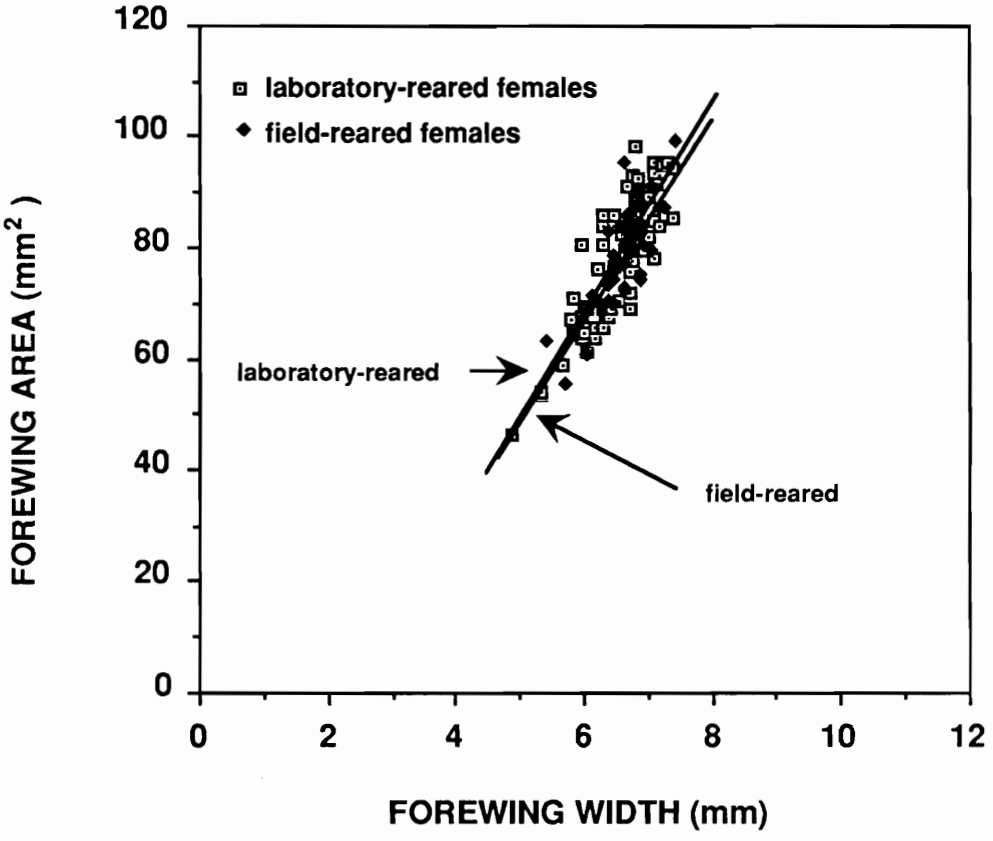

Figure 28: The relationships of estimated forewing area to measured forewing width of female fall armyworm moths from laboratory- and field-reared larvae. Combined regression: $y = -45.01 + 18.83x$, $r^2 = 0.69$, $n = 118$.

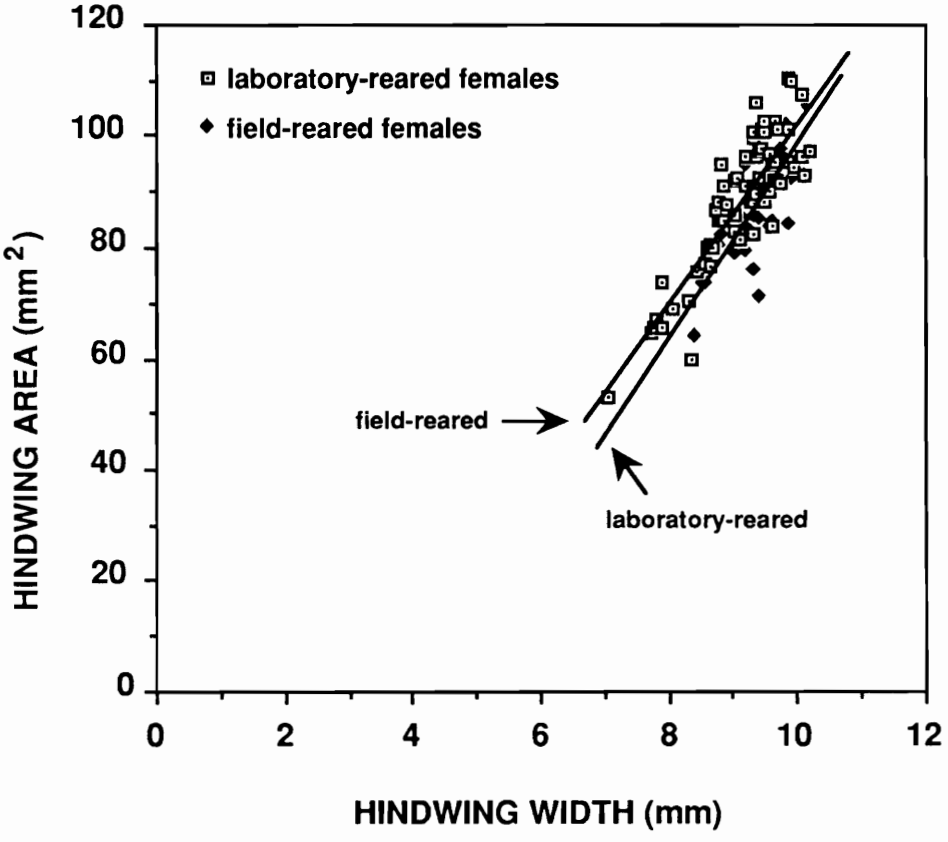

Figure 29: The relationships of estimated hindwing area to measured hindwing width of female fall armyworm moths from laboratory- and field-reared larvae. Combined regression: $y = -55.68 + 15.61x$, $r^2 = 0.68$, $n = 114$.

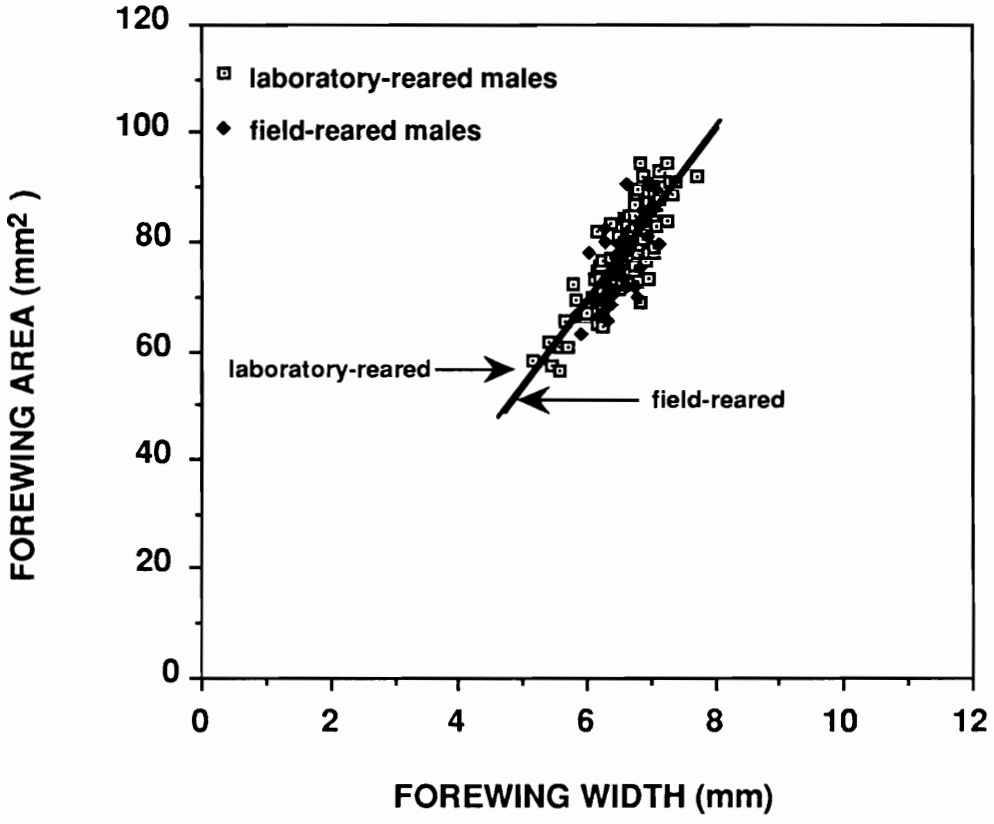

Figure 30: The relationships of estimated forewing area to measured forewing width of male fall armyworm moths from laboratory- and field-reared larvae. Combined regression: $y = -26.63 + 15.88x$, $r^2 = 0.68$, $n = 147$.

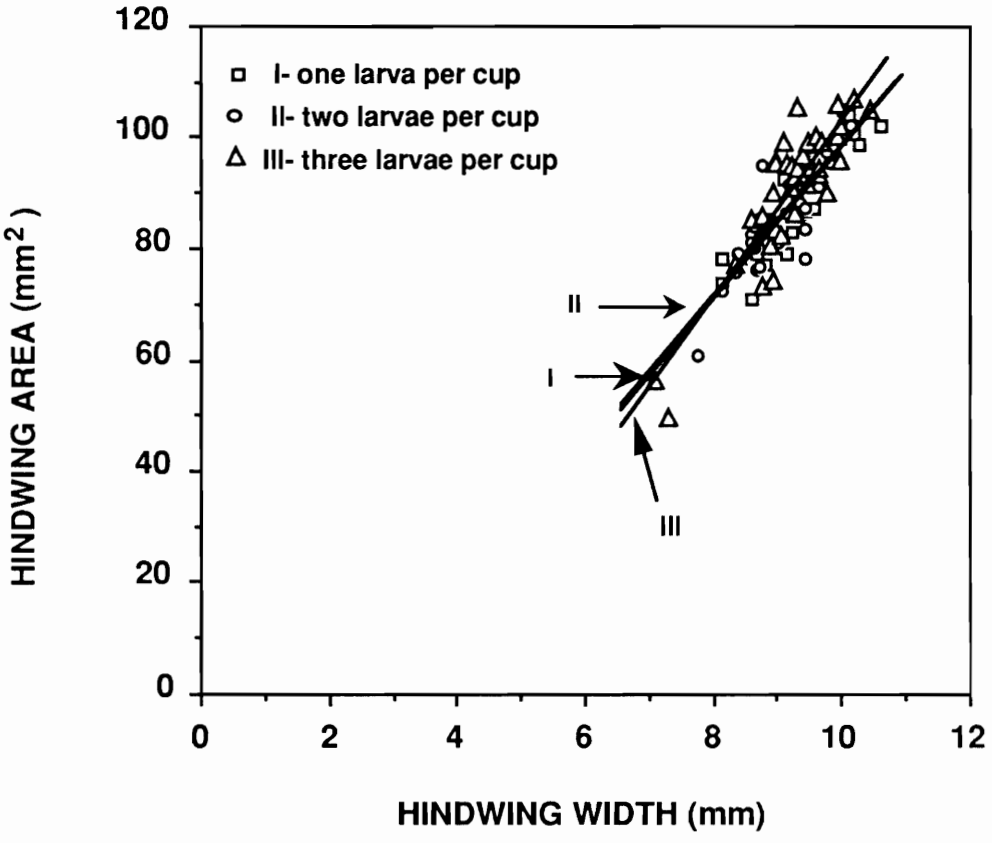

Figure 31: The relationships of estimated hindwing area to measured hindwing width of male fall armyworm moths from laboratory-reared larvae. One larva per cup: $y = -41.36 + 13.95x$, $r^2 = 0.81$, $n = 23$; two larvae per cup: $y = -37.95 + 13.65x$, $r^2 = 0.69$, $n = 34$; and three larvae per cup: $y = -60.06 + 16.34x$, $r^2 = 0.77$, $n = 37$.

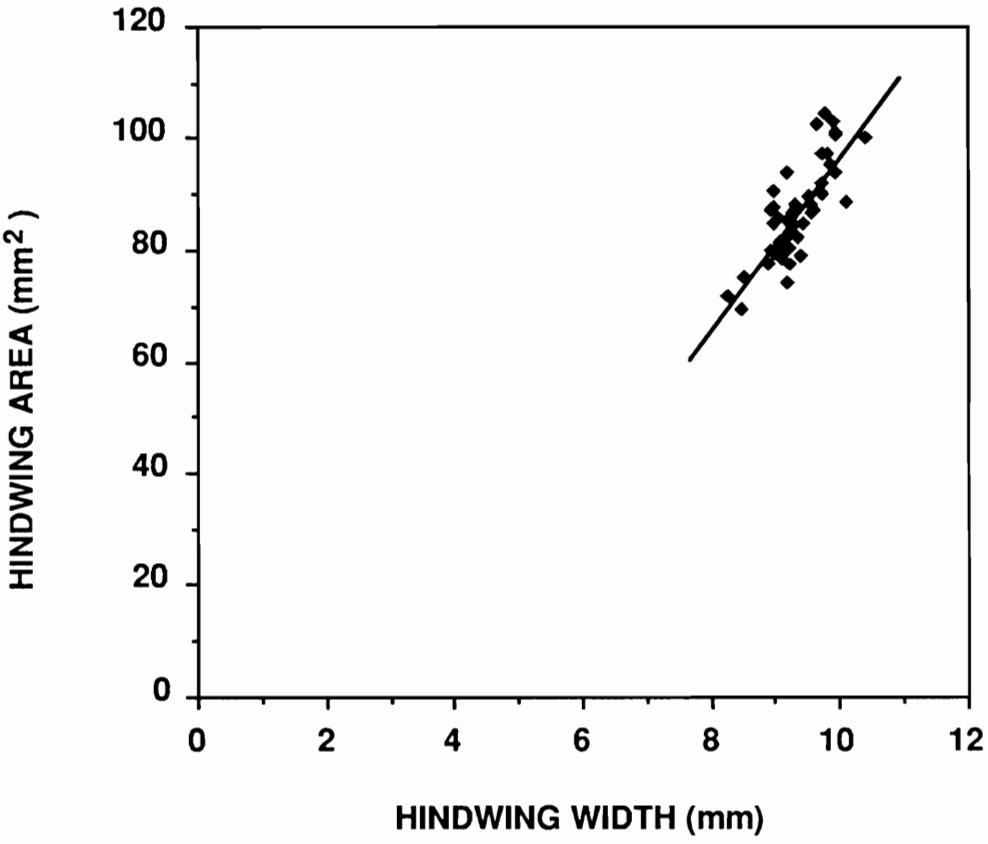

Figure 32: The relationship of estimated hindwing area to measured hindwing width of male fall armyworm moths from field-reared larvae. Regression: $y = -60.42 + 15.74x$, $r^2 = 0.64$, $n = 48$.

in laboratory- and field-reared male forewing data ($P > 0.05$, combined regression: $y = -26.63 + 15.88x$, $r^2 = 0.68$, $n = 147$) or in field-reared male hindwing data ($P > 0.05$, $y = -60.42 + 15.74x$, $r^2 = 0.64$, $n = 48$) (Figures 30 and 32). Rearing density affected the relationship of laboratory-reared male hindwing area on hindwing width (Figure 31) ($P < 0.05$, one larva per cup: $y = -41.36 + 13.95x$, $r^2 = 0.81$, $n = 23$; two larvae per cup: $y = -37.95 + 13.65x$, $r^2 = 0.69$, $n = 34$; three larvae per cup: $y = -60.06 + 16.34x$, $r^2 = 0.77$, $n = 37$). This statistically significant difference may not be biologically significant, considering the narrow range of hindwing area and hindwing width values in the data in Figure 31.

Forewing and hindwing area estimates were used to obtain total wing areas for the wing-loading study. Many different approaches have been used to evaluate wing-loading (Miller 1977, Angelo & Slansky 1984, Danthanarayana 1976, Parker & Gatehouse 1985a). Parker & Gatehouse (1985a) concluded that density did not affect wing-loading in *S. exempta*, but their regression analyses appear to be flawed. They regressed wing-loading (moth weight/wing area) on moth weight and reported that wing-loading was a function of moth weight. I followed the methods of Angelo & Slansky (1984) and regressed total wing area on adult dry body weight to eliminate the dependency problem in the *S. exempta* study. There were no significant differences in wing-loading among laboratory density treatments for either sex or between field density treatments for either sex ($P > 0.05$). It can be concluded that rearing density did not affect wing-loading in FAW adults in this study.

Angelo & Slansky (1984) determined that *S. frugiperda*, subjected to varying degrees of starvation during the last instar, had lower than theoretically predicted wing-loading ratios based on the following logic. If wing area is the square and body weight is the cube of linear dimensions (i.e., the wing length), then linearizing wing area against body weight through a logarithmic (base 10) transformation would yield a straight line with a slope of 0.67. Indeed, Miller (1977) determined empirically that biomass was related to forewing length cubed in a pooled group of olethreutid moth species. The wing area to body weight log-log relationship of the FAW in Angelo & Slansky's (1984) study yielded a slope of 0.22 which was considerably lower than the theoretical slope of 0.67. This led Angelo & Slansky (1984) to conclude that as body weight is

reduced in the fall armyworm, a relatively larger wing area than theoretically expected occurs. I traced the theoretical line from their graph on log-log paper, not knowing how they determined the y-intercept of the line, and compared it to my wing area to body weight relationships for male and female moths from laboratory- and field-reared larvae (Figures 33 and 34). Slopes of both female and male lines were significantly less than the theoretical slope of 0.67 ($P < 0.05$, using base 10 log values, theoretical line: $y = 1.59 + 0.67x$; females from laboratory-reared larvae: $y = 1.83 + 0.41x$, $r^2 = 0.62$, $n = 70$; females from field-reared larvae: $y = 2.02 + 0.30x$, $r^2 = 0.54$, $n = 45$; males from laboratory-reared larvae: $y = 1.75 + 0.46x$, $r^2 = 0.56$, $n = 96$; males from field-reared larvae: $y = 2.14 + 0.24x$, $r^2 = 0.62$, $n = 48$). However, all the data lie below the theoretical line, indicating that the FAW in my study had higher than expected wing-loadings. Interestingly, if I redefined the y-intercept of the theoretical line so that the line fell below most of the data points, then I could conclude that the fall armyworm had lower than expected wing-loadings, supporting the hypothesis that migrant species have low wing-loadings for more efficient flight (Figure 35) (using base 10 log values, new theoretical line: $y = 1.33 + 0.67x$). Likewise, Angelo & Slansky (1984) would have had to drop their theoretical line down in order to make the conclusion that their starving FAW had lower than expected wing-loading ratios. Lower wing-loadings have been recorded for other migrant species, such as *Epiphyas postvittana* (Walk.), the light brown apple moth (Danthanarayana 1976), and *Plusia gamma* L. (Long 1959).

I have presented evidence that an increase in larval rearing density in the laboratory and in one field replicate significantly increased lipid reserves in the FAW. However, the increases due to rearing density may not be biologically significant, and the premise that higher lipid reserves are related to a higher activity potential is not supported by my actograph data. In fact, one group of field-reared female moths kept in the actograph for six days gained weight and lipids, presumably by feeding on sucrose solution, while their activity level decreased with age. Triglycerides, the primary storage lipids used for flight energy, were the dominant neutral lipids detected in whole body lipid samples. Larval rearing density did not affect wing-loading in the fall armyworm. Yet, as a migratory species, it showed lower than theoretically expected wing-loadings,

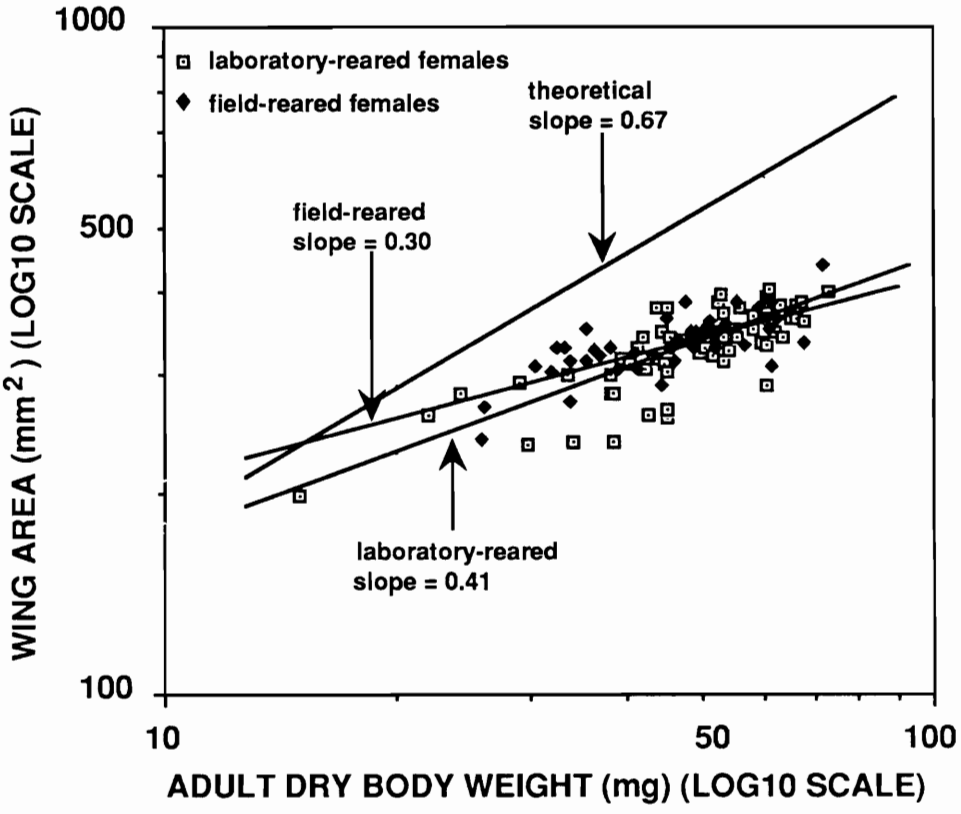

Figure 33: Comparison of wing area to dry body weight relationships of female fall armyworm moths from laboratory- and field-reared larvae. Theoretical line: $y = 1.59 + 0.67x$; laboratory-reared females: $y = 1.83 + 0.41x$, $r^2 = 0.62$, $n = 70$; field-reared females: $y = 2.02 + 0.30x$, $r^2 = 0.54$, $n = 45$.

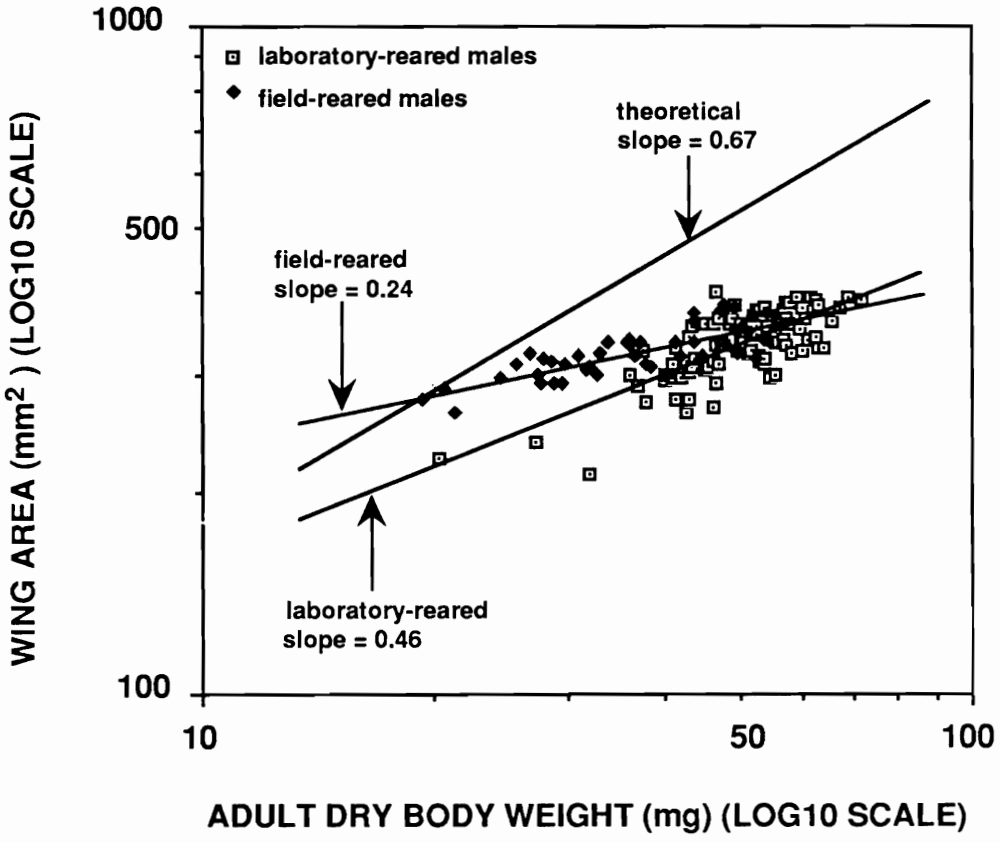

Figure 34: Comparison of wing area to dry body weight relationships of male fall armyworm moths from laboratory- and field-reared larvae. Theoretical line: $y = 1.59 + 0.67x$; laboratory-reared males: $y = 1.75 + 0.46x$, $r^2 = 0.56$, $n = 96$; field-reared males: $y = 2.14 + 0.24x$, $r^2 = 0.62$, $n = 48$.

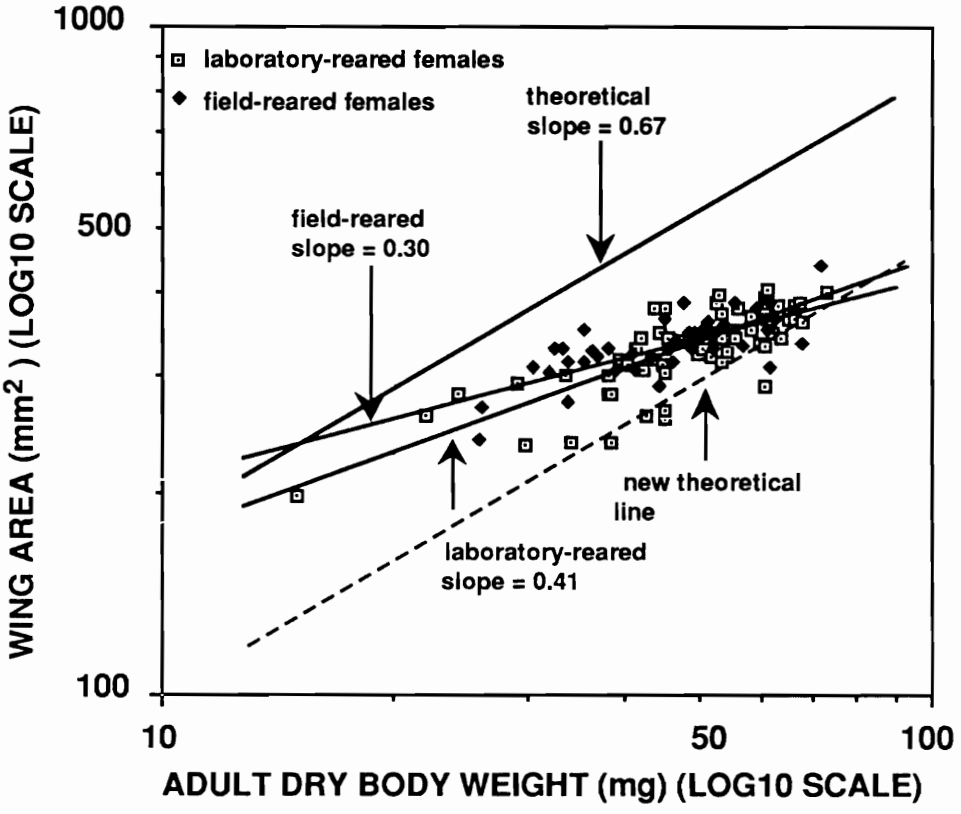

Figure 35: Comparison of wing area to dry body weight in female fall armyworm moths with new theoretical line: y = 1.33 + 0.67x.

based on the fact that wing area to body weight relationships had significantly smaller slopes than a theoretical slope of 0.67.

The density effect on lipid stores needs to be examined further to attempt to answer why, how, and when crowded individuals accumulate more lipids. Nonetheless, these data support the belief that the FAW is capable of long-range migration, given the large amount of lipid reserves found in this species as a whole and the lower than theoretically expected wing-loadings. However, the lack of density-dependent increases in lipid reserves associated with higher adult activity and the lack of density effects on developmental time and size indicates that a premigrant phase induced by high larval density is questionable in the fall armyworm. Components of weather (wind, temperature, photoperiod, etc.) may be more important than larval density in initiating FAW migrant behavior (Pair et al. 1986, Westbrook & Sparks 1986, Johnson 1987).

Chapter 7

Summary and Conclusion

An investigation was undertaken to test a hypothesis that crowding produces premigrant traits in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (FAW). High population density, probably acting in conjunction with other factors, including a decrease in food quality and food quantity, could produce two or more groups of adults, which may differ in body weight, wing size, lipid reserves, developmental time, and/or flight behavior (Johnson 1969). Putative premigrant traits include small size, high flight potential, low wing-loading (body weight/wing area), and high lipid reserves (see review, Angelo & Slansky 1984). Density-dependent phase variation is present in a congeneric migratory species, *Spodoptera exempta* (Walker), the African armyworm (Faure 1943), and we proposed that the same might occur in the FAW. Differences have been recorded between the *S. exempta* low density and high density phases in size, developmental time, lipid reserves, and adult activity (Parker & Gatehouse 1985a and b, Simmonds & Blaney 1986, Gunn & Gatehouse 1986 and 1987). The effects of rearing density on the production of premigrant traits have been examined in other migratory noctuids (*Anticarsia gemmatalis* Hubner, *Alabama argillacea* Hubner, *Trichoplusia ni* (Hubner), and *Agrotis ipsilon* (Hufnagel)) (Anazonwu & Johnson 1986, Fescemyer & Hammond 1986, Johnson et al. 1985, Tignor & Eaton 1986, Lewis & Keaster 1989, Sappington & Showers 1992), but no such studies have been accomplished for the FAW. The main objective of the laboratory and field experiments was to determine if a higher larval rearing density produces premigrant traits, and if these traits could be used as criteria for separating non-premigrant from premigrant groups of the FAW.

An increase in larval rearing density in the laboratory, but not in the field, significantly decreased pupal weight and forewing width, both of which are indicators of size. However, adult dry body weight was not affected by an increase in rearing density. Decreased body size as a result of increased density could be irrelevant to migratory potential, as similar results have been obtained with non-migrant species (see review, Peters & Barbosa 1977).

Moreover, Rankin & Burchsted (1992) found no correlation between body size and long-duration (presumed migratory) flight in non-Lepidopteran migrants such as *Oncopeltus fasciatus* (Dallas).

The greatest differences among density treatments in the developmental times for FAW from laboratory-reared larvae were 0.6 d for females and 1.07 d for males. Rearing density did not affect duration of pupal stage in the field-reared FAW. Crowding in other migratory species resulted in both longer (Tignor & Eaton 1986, Fescemyer & Hammond 1986) and shorter developmental times (Simmonds & Blaney 1986) with differences among treatments much greater than 1 day. Therefore, developmental time is not a good variable to use in differentiating between non-premigrant and premigrant FAW.

Although field rearing density did not affect pupal weight, wing width, and duration of pupal stage, the length of time spent on artificial diet before pupation significantly affected these parameters. Larvae were either fifth or sixth instar at the time of field collection, and they were supplied with artificial diet until pupation. Feeding on artificial diet caused increases in pupal weight and decreases in duration of pupal stage. The confounding effect of the artificial diet along with high dispersal and mortality of early instar larvae in the field after artificial infestation (Morrill & Greene 1973a and b, 1974) were the primary causes of the lack of a crowding effect on size and developmental time in the field-reared FAW.

The actograph designed at V. P. I. & S. U. was used to measure adult FAW activity. This actograph is unique among currently operating actographs in that it does not involve tethering moths. Also, validation of the other actographs has not been reported in the literature, whereas the V. P. I. & S. U. actograph was validated with videotape recordings of moths moving about in actograph cages. The actograph study with the FAW represents one of the numerically largest laboratory investigations of migratory noctuid adult activity, rivaled only by the tethered flight experiments with the African armyworm. Data were gathered on 134 unmated and 77 mated females from laboratory-reared larvae, 90 unmated females from field-reared larvae, 119 unmated males from laboratory-reared larvae, 12 unmated males from field-reared larvae, and 13 unmated females exposed to males, both from laboratory-reared larvae. While

other researchers examined only one or two nights of activity for each moth (Wales et al. 1985, Woodrow et al. 1987, Parker & Gatehouse 1985a, Lewis & Keaster 1989, Sappington & Showers 1992), I analyzed activity from the first through the sixth nights after emergence. Usually, there is more emphasis on unmated female moth activity in other similar behavior studies, but presented here are companion data on males and mated females as well.

From the videotape recordings, it was observed that FAW moths engaged in behavior other than flying; hence computer-recorded counts were termed activity counts. The usual activity, besides feeding, was flying. The number of activity counts was determined to be a valid measure of the amount of time a moth was active. Also, it was noted that male moths consistently showed greater activity than females by having a greater proportion of long-duration activity bouts (spells of activity), a greater activity bout duration, and a generally greater magnitude of activity during peak periods. Greater activity in males, probably associated with mate-finding, is also seen in *T. ni* (Sprint & Eaton 1987), the African armyworm (Woodrow et al. 1987), and the corn earworm (Judge et al. 1991).

The actograph analyses showed that unmated male FAW moths could migrate on the first or second night after emergence concurrent with the unmated females during the initial evening peak. Indeed, these data are supported by the fact that both sexes of FAW (presumed migrants) were caught in light traps in the Gulf of Mexico (Sparks et al. 1986). An increase in larval rearing density did not affect the incidence of mating in females. The majority of the females mated when given the opportunity. The actograph results from the mated and unmated female FAW experiments neither support nor refute Kennedy's (1961) migration hypothesis. Kennedy (1961) proposed that insects migrate before they mate, mature during the migratory flight, and mate upon arrival of the new habitat. Mated females showed very little actograph activity compared to unmated females not exposed to males in earlier studies, supporting Kennedy's (1961) hypothesis. Also supportive is the study of Leppla et al. (1979) which demonstrated reduced levels of activity in mated female FAW, but multiple pairs per actograph cage were used in their study. However, in the present study, the few unmated female moths which had been exposed to males showed similar suppressed actograph activity, refuting Kennedy's

hypothesis. These unmated moths did not behave as if they were migrants which postpone mating to undergo migratory flight. Additionally, published evidence indicates that both mated and unmated moths migrate. Forty to 50% of presumed migrant FAW females caught in light traps in the Gulf of Mexico were mated (Sparks et al. 1986), and mated FAW females traveled wind-borne from the U.S. Gulf Coast to Canada (Rose et al. 1975).

Activity counts differed greatly among groups of FAW tested. A comparison of highest mean numbers of activity counts from any density treatment reveals that the lowest activity levels were found in females exposed to males, unmated (86.50) and mated (161.38), followed by laboratory-reared unmated females not exposed to males (453.60), field-reared unmated females (574.25), field-reared unmated males (770.50), and laboratory-reared unmated males (1594.70) with the highest activity. There is little evidence that adult activity was significantly affected by larval rearing density.

Most individuals showed low levels of activity, and a few individuals showed relatively high levels of activity. The problems of great individual variability and skewed data distributions in behavioral data have been reported by other researchers (Parker & Gatehouse 1985a, Woodrow et al. 1987, Lewis & Keaster 1989, Sappington & Showers 1992). Davis (1980) speculated that right-skewed flight data distributions were the result of short flights for feeding, mating, and oviposition being adaptive most of the time; long flight, being costly, is undertaken only in the case of unfavorable habitat conditions. Despite these problems, significant density effects on adult activity were found for the African armyworm (Woodrow et al. 1987, Parker & Gatehouse 1985a), contrasting the present findings for the FAW. Studies on the African armyworm involved crowding the larvae at higher levels than I did with the FAW. Perhaps the larvae were not crowded enough in my experiments to produce significant changes in adult FAW activity.

The presumed migrant phase of several lepidopteran species was found to have greater lipid reserves and lower wing-loadings (body weight/ wing area) (see review, Angelo & Slansky 1984). Generally, lipid reserves of FAW moths from field-reared larvae were not affected by an increase in field density but were increased by feeding on artificial diet (see above). My investigations determined that an increase in rearing density in the laboratory significantly

increased lipid reserves in the FAW, but only by 8.4 to 15.1%. Larger increases in lipid reserves (250-610% increase) as a result of an increase in larval rearing density were obtained for the African armyworm by Gunn & Gatehouse (1986 and 1987). However, Gunn & Gatehouse's (1986 and 1987) lipid data are questionable because the spectrophotometric methods used to quantify lipids may greatly underestimate actual lipid amounts (D. N. Judge pers. comm.). Gunn & Gatehouse (1986 and 1987) contended that the increase in lipid reserves in the moths reared under crowded conditions was related to their dispersal potential, which was supported by their tethered flight data (Parker & Gatehouse 1985a, Woodrow et al. 1987). However, no such relationship was found between lipid reserves and actograph activity in my study. In fact, one group of actograph-tested females from field-reared larvae gained weight and lipids when fed 10% sucrose solution while their activity level decreased with age. The range of % whole body lipid content in newly emerged field-reared FAW males (17.5 - 48.1%) falls within the range for free-flying pheromone-trapped FAW moths presumed to be migrants (12.2 - 71.4%). This could indicate that moths utilized very little of their lipid reserves for flight, relying mostly on wind assistance, and/or they synthesize lipids from nectar sources before undergoing migratory flight. FAW moths and other migrant noctuids are known to feed on nectar soon after emergence (Sparks 1979, Rose & Dewhurst 1979, Rose et al. 1985). In HPTLC studies, triglycerides, the primary storage lipids used for flight energy, were the dominant neutral lipids detected in whole body samples, as was expected from other similar studies in insects (Beenakkers et al. 1985). Although larval rearing density did not affect wing-loading in the FAW, as a migrant species, the moth showed lower than theoretically expected wing-loadings. This is based on the fact that wing area to body weight relationships had significantly smaller slopes than a theoretical slope of 0.67 (Angelo & Slansky 1984).

Given that some premigrant traits were produced by increasing the larval rearing density while other premigrant traits were not induced, the evidence is inconclusive that true premigrants were produced by my rearing methods. Morphological (decreased pupal weight) and physiological (higher lipid reserves) changes as a result of an increase in larval rearing density would indicate a separable premigrant phase. However, developmental time, wing

width, wing-loading, and adult activity were not appreciably affected by changes in larval rearing density. Additionally, the abundant evidence from the literature that weather conditions (temperature, photoperiod, wind, precipitation) influence FAW dispersal and outbreaks (Pair et al. 1986, Westbrook & Sparks 1986, Johnson 1987) supports the hypothesis that there are no distinct premigrant and non-premigrant phases in this species. If FAW moths can be picked up via convective and turbulent air forces during their normal nightly flight activity, then the existence of a premigrant phase is not necessary for successful long-range dispersal.

Based on the evidence in this dissertation, it can be concluded that the FAW does not have distinct density-dependent phases as found in the African armyworm. More attention should be directed toward the effects of components of weather, such as temperature and photoperiod, on FAW premigrant and migrant behaviors in order to better predict outbreaks of this sporadic insect pest.

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VITA

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