Physiological and Ecological Aspects of Migration and Diapause in the Cotton Bollworm *Helicoverpa armigera*

Thesis submitted for the degree of

Doctor of Philosophy

By

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Submitted to the Senate of the Hebrew University of Jerusalem

February, 2000

ABSTRACT

The cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is one of the most widely distributed agricultural pests. Its distribution is within the boundaries of roughly 45 degrees latitude north and south, excluding the Americas. Its ability to undergo facultative diapause together with a seasonal migration is considered responsible for its pest status throughout the subtropical and tropical regions of the Old World. A laboratory colony reared from Israeli *H. armigera* populations was found to undergo diapause in response to low temperature and short day conditions. However, it is not clear how diapause occurs under field conditions. The migratory status of *H. armigera* in the eastern Mediterranean region is also unclear.

In order to clarify the relationships between overwintering and spring migration in the cotton bollworm in Israel, larvae were caged in this study under field conditions in the autumn, and sex pheromone traps were used to monitor field populations during the following spring. No diapause was detected in October-exposed individuals. Overall, 67.7% and 89.2% of individuals exposed to field conditions during November and December, respectively, entered pupal diapause. It can therefore be concluded that most *H. armigera* individuals in Israel overwinter as diapausing pupae. There was no significant interaction in pupal diapause incidence between introduction date and insect sex, and no significant difference was detected between the sexes in diapause incidence. However, the rate of pupal diapause was significantly higher in the December population than in the November population. Most nondiapausing individuals eclosed at the beginning of the following January. It appears that no adults were able to eclose from nondiapausing

individuals that had pupated in the winter. In contrast, post-diapause adults started to eclose synchronously during the following late April, despite differences in diapause duration. The median of post-diapause female eclosion was before 6 May, 9 days prior to the median of male eclosion. By 5 June, all moths of both sexes had eclosed.

Field capture of *H. armigera* moths in sex pheromone traps occurred one month before the eclosion of caged overwintering diapausal pupae. A second peak of pheromone trap captures coincided with the eclosion of the local overwintering population in mid-May. The third peak of captured moths appeared in mid-June, after all post-diapause pupae had eclosed. Moths trapped before the eclosion of diapausing individuals had significantly fewer wing scales (i.e. greater number of scales lost) and lower wing loading than those trapped later on. The relative thorax size of moths caught after the eclosion of local post-diapause pupae was significantly smaller than that of moths collected before. Because a loss of wing scales is associated with moth activity; a low value of wing loading tends to reduce the power output demands of sustained flight; and a large relative thorax size implies containing relatively large volume of flight muscles, the morphometric data of the early season field moths strongly suggest that these moths had undergone a long distance flight. Relatively high levels of wing loading and wing scale count recorded in the moths trapped during local post-diapause eclosion suggest that only a few immigrants were present in this group of moths. However, their high degree of relative thorax size implies strong flight capability. Taken together, the phenological and morphometrical data support the notion that *H. armigera* exhibits early-spring migratory behavior in Israel.

If migration of *H. armigera* does occur and the migrants breed successfully, the gene flow in this region should be high and there should be very low genetic differentiation among individuals within the geographical scope of migration. To

estimate levels of gene flow in the eastern Mediterranean region, moths were sampled at 6 locations (5 in Israel and one in Turkey). A small number of moths from Egypt and Ethiopia were also used. Gnomic DNA of all samples was extracted individually. The genetic relationships among these populations were analyzed using the RAPD-PCR technique. Three primers which generated consistent and reproducible polymorphic bands were selected out of fifty-five 10-oligonucleotides. Each band was scored as a separate locus. A RAPD-PCR database was established as the presence (1) or absence (0) of every polymorphic band for all of the individual samples. A total of 84 presumptive polymorphic loci were revealed to estimate population structure. Low genetic distances were found among the Israeli and Turkish populations (0.0029 ± 0.00079 S.D.). The estimated values of F_{ST} and θ across all populations in the eastern Mediterranean and East Africa were very low, but significant (θ = 0.015 ± 0.020 S.D. for overall populations, and θ = 0.011 ± 0.019 S.D. for Israeli and Turkish populations), indicating a low genetic differentiation and a high level of gene flow in this region. θ value decreased to 0.004 and 0.009 (both are not significantly larger than zero) for the regional levels of eastern Israeli and Turkish populations, and western Israeli populations, respectively. No isolation by geographic distance was detected, but topographical barriers may play a role in such isolation. Four distinct RAPD-product profile types were defined; these were found in all Israeli and Turkish populations. The mixture of individuals representing all four RAPD types in all populations further suggests frequent movement over this geographic range.

The induction of *H. armigera* migration has been a subject of much debate. Environmental changes are likely to serve as stimuli, causing a physiological switch

to a migratory phase. Migration by many insect species, including the Noctuidae, is usually associated with a delay in reproductive development. Therefore, laboratory studies investigating the environmental stimuli focused on this physiological difference between presumptive migrants and nonmigrants. *H. armigera* were reared in the laboratory under several combinations of constant temperature and photoperiod which simulate average conditions encountered in the spring, summer, early autumn and late autumn in Israel. Juvenile hormone (JH) biosynthesis, the onset of calling behavior, sex pheromone production and ovarian development were examined in virgin female moths subsequent to eclosion. There was a significant interaction in JH production between seasonal conditions and moth age. Virgin D0 females produced very little JH under all seasonal simulations. Spring moths produced a significantly smaller amount of JH on D1, suggesting a delay in allatal maturation. This was probably the cause of the observed delay in ovarian development and the onset of calling behavior, as well as the reduction in sex pheromone biosynthesis. There was a significant interaction in ovary weight between seasonal conditions and female age. The overall ovary weight increased significantly from D0 to D5, and all virgin female moths attained a similar ratio of ovary weight / body mass (O/B) by D5. On D0 and D1, ovarian development was most advanced in summer moths. In contrast, the rate of ovarian maturation in the spring moths was slower; on D2, spring moths had significantly lower O/B values than moths from other seasonal simulations, except post-diapause females. Spring-reared *H. armigera* had a significantly longer pre-calling period than that of moths under the other rearing conditions. By the third night, less than 30 % of the spring moths initiated calling, compared to about 75% of the summer and post-diapause moths. By that time, 60% of the nondiapausal late-autumn moths had called for the first time. Furthermore, moths reared under

spring conditions produced significantly less sex pheromone throughout the experiment. No significant interaction was detected in pheromone production between treatment and moth age. The delay in female sexual maturation in moths reared under spring-like conditions, commonly associated with migratory flight, is consistent with the spring migration observed among *H. armigera* in the field.

In summary, this study elucidated the overwintering and spring abundance of Israeli *H. armigera* populations; provided both direct and indirect evidences of spring migration in this insect; suggested the stimuli inducing migration; examined the role of juvenile hormone (JH) in regulating reproduction and migration; and developed a morphometric analysis to identify specific groups of field-collected moths likely to be migrants. My study revealed that most *H. armigera* individuals in Israel enter overwintering pupal diapause in the autumn and eclose as adults in late April and early May. Results strongly suggest that most moths observed flying in the early spring are immigrants. To some extent, individuals complete their development during spring may be inclined to emigrate further. The range of migration may extend from East Africa (10°N) to Turkey (40°N).