Maize Resistance to *Chilo partellus* (SWINHOE) (Lepidoptera: Pyralidae): Behaviour of Newly Hatched Larvae and Movement from Oviposition Sites to Feeding Sites

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The effect of resistant and susceptible maize lines on behaviour and establishment of the neonate *Chilo partellus* larva was studied. Larval behaviour was categorized according to plant acceptability. More larvae showed a higher acceptance for the susceptible Inbred A than the resistant Inbred G and ICZ2-CM lines. Larval behaviour suggested that certain conditioning stimuli were central to larval acceptance or rejection of the plant on which eggs were laid. Other stimuli guide larvae to the whorls where feeding usually commences. Analyses of plant surface extracts suggested that different chemical characteristics played a certain role in larval acceptance of these plants. Implications of the possible use of such characteristics as indices in the selection of maize material resistant to *Chilo partellus* are discussed.

INTRODUCTION

Observations on oviposition behaviour of *Chilo partellus* (SWINHOE) moths on young maize plants show that the lower and older leaves are preferred (DABROWSKI and NYANGIRI, 1983). However, the newly hatched larvae initiate feeding in the whorl region where they may later become established (CHATTERJI et al., 1971). Emerging larvae are therefore faced with the task of migrating from the eclosing sites to the feeding sites, a journey which seems to be a critical phase in larval survival and establishment on the plant. This suggests that suitability to neonate larval feeding is not a major determining factor in the choice of oviposition sites.

Any plant characteristic which slows down the larval movement or causes larvae to leave the plant, exposes them to the dangers of increased predation and elemental factors such as heavy rain, excessive wind or heat (SINGH et al., 1974).

Resistance to *C. partellus* in maize may therefore occur simply as a result of failure by the larvae to reach the whorls due to the presence or absence of certain plant characteristics. Such characteristics may be influenced by genotype and are therefore open to manipulation through breeding processes.

This paper reports on a study of the influence of certain maize lines on the behaviour of the neonate *C. partellus* larvae and their movement from the lower leaves to the whorls.
MATERIALS AND METHODS

Four maize lines showing varying levels of resistance (Dabrowski and Nyangiri, 1983) were selected for examination. These were ICZ2-CM (CMT 324) (high resistance), ICZ1-CM (CMT 33), Inbred G (moderate resistance), and Inbred A (susceptible). Observations were made on both greenhouse (potted) and field plants at the whorl stage (6–8 leaves: 3–4 weeks after germination). This is the most preferred stage for oviposition (Dabrowski and Nyangiri, 1983; Singh and Sandhu, 1978).

The C. partellus larvae used were from a laboratory culture which was constantly supplied with moths from the field population to prevent inbreeding and subsequent deviations from normal behaviour. The culture was raised on an artificial diet under ambient temperature and humidity and natural light conditions.

Larval behaviour and movement from sites of release. An egg mass in the black head stage was fixed at about 3 cm from the axil on the lower surface of leaf 2 from the ground. Where this leaf was loosely attached to the stem, the egg-mass was fixed on leaf 3; this prevented the entrapment of larvae between the leaf sheath and the stem. The exact size of each egg-mass was recorded for each plant and the number left unhatched was used to calculate the actual number of larvae released. The infestations were done in the late afternoon and the plants were set aside until ca. 600 hr the following morning when hatching commenced. Larvae for the individual inoculation were placed singly in the middle of the upper surface of the leaf.

Larvae from both the egg-masses and single releases were continuously observed and their behaviour monitored and recorded until they reached the inner parts of the whorl, settled elsewhere or left the plant.

Observations on individual larval behaviour and movement were also made on an artificial plant (constructed with white duplicating paper (Xerox 80 g/m²) and on Inbred A plants with leaf margins removed. These were to verify some of the results from larval behaviour on intact plants. The Inbred A plants were set aside for 24 hr after removal of the leaf margins to allow the wound to heal before use. Based on these observations, larval movement behaviour was classified into different acceptance patterns. There were five replications, each of 10 observations per line and no more than five observations were made on a single plant.

Plant surface characteristics. To correlate larval behaviour with plant surface characteristics, excised leaves were examined under the stereo-microscope for trichome type and density counts. Aerial portions of the plants were intermittently agitated in 250 ml of distilled water (per plant) for 4 hr. The aqueous wash was filtered to remove soil and other particles and absorption spectra measured in an automatic recording u-v spectrophotometer. This was to determine possible differences in leaf surface chemical characteristics.

RESULTS

Larval behaviour and movement

Larval behaviour and movement from egg masses or individual releases were similar. On emergence or release, the larva moved to the leaf margin and crawled toward the axil, or toward the leaf tip. A larva crawling toward the leaf tip, on arrival
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spun a thread and swung from it or turned around and crawled toward the axil again. The larva appeared to be able to control the length of the thread and sometimes used this mechanism to repeatedly move on and off the plant. On the leaf surface, the larva was observed to make repeated head raising movements with high antennal activity. This behaviour was apparently in search of an acceptance or rejection cue, and was more pronounced on the artificial plant. At the leaf margin, the larva was frequently observed to hold to the plant with its mandibles but it was not clear whether this was a biting activity or an effort to avoid falling. Microscopic examination of such surfaces, however, revealed no bite marks. On field plants, a larva swinging from the lower leaves or on long threads invariably landed on the ground, on nearby weeds or on other maize plants. This way, dispersal or migration from unacceptable plants may be effected.

On arrival at the leaf axil, the larva immediately started the upward climb along the culm to the inner whorl leaves. However, a larva that has been roaming the leaf surface for over 30 min tended to rest for short periods before commencing the upward climb. Generally, more larvae stayed on the plant exterior of ICZ2-CM and Inbred G for longer periods than on ICZ1-CM and Inbred A. A few larvae on ICZ2-CM and Inbred G were observed descending the plant (i.e. on leaves lower than they were originally placed or crawling down the culm). This behaviour was rare on Inbred A. Percent larval recovery from the plants 24 hr after eclosion was higher on Inbred A (67) than on the other lines (which ranged from 40–54) (Fig. 1).

Larval movement and behaviour on the leaf surface was categorized according to its acceptance of the plant:

(i) High acceptance: the larva moved in the direction of the axil and reached it within 10 min of release.

(ii) Low acceptance: the larva roamed the leaf surface for 11–30 min before reaching the axil.

(iii) Tentative acceptance: the larva roamed the leaf surface for more than 30 min. It sometimes repeatedly spun on and off the leaf before reaching the axils or spinning off the plant entirely.

(iv) Non-acceptance: the larva spun off the plant within 15 min of release.

*Plant characteristics*

(i) **Trichomes**

Trichome density appeared very sparse on the surface of the lower (older) leaves. There were no significant differences among the different maize lines in this regard. Higher (younger) leaves, however, were more hirsute. There was a trichome density gradient along the leaf margin which increased from the base toward the tip (Fig. 2). This was similar in all the maize lines.

(ii) **Pigmentation**

Two-to-three-week old plants developed a purple pigmentation which varied in intensity among the lines: ICZ2-CM > Inbred G > ICZ1-CM > Inbred A. The pigmentation disappeared from the leaf surfaces by the fourth week leaving only traces at the leaf margins and the lower culm. A Spearman’s correlation between rank order of pigmentation intensity and level of resistance (larval acceptance) gave a perfect fit ($R_s=1$, $t\to\infty$).
(iii) Absorption spectra of plant surface extracts

Figure 3 shows the U-V absorption patterns of aqueous washes from the maize lines. Inbred A and ICZ1-CM absorbed at 195 nm with the extract from ICZ1-CM showing a higher optical density. Inbred G absorbed at 192 nm with another (weaker) absorption centred around 225 nm suggesting the presence of more than one absorbing characteristic in the extract. ICZ2-CM absorbed at 210 nm after a long shoulder.

Table 1 gives a summary of chi-square analysis of frequency distribution of neonate C. partellus larval acceptance patterns for the maize lines and the artificial plant. The larvae appeared to follow a set pattern (higher acceptance) on Inbred A leaves. The chi-square value of 66.8 was highly significant. The acceptance pattern on the other lines appeared random. Chi-square values ranged from 0.72 on the artificial plant to 6.24 on Inbred A plants with leaf margins removed.

Analysis of variance of larval behaviour and acceptance levels (Table 2) also showed Inbred A plants to be the most acceptable. More larvae (67.3%) showed high acceptance for Inbred A compared with Inbred G (25%) and ICZ2-CM (29.5%). By contrast, fewer larvae (9.2%) rejected Inbred A surfaces compared with Inbred G (35.5%) and ICZ2-CM (33.9%). ICZ1-CM was intermediate in these comparisons. Larvae on Inbred A plants with leaf margins removed showed a significant decrease
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Fig. 3. Ultraviolet absorption spectra of aqueous washes from the maize genotypes (solid line: ICZ-CM, broken line: Inbred A, dotted line: ICZ2-CM, broken line with dots: Inbred G).

**Table 1.** Chi-square values of frequency distribution of neonate *C. partellus* larval acceptance patterns on the maize lines

<table>
<thead>
<tr>
<th>Maize line</th>
<th>$\chi^2$-value</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbred A</td>
<td>66.80</td>
<td>0.001</td>
</tr>
<tr>
<td>Inbred A-X$^a$</td>
<td>6.24</td>
<td>0.10</td>
</tr>
<tr>
<td>Inbred G</td>
<td>1.04</td>
<td>0.50</td>
</tr>
<tr>
<td>ICZ1-CM</td>
<td>3.44</td>
<td>0.25</td>
</tr>
<tr>
<td>ICZ2-CM</td>
<td>3.60</td>
<td>0.25</td>
</tr>
<tr>
<td>Artificial plant</td>
<td>0.72</td>
<td>0.90</td>
</tr>
</tbody>
</table>

$^a$ Inbred A-X=Inbred A plants with leaf margins removed.

in acceptance levels. On such leaves, only 22% of the larvae exhibited high acceptance compared with 67.3% on intact leaves. However, there was no difference in the number of larvae that rejected the surfaces outright. Most larvae on this surface followed the low or tentative acceptance (confused behaviour) patterns. Larval behaviour on the artificial plant was random.

The major differences in larval behaviour appeared to occur on the leaf surfaces. Once the larvae arrived at the axils they all pursued a similar route and pattern in climbing towards the whorls. On ICZ1-CM, however, owing to the apparent higher
Table 2. Mean percentage of *C. partellus* larvae following different patterns of behaviour on the different maize lines

<table>
<thead>
<tr>
<th>Maize line</th>
<th>Larval acceptance behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High acceptance</td>
</tr>
<tr>
<td>Whole plants</td>
<td></td>
</tr>
<tr>
<td>Inbred A</td>
<td>67.34 (55.15) a</td>
</tr>
<tr>
<td>Inbred G</td>
<td>25.00 (29.95) b*</td>
</tr>
<tr>
<td>ICZ1-CM</td>
<td>39.60 (39.00) ab</td>
</tr>
<tr>
<td>ICZ2-CM</td>
<td>29.50 (32.91) b</td>
</tr>
<tr>
<td>Artificial/modified plants</td>
<td></td>
</tr>
<tr>
<td>Inbred A- ( \approx ) b</td>
<td>22.00 (28.03) b</td>
</tr>
<tr>
<td>Artificial plant</td>
<td>29.30 (32.79) b</td>
</tr>
<tr>
<td>Standard error</td>
<td>4.55</td>
</tr>
</tbody>
</table>

* Figures in parentheses are means of angular transformations.
* Inbred A- \( \approx \) Inbred A plants with leaf margins removed.
* Means followed by the same letters are not significantly different at the 1% level of probability.

Table 3. Mean percentage of larvae reaching the leaf axils and settling in whorls (individual larval releases)

<table>
<thead>
<tr>
<th>Maize line</th>
<th>Percentage of larvae reaching axils ((n=50))</th>
<th>Percentage larvae(^a) settling in whorls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbred A</td>
<td>74.17 (59.45) (^b) a*</td>
<td>89.08 (70.75)</td>
</tr>
<tr>
<td>Inbred G</td>
<td>56.27 (48.64) bc</td>
<td>92.22 (73.78)</td>
</tr>
<tr>
<td>ICZ1-CM</td>
<td>70.50 (57.09) ab</td>
<td>70.97 (57.41)</td>
</tr>
<tr>
<td>ICZ2-CM</td>
<td>50.00 (45.00) c</td>
<td>84.10 (66.55)</td>
</tr>
<tr>
<td>Standard error</td>
<td>3.35</td>
<td>6.02</td>
</tr>
<tr>
<td>Level of significance</td>
<td>.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Expressed as number reaching axils.
* Figure in parentheses are means angular transformation.
* Means followed by the same letter(s) are not significantly different at the expressed level of probability.

wax deposits on the culm, there was a tendency for larvae to slip and swing for brief periods. Some larvae were blown off in mild windy conditions and did not reach the whorls. Figure 4 shows the relationship between larval climbing times (axil of leaf 2 to whorl) and plant height. Climbing rate was the fastest on ICZ2-CM \((b=5.58)\) and the slowest on ICZ1-CM \((b=1.71)\). Inbreds A and G had intermediate rates. Eighty-four, 89 and 92\% of larvae arriving at the axils of ICZ2-CM, Inbred A and Inbred G respectively settled in the whorls successfully compared with 71\% in ICZ1-CM. A similar pattern of larval successes was observed in the larval releases from egg batches (Fig. 1 and Table 3). There was a positive correlation in larval establishment (larval recovery) \((R=0.92)\) between releases from egg batches and individual larval releases on the maize lines.
DISCUSSION

The neonate *C. partellus* larval behaviour on the maize plants suggested a succession of reactions in its movement from the eclosion sites to the initial feeding sites. This consisted of (i) acceptance of plant; a reaction which occurred on the leaf surface, (ii) orientation on the leaf surface and (iii) orientation on the culm. The presence of favourable or adverse stimuli on the leaf surface seemed to be the major factor at play in the larval journey. These factors may be altogether absent or concurrent and may interact to elicit a particular behaviour pattern from the insect.

The results showed a direct relationship between the intensity of the purple pigmentation in the plants and the level of resistance. Spectroscopic analyses also showed extracts from the more susceptible lines to absorb at a particular wavelength which was different from the absorbance pattern of the more resistant lines. These observations indicate further the presence of different chemical characteristics on the leaf surfaces of the different maize lines. Such characteristics are likely to condition the plants for acceptance or rejection by the larvae as discussed by Dethier (1982), and hence require further investigation.

On arrival at the leaf margin, trichome density gradient appeared to play a role in guiding the larva to reach the axil. This was made clear by observations on larval behaviour on Inbred A plants with leaf margins removed. The larvae stayed on such leaf surface and moved up and down the margins for longer periods than on plants with intact leaves. The observations suggest the presence of adequate favourable stimuli (arrestant) on the demargined leaf surface but inadequate guiding stimuli to reach the axils. Larval behaviour on the artificial plant was random. This surface lacked both the chemical and physical characteristics to guide the larva.

An ability to recognize and assess various biochemical and/or biophysical characteristics on the plant surface is probably of great value to the larva. This would facilitate the recognition of acceptable and non-acceptable plants and guide larvae to preferred feeding sites. Schoonhoven (1973) discussed the role of chemoreceptors on the antennae and maxillary palpi of lepidopteran larvae in the discernment of various volatiles from the plant surface and host plant recognition.

Roome (1980) observed that the neonate *C. partellus* larva does not feed prior to dispersal. This implies that gustation is not an influential factor in the neonate larval acceptance of an oviposited plant. However a touch on the plant surface with the maxillary palpi or sensilla styloconica could help assess the complexity of chemical stimuli on the surface. The summation of such stimuli would aid in decision-making for the larva. Robinson et al. (1978) demonstrated greater dispersal of *Ostrinia nubilalis* (Hubner) neonate larvae from resistant maize hybrids than from the susceptible lines. They related this observation to the high content of DIMBOA in the resistant plants.

The stimuli guiding larval orientation on the leaf surface and the culm appear to be different. Surface characteristics seemed to be responsible for orientation on the leaf surface, while environmental characteristics seemed to be major stimuli in the orientation on the culm. Bernays et al. (1983) suggested positive phototaxis as the primary factor on the culm; their studies, however, were carried out under artificial conditions. Negative geotaxis also appeared to play a role in the present studies. Some larvae, emerging from egg batches in the dark were recovered from the whorls...
when plants were dissected, and there was no relation between the incidence of the early morning sun and the preferred side of the culm in the upward climb. On arrival at the spiral roll of leaves in the whorl, thigmotactic stimuli probably cause the larvae to settle and feed.

Despite the distance between the oviposition and initial larval feeding sites, the neonate C. partellus larva appears well adapted for a successful migration between these sites. Results from this experiment indicate a set of stimuli and responses which guide the larva to discern between acceptable and unacceptable plants. Another set of stimuli guide the larva in its movement towards the whorls of acceptable plants where feeding usually commences.

The findings also indicate real differences among the maize lines according to the level of successful larval establishment. The resistance reported in ICZ2-CM and Inbred G (DABROWSKI and NYANGIRI, 1983) was probably occasioned by the presence of repellent factors on the plant surface which led to poor larval establishment on such plants. Further investigations are necessary to confirm and characterize these factors. Information on the heritability of these characteristics could lead to a simple index for the selection of maize lines which are resistant to Chilo partellus.

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