Characteristics of the Larval Development of the Rice Stem Borer, *Chilo suppressalis* Walker (Lepidoptera: Crambidae) in the Ebro Delta (Northeastern Spain)

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The larval development in the first and the second generation of *Chilo suppressalis* from the rice fields of the Ebro Delta was characterized by the number of larval instars and their mean duration. The results showed clear differences between the two generations: larvae of the first generation tended to undergo higher number of molts and to develop more slowly than did larvae of the second generation. The air temperature was probably the most important environmental parameter to cause such differences.

Key words: *Chilo suppressalis*, rice stem borer, life cycle, larval development, Ebro Delta

INTRODUCTION

The rice stem borer *Chilo suppressalis* Walker is an important pest in Old World countries that produce rice, in spite of the fact that in some areas a regressive trend has been observed in the past 20 years (Kim et al., 1988; Kiritan, 1988).

The Ebro Delta (Catalonia) is the main rice producing area of Northeastern Spain, with about 18,000 ha cultivated. The irregular pattern of infestation by *C. suppressalis*, where seriously damaged rice fields are found beside unaffected ones, and stem boring reduces the effectiveness of pesticide application. This prompted us to study the larval development patterns of *C. suppressalis* in the Ebro Delta to develop a method of predicting when the majority of the pest population will occur as early larval instars, the most sensitive stages to the pesticide application (Audemard, 1971).

The present study analyzed the following items using two generations of *C. suppressalis* that complete development in the Ebro Delta during the rice season (May to September): a) the number of larval instars to complete their development, b) the characterization of these instars, and c) the mean duration of each instar. In the Ebro Delta *C. suppressalis* produces a third generation that overwinters (Ramoneda and Roig, 1989b).

MATERIAL AND METHODS

The larval development of *C. suppressalis* in the Ebro Delta had been characterized

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in the first and second generations during the 1987 and 1988 rice seasons by the same methodology.

Fourteen 1 m² plots were cordoned with cubic cages covered with fine mesh, according to KOYAMA (1977), to protect the sampling plots from foreign adults. In one of the central stems a laboratory-reared egg mass that would soon hatch was attached by means of two adhesive bands. In 1988, groups of 30 newly hatched larvae placed onto small filter-paper cups and attached to a leaf by means of a clip were used for the study in the first generation. We could verify that both systems allowed a normal dispersion of the larvae.

Every seven to ten days after egg hatching, all the rice plants enclosed in two plots were uprooted. The stems showing some trace of borer attack were brought to the laboratory and all the head capsules were measured under a binocular microscope.

**Determination of the number of larval instars.** To determine the number of instars of the larvae of *C. suppressalis* in the Ebro Delta we followed PETERSON and HAEUSLER (1928 in GAINES and CAMPBELL, 1935) method. It is based on the fact that the increase in areas of sclerotized parts of insects during larval development occurs only at ecdysis. When all members of a population go through the same number of molts, the measurements of the head capsules may fall into more or less discontinuous groups, each group representing one instar. Visualizing the frequency distribution of the head capsules’ maximum widths in the form of a bar diagram we may obtain a plurimodal distribution, the number of instars being equal to the number of peaks in the diagram.

**Characterization of the larval instars.** To determine the head capsule size distribution for each instar we employed the method of CALTAGIRONE et al. (1983), with a starting hypothesis that in every instar the head capsule widths are normally distributed. We performed a normality test for every possible frequency distribution that could define individual instars. To characterize each instar we adopted distributions that had a better fit with a normal curve.

In 1988, to verify the assumption that the results obtained from the 1 m² plots are representative of the features of wild populations, ten aleatory samples in several rice fields were taken. The sampled larvae, kept in 70% alcohol, were measured to provide the frequency distribution of the head capsule maximum widths in each field. A normality test was applied to every part of the distribution corresponding to every instar and each characteristic mean width was calculated. Then, all those mean values were tested against the mean widths of the instars characterized in the 1 m² plots by the analysis of variance (ANOVA).

**Mean duration of each larval instar.** To determine the mean duration of each larval instar we partly used the KOYAMA method (1977) which calculated the mean instar of the population, at successive time intervals from the hatching by \( E = \frac{\sum (i \cdot n_i)}{\sum n_i} \), where \( E \) = mean instar, \( i \) = number of the instar, \( n_i \) = number of larvae belonging to instar \( i \). To elucidate the development pattern of the borer through its life cycle, the mean instar was plotted as a function of time from hatching in coordinate. When the fitness is good the mean duration can be calculated by this equation for full development.

Air temperatures were provided by a meteorological station in the rice area, about 5 km east of the study field. The physiological state of the rice plant was estimated based on the classification of vegetative, flowering and mature plants (GRIST, 1982).
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**RESULTS AND DISCUSSION**

The number of larval instars

Figure 1 shows the frequency distributions of the head capsule widths of the first and second generation larvae. They showed two distinctive features: a) the larvae of the first generation molted, prevalingly, six times in the course of its development, whereas the second generation underwent only five molts, and b) the peaks are less clear in the first generation than in the second; an overlapping between capsule widths as obtained by us in the first generation larvae has been reported also by Gaines and Campbell, 1935, Kishi, 1971, Schmidt et al., 1977 and Garcia del Pino and De Haro, 1987 in other insects. These authors attributed it to the polymorphic development pattern.

KatsuMata (1934 in Poitout and Bues, 1978) and LOMA (1974), referring to the importance of the supernumerary molts in the larval development of *C. suppressalis*, considered that in contrast with the normal five or six instar pattern, some second generation larvae molted seven, eight and even nine times. Koyama (1977), however, distinguishes six instars from the distribution of the head capsule widths in both generations of *C. suppressalis* in Akita Prefecture (North Japan).

To explain the differences in the number of larval instars between the two gen-

![Image of frequency distribution of head capsule widths of the larvae of *C. suppressalis* of the first and second generations in the Ebro Delta (bar diagrams). The most probable limits between instars are indicated with arrows. The curves corresponding to the expected frequency into every instar, given that those widths be normally distributed (curves overlapped to the bars), are included.](image-url)
Table 1. Mean air temperatures measured during the developmental period of the larvae of *C. suppressalis* of the first and second generations in the Ebro Delta (mean of the two rice seasons in the study)

<table>
<thead>
<tr>
<th></th>
<th>Maximum temperature (°C)</th>
<th>Minimum temperature (°C)</th>
<th>Mean temperature (°C)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>First generation</td>
<td>24.4</td>
<td>19.5</td>
<td>22.0</td>
<td>1.89</td>
</tr>
<tr>
<td>Second generation</td>
<td>28.1</td>
<td>21.8</td>
<td>24.9</td>
<td>1.32</td>
</tr>
</tbody>
</table>

In the second generation, the climatic conditions were analyzed. Peterson and Haeussler (1928 in Gaines and Campbell, 1935) and Poitout and Cayrol (1969) pointed out that temperature was one of the factors that influenced the number of larval instars, low temperatures favouring supernumerary molting. Loma (1974) showed that larvae of *C. suppressalis* reared in the laboratory on artificial diet molted five to nine times, depending on the temperature; at 25°C they molted, on average, fewer times than at 21°C.

The mean temperature of the first generation (22.0°C) was lower than that of the second generation (24.9°C) (Table 1). Such a difference (ca. 3°C) could induce additional molts in the first generation.

Close relationships between the supernumerary molts and nutritive deficiencies are well known in lepidopterans (Gaines and Campbell, 1935; Poitout and Cayrol, 1969; Loma, 1974). Hirano (1964) showed that the plants in the vegetative stage were better quality food (the carbohydrates/proteins ratio was lower) than those in the mature stage; the former favored the larval development. In exchange, in the Ebro Delta the first generation larvae (that molt six times on the average) develop on plants in vegetative stage, whereas the second generation (which molt five times) do so on plants in flowering-maturity stage. These results indicate that the effect of temperature apparently overrode the effect of nutritional conditions.

Characterization of the larval instars

Figure 1 shows the frequency of the head capsule maximum widths of the larvae of the first and second generations (bar diagrams), with the frequency curves expected from normal distribution. The application of this method was based on the results obtained in a previous study (Ramoneda and de Haro, 1988), which was carried out in the laboratory with larvae reared on an artificial diet, by direct and individual observations.

In the first generation the fitting to a normal distribution was significant (Kolmogorov-Smirnov test *p* = 0.05) only for the fifth and sixth instars. In the second generation, this was the case only for the fifth instar. The distributions with the worst fits to normal were those of the initial instars. A possible explanation is that since all the head capsules were measured with the same accuracy (0.5 mm), the sizes of initial instars are more concentrated in lower width classes than are the final ones. To truly apply the normality test to the initial instars we should increase the number of class intervals per instar, that is to say, to measure the head capsules with greater accuracy (10^-4 mm for instance).

Table 2 characterizes the instars of the larvae of the first and the second generations. To test these data, ten aleatory samplings of larvae of *C. suppressalis* were made in several rice fields of the Ebro Delta during the second generation. A com-

Table 2. Frequency distribution of the larval instars of *C. suppressalis* in the first and second generations

<table>
<thead>
<tr>
<th>Instar</th>
<th>First generation</th>
<th>Second generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Main characteristics of the larva of *C. suppressalis* of the first and second generations in the Ebro Delta (1987–88). The ranges indicated by the percentages followed the normal distribution.

<table>
<thead>
<tr>
<th>Instar</th>
<th>Widths of Head Capsule (mm)</th>
<th>Growth Ratio</th>
<th>Head Capsule Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td><strong>First Generation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0.25±0.00</td>
<td>0.25</td>
<td>1.42</td>
</tr>
<tr>
<td>II</td>
<td>0.35±0.03</td>
<td>0.30–0.40</td>
<td>1.35</td>
</tr>
<tr>
<td>III</td>
<td>0.48±0.04</td>
<td>14% 0.40–67%</td>
<td>1.34</td>
</tr>
<tr>
<td>IV</td>
<td>0.64±0.06</td>
<td>33% 0.55–80%</td>
<td>1.41</td>
</tr>
<tr>
<td>V</td>
<td>0.90±0.10</td>
<td>20% 0.75–33%</td>
<td>1.57</td>
</tr>
<tr>
<td>VI</td>
<td>1.41±0.11</td>
<td>67% 1.15–1.70</td>
<td>Brown</td>
</tr>
<tr>
<td><strong>Second Generation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0.25±0.00</td>
<td>0.25</td>
<td>1.41</td>
</tr>
<tr>
<td>II</td>
<td>0.35±0.03</td>
<td>0.30–0.40</td>
<td>1.62</td>
</tr>
<tr>
<td>III</td>
<td>0.57±0.06</td>
<td>45–98% 0.65</td>
<td>1.46</td>
</tr>
<tr>
<td>IV</td>
<td>0.84±0.09</td>
<td>2% 0.65–43%</td>
<td>1.59</td>
</tr>
<tr>
<td>V</td>
<td>1.33±0.11</td>
<td>57% 1.10–1.65</td>
<td>Brown</td>
</tr>
</tbody>
</table>

Comparison of the mean head capsule widths between the fifth instar larvae collected in such sampling and those developed in the experimental plots (ANOVA) showed no difference (SCHEFFE test $p=0.05$). This ensured that the methodology applied in this study for the characterization of the larval development of *C. suppressalis* correctly represented the natural situation in the rice fields of the Ebro Delta.

**The mean duration of each larval instar**

Figure 2 shows the rate of larval development in the two generations of *C. suppressalis*. Both linear regression lines resulted in good correlations. Starting from these lines, the mean time spent in every instar has been calculated for every generation (Table 3). The larvae of the first generation spent about 2.5 more days (on average) in every instar, and about 20 more days for the whole development in relation to the second generation. We looked then for the environmental factors attributable to the differences.

Table 1 shows that the difference in the mean temperatures between the generations was about 3°C. Since GÓMEZ CLEMENTE (1948) indicates that the optimum temperature for the larval development of *C. suppressalis* is about 26°C, in the Ebro Delta the larvae of the second generation must develop under very favorable temperature conditions (about 25°C on average), whereas the larvae of the first generation develop at a lower temperature, which requires more time for completion. TÓR (1971), LOMA (1974)
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Fig. 2. Mean instars of the larvae of *C. suppressalis* of the first and second generation throughout their time of development in the Ebro Delta. The regression lines and the corresponding correlation coefficients are included.

Table 3. Mean time required by the larvae of *C. suppressalis* of the first and second generation in the Ebro Delta to reach full development

<table>
<thead>
<tr>
<th>Developmental period (days)</th>
<th>Generation</th>
<th>Larvae</th>
<th>Pupae</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>48 (6 instars)</td>
<td>8–9</td>
<td>6–7</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>28 (5 instars)</td>
<td>8–9</td>
<td>6–7</td>
</tr>
</tbody>
</table>

and Portour and Bues (1978) pointed out that lower temperatures increased the mean duration of the larval development in *C. suppressalis*.

The fact that the rate of development in the second generation larvae in the Ebro Delta was higher (the rice plant in maturity) than that of the first generation (the rice plant in vegetative stage) showed that environmental temperature must be a key factor affecting the mean duration of the larval development of *C. suppressalis*.

In conclusion, the development of the larvae of *C. suppressalis* in the Ebro Delta differs according to generation. The larvae of the first generation underwent additional molt and about 79% more time was spent in completing their development compared with those of the second generation.

Of the two environmental factors studied (i.e., the air temperature and the physiological state of the rice plant), the temperature seemed to be the most important parameter for the observed differences between the two generations.

Contrary to the effect of the physiological state of the rice plants determined by Hirano (1964) in laboratory conditions, in the Ebro Delta rice fields the air temperature overrode the effect of other factors such as nutrition.

The characterization of the larval development of *C. suppressalis* in the Ebro Delta
has improved the method of forecasting the suitable time periods for the application of pesticides against this pest (Ramoneda and Roig, 1989a).

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REFERENCES


