NOTE

Natural Mortality among Indianmeal Moth Larvae with Resistance to Bacillus thuringiensis

The selection of Indianmeal moth (Plodia interpunctella, IMM) strains that are resistant to Bacillus thuringiensis has provided opportunities for studying insecticidal crystal protein (ICP) mode of action as well as resistance mechanisms and toxin specificity (Van Rie et al., 1991; McGaughey and J ohnson, 1992; McGaughey and J ohnson, 1994). However, the acquisition of resistance may not come without cost to the insect. A number of studies have shown changes in fitness resulting from selection with insecticidal agents. For example, Longstaff (1991) demonstrated higher fecundity and reduction in the developmental period of rice weevil, Sitophilus oryzae, when exposed to sublethal doses of the chemical insecticide pyrimiphos-methyl. In the IMM, resistance to a granulosis virus resulted in reduced egg viability, larvae that took longer to develop, and an increase in pupal weight (Boots and Begon, 1993). In the diamondback moth, Plutella xylostella, a strain from Hawaii resistant to B. thuringiensis had lower survival from egg to adult, lower fecundity, and lower percentage egg hatch when compared to a susceptible strain (Groeters et al., 1994). Moreover, a pronounced difference in fitness of Heliothis virescens was found under conditions in which there was a choice between feeding on B. thuringiensis-treated or untreated food (Gould and Anderson, 1991). The relationship between ICP specificity, insect resistance, and low-level ICP susceptibility is fundamental to planning management strategies for insect control using B. thuringiensis. In this study, we assessed the vigor and fitness of Indianmeal moth larvae selected for resistance to B. thuringiensis. Five separate colonies of B. thuringiensis-resistant Indianmeal moths were used (McGaughey and J ohnson, 1992). They were selected from the same parent colony (RC688/unt) for resistance to five different spore-crystal preparations of B. thuringiensis: RC688/112R, subsp. aizawai HD-112; RC688/133R, subsp. aizawai HD-133; RC688/198R, subsp. entomocidus HD-198; RC688/DipR, subsp. kurstaki HD-1; and RC688/DipR + 133R, a 50:50 mixture of Dipel and HD-133. Selection procedures involved rearing the insects on a diet in which the appropriate B. thuringiensis isolate had been incorporated using the methods reported in earlier studies (McGaughey and J ohnson, 1992). Resistance levels on this cracked wheat bioassay system were 21-fold for RC688/198R and RC688/DipR + 133R, 29-fold for RC688/112R, 62-fold for RC688/133R, and 140-fold for RC688/DipR. The resistant IMM strains were maintained under continuous selection pressure during this study.

The known cry gene composition for each strain of B. thuringiensis used in this study was as follows: HD-1 (subsp. kurstaki) contained cryIA(a,b,c), cry1A, and cry1B; HD-112 (subsp. aizawai) contained cry1A(a,b), cry1C, cry1D, cry1G, and cry1I; HD-133 (subsp. aizawai) contained cry1A(a,b), cry1C, and cry1D; and HD198 (subsp. entomocidus) contained cry1A(a,b), cry1C, and cry1D (Höfte and Whiteley, 1989; McGaughey and J ohnson, 1994). However, all of the genes may not be fully transcribed in each strain, as some are not efficiently expressed or may be cryptic (i.e., cry1IB; Aronson, 1993).

We routinely use two different bioassay methods to measure the toxicity of various ICP preparations toward the IMM strains. The apple slice bioassay system employs individual larvae in compartmented trays, each treated and contained separately from the others (J ohnson et al., 1991). The apple cubes are dosed with 1:2 dilutions of aqueous suspensions of spore-crystal formulation in 1% yeast extract and are replaced with undosed cracked wheat diet once the apple cube is totally consumed. The cracked wheat bioassay system involves the treatment of a series of ten 30-g samples of cracked wheat rearing medium in jars with 1:2 dilutions of aqueous suspensions of spore-crystal formulation (McGaughey and J ohnson, 1987).

During regular toxicity trials using each of the B. thuringiensis-resistant IMM strains, we observed an abnormally high natural mortality among the untreated control larvae. A more thorough study revealed an average control mortality of 11.3 and 11.6% for the untreated susceptible colony, as measured by either the apple slice or the cracked wheat bioassay methods, respectively (Table 1). The means of the control mortalities by the two different bioassay methods were not statistically significantly different (ANOVA, P = 0.903). Consequently, subsequent data collected for the resistant IMM strains by each bioassay method were analyzed separately and with pooled data.
Average pooled control mortalities for the resistant colonies ranged from 23.4 to 29.1% (Table 1). When analyzed as a group compared with susceptible IMM measured by both bioassays, the difference in the mean control mortality between groups was significantly different (ANOVA, P < 0.0001). When analyzed individually by pairwise multiple comparison procedures (Dunn’s method), all comparisons of control mortality between RC688/unt resistant IMM strains were significantly different except for the comparison between RC688/unt and RC688/112R (P < 0.05). There were no significant differences between control mortalities of the BT-resistant IMM strains (data not shown).

Nearly all natural mortality among larvae employed during the apple slice bioassay occurred in the larval stage, since those larvae that underwent pupation exhibited nearly 100% adult emergence by 6–10 days following pupation. This was true for the BT-resistant IMM strains as well as for susceptible IMM larvae. Consequently, there was little significant difference between bioassay methods, which could account for the control mortality variation observed.

Larval development times between stadia for resistant Indianmeal moths were equivalent to comparable stages in the susceptible strain. Pupation of resistant larvae occurred within ±1.5 to 2 days of the susceptible strain (approximately 26 days after egg laying). Emerging adults were normal in appearance and indistinguishable from the susceptible strain. Average weight of late second to early third instars ranged from an average of 68 to 78 µg (based upon a sampling size of 100 larvae) and did not vary significantly between susceptible (average larval weight 0.72 ± 0.08 mg) and resistant (average larval weight 0.69 ± 0.04 mg) strains.

### Table 1

<table>
<thead>
<tr>
<th>IMM strain</th>
<th>Bioassay</th>
<th>No. larvae</th>
<th>% Mortality mean</th>
<th>Confidence limits (25–75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC688/Unt</td>
<td>Cracked wheat</td>
<td>750</td>
<td>11.3 (a)</td>
<td>6.4–14.8</td>
</tr>
<tr>
<td></td>
<td>Apple slice</td>
<td>832</td>
<td>11.6 (a)</td>
<td>6.4–14.8</td>
</tr>
<tr>
<td>RC688/112R</td>
<td>Pooled</td>
<td>1690</td>
<td>23.4 (a, b)</td>
<td>14.0–27.8</td>
</tr>
<tr>
<td>RC688/133R</td>
<td>Pooled</td>
<td>1968</td>
<td>28.1 (b)</td>
<td>20.2–32.6</td>
</tr>
<tr>
<td>RC688/DipR</td>
<td>Pooled</td>
<td>1668</td>
<td>29.1 (b)</td>
<td>22.7–35.3</td>
</tr>
<tr>
<td>RC688/DipR + 133R</td>
<td>Pooled</td>
<td>1840</td>
<td>26.9 (b)</td>
<td>22.6–31.3</td>
</tr>
</tbody>
</table>

RC688/Unt Cracked wheat 750 11.3 (a) 6.4–14.8
RC688/112R Pooled 1690 23.4 (a, b) 14.0–27.8
RC688/133R Pooled 1968 28.1 (b) 20.2–32.6
RC688/DipR Pooled 1668 29.1 (b) 22.7–35.3
RC688/DipR + 133R Pooled 1840 26.9 (b) 22.6–31.3

Average larval weight 0.72 ± 0.08 mg and did not vary significantly between susceptible and resistant larvae. This was true for the BT-resistant IMM strains as well as for susceptible IMM larvae. Consequently, there was little significant difference between bioassay methods, which could account for the control mortality variation observed.

Larval development times between stadia for resistant Indianmeal moths were equivalent to comparable stages in the susceptible strain. Pupation of resistant larvae occurred within ±1.5 to 2 days of the susceptible strain (approximately 26 days after egg laying). Emerging adults were normal in appearance and indistinguishable from the susceptible strain. Average weight of late second to early third instars ranged from an average of 68 to 78 µg (based upon a sampling size of 100 larvae) and did not vary significantly between susceptible (average larval weight 0.72 ± 0.08 mg) and resistant (average larval weight 0.69 ± 0.04 mg) strains.

### Table 2

Decrease in Larval Response to Crystal Toxins of B. thuringiensis Due to Increasing Larval Developmental Stage

<table>
<thead>
<tr>
<th>IMM colony</th>
<th>Instar</th>
<th>LD50</th>
<th>95% FL</th>
<th>Slope Ratio</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC688/Unt</td>
<td>2</td>
<td>224</td>
<td>1.94</td>
<td>1.56–2.42</td>
<td>2.58</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>240</td>
<td>1.99</td>
<td>1.52–2.58</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>240</td>
<td>2.92</td>
<td>1.95–4.30</td>
<td>2.28</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>240</td>
<td>10.45</td>
<td>7.53–16.20</td>
<td>1.38</td>
</tr>
<tr>
<td>RC688/112R</td>
<td>2</td>
<td>240</td>
<td>17.98</td>
<td>10.15–34.79</td>
<td>2.29</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>240</td>
<td>26.73</td>
<td>20.46–37.39</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>240</td>
<td>35.16</td>
<td>24.95–57.80</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>240</td>
<td>54.03</td>
<td>41.17–84.01</td>
<td>2.58</td>
</tr>
<tr>
<td>RC688/133R</td>
<td>2</td>
<td>256</td>
<td>33.21</td>
<td>24.79–46.19</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>256</td>
<td>76.81</td>
<td>62.44–110.05</td>
<td>3.72</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>256</td>
<td>87.18</td>
<td>60.32–171.62</td>
<td>1.94</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>256</td>
<td>179.9</td>
<td>99.32–1358.19</td>
<td>1.97</td>
</tr>
</tbody>
</table>

NOTE

- IMM larvae of the appropriate stadia were collected from colonies maintained under selection conditions. They were placed on apple slices containing a graded series of spore-crystal mixture.
- Micrograms dry weight/larva.
- Dipel response ratio of LD50 of second instar vs LD50 of each instar in question.
- Resistance ratio: LD50 of resistant larva (any instar) vs LD50 of susceptible larva (same instar).

McGaughey (1978) reported that the sensitivity of susceptible IMM larvae to B. thuringiensis crystal toxins diminished with an increase in larval age. However, the effect of increasing larval age on resistant IMM larvae to elevated levels of B. thuringiensis toxins has not been determined. In order to answer this question, we bioassayed resistant IMM larvae of each stage against Dipel (B. thuringiensis subsp. kurstaki HD-1, Abbott Laboratories, Chicago, IL). The sensitivity of three RC688 colonies (unselected, 112R, and 133R) to Dipel decreased as larval size increased (Table 2). However, resistance ratios according to instar were relatively constant when compared to the same instar of susceptible larvae. Also, the physical size of B. thuringiensis-resistant late fifth instars (either RC688/112R or RC688/133R) was similar to sensitive larvae at the same stage (2.65 ± 0.12 mg vs 2.62 ± 0.11 mg, respectively). Thus, the relative difference in sensitivity to Dipel among the susceptible and resistant IMM colonies was similar at each developmental stage; the level of sensitivity was not affected by resistance to B. thuringiensis, but larval sensitivity to Dipel did decrease with increasing instar.

It is critical that we understand the physiological pressures imposed upon the insect after selection for resistance, in order to assess the competitiveness and durability of the resulting strain. The consequences of acquired resistance to the endotoxin of B. thuringiensis among various insects range from an impact upon...
fitness (Tabashnik, 1994) to physiological effects such as receptor site alterations (Van Rie et al., 1990; Ferré et al., 1991). Selection pressure generated from large fields of genetically transformed plants may result in production of insecticide-resistant insects, which will then compete with dwindling numbers of the susceptible population. In the laboratory, B. thuringiensis-resistance has been shown to be stable (McGaughey and Beeman, 1988). We do not know the extent to which reduced fitness among B. thuringiensis-resistant insects will compensate for increased survival among plantings protected with transformed B. thuringiensis cry genes. Thus, more extensive information concerning fitness costs due to resistance acquisition is needed in order to predict with any certainty the fate of the resistant insect in the field.

Key Words: Indianmeal moth; Plodia interpunctella; Bacillus thuringiensis; larvae; resistance; δ-endotoxin; mortality.

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References


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