Decreased susceptibilities of four field populations of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), to acetamiprid

Kodwo D. Ninsin, Jianchu Mo and Tadashi Miyata*

Laboratory of Applied Entomology, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464–8601, Japan

(Received 11 April 2000; Accepted 12 May 2000)

**Abstract**

The efficacy of acetamiprid, a neonicotinoid insecticide, against four field populations of the diamondback moth, *Plutella xylostella* (L.), from Aichi, Hyogo and Kagoshima Prefectures in Japan was determined using the leaf-dipping method. The data obtained indicated that, compared to the reference Osaka susceptible strain (OSS), the four field populations were tolerant to the insecticidal effect of acetamiprid, displaying LC$_{50}$ resistance ratio (RR) of 4.5–9.0. Maintaining the population from Aichi Prefecture in the laboratory without exposure to acetamiprid resulted in a decline of the LC$_{50}$ RR from 9.0 to 1.6 over seven generations, suggesting that acetamiprid-resistance is not stable.

**Key words**: *Plutella xylostella*, diamondback moth, acetamiprid, neonicotinoid, resistance

---

**INTRODUCTION**

The diamondback moth (DBM), *Plutella xylostella* (L.), has become, in the past 40 years, the most destructive pest of crucifers (Shelton et al., 1997). The larvae attack and severely damage 42.2 million tons of cabbage, cauliflower and broccoli grown worldwide, requiring an estimated annual cost of US$1 billion to manage it (Talekar, 1992).

Despite the large investment in the management of DBM, a sustainable strategy remains elusive because of some limiting factors such as the development of insecticide resistance in the DBM (Miyata et al., 1986). Insecticide resistance in the DBM has been cited as one of the factors that have contributed to the notorious pest status of the insect in Japan (Yamada, 1977), in addition to greatly limiting the use of some otherwise useful insecticides (Sinchaisri et al., 1995; Cheng and Kao, 1999). Resistance in the DBM is still a major problem because consumers continue to attach high cosmetic value to cruciferous vegetables, thereby compelling farmers to sometimes rely on the exclusive use of insecticides in the absence of other effective control methods.

Acetamiprid is a neonicotinoid insecticide registered for use in Japan in 1995, and formulated for both soil and foliar applications (Matsuda and Takahashi, 1996). It possesses characteristic properties different from other neonicotinoid insecticides, notably a comparatively high activity against lepidopterous pests (Takahashi et al., 1998b). A single application of acetamiprid at transplanting, by planting hole application, provides lasting protection to cabbage plants from DBM damage (Takahashi et al., 1999). The compound is also effective against resistant insects (Matsuda and Takahashi, 1996), and has become an integral component of many DBM management programs in Japan.

Even though the desired control of DBM is presently achieved with acetamiprid in Japan, the history of resistance development in this insect and the importance of detecting resistance at an early stage of development prompted this study. We evaluated the efficacy of acetamiprid against four populations of DBM from three prefectures in Japan where the insecticide is used at different levels. When the results indicated decreased susceptibilities to acetamiprid in the field populations, we maintained and observed susceptibility changes to the insecticide in one of the populations, Toyohashi.

**MATERIALS AND METHODS**

**Insects and insecticide.** The Osaka susceptible strain (OSS) (reference laboratory strain) and four
field populations [Inami (INA), Kagoshima (KAG), Kobe (KOB) and Toyohashi (TYH)] were used for this study (Table 1). The rearing condition and method used to culture the DBM was as described by Noppon et al. (1983), but with some modifications. The DBM were reared at 25±1°C and 50% RH, under 16L:8D photoperiod. Moths were fed on 5% honey solution, and larvae on 2–3 day-old radish (Raphanus sativus var. Osaka 40 nichi) seedlings.

Commercial formulation of acetamiprid (Mospilan® 200 g·kg⁻¹ SP) received from Nippon Soda Company Ltd. (Tokyo, Japan) was used for this study. Acetamiprid concentrations were prepared with distilled water containing 200 µL⁻¹ Linoh® spreading agent (Nihon Noyaku Co. Ltd.).

Bioassay

Susceptibilities of field populations. The method used was a modification to the leaf-dipping technique described by Fahmy et al. (1991). Cabbage, Brassica oleracea var. capitata subvar. Chuseikanran, leaves measuring 5 cm×5 cm were dipped for 10 s in acetamiprid solutions. Control test cabbage leaves were dipped in distilled water containing only the spreading agent. The treated leaves were left to air-dry at 25°C. Each leaf was put into a 200 ml plastic cup, padded with a moistened 70 mm Advantec® filter paper (Toyo Roshi Kaisha Limited). Ten 12 to 24-h-old third stadium larvae were introduced into each cup. Four replicates for each treatment of insecticide concentration and control were made. Mortality was recorded at 72 h after treatment (results of preliminary experiments showed that 72 h after treatment is the end point for the bioassay). Larvae that did not respond to pencil tip prodding were considered dead. The data obtained was analyzed by probit analysis (Finney, 1971), with a personal computer, EPSON PC-486DF.

Stability test. Susceptibility of TYH was determined at each generation and compared to OSS. The bioassay method used is as described above. Concentrations of acetamiprid used at each generation were based on results of the bioassay for the preceding generation. A minimum of six concentrations was prepared, and four replicates for each concentration and control made. The data obtained after 72 h was analyzed by probit analysis as mentioned above.

RESULTS AND DISCUSSION

Determination of susceptibilities of INA, KAG, KOB and TYH, compared to the reference OSS indicated decreased susceptibilities in all the field populations of DBM, although susceptibilities varied from field to field (Table 2). The highest LC50 and LC95 values and their corresponding resistance ratio (RR) were observed in the TYH population. The observed levels of tolerances to acetamiprid, the first to be documented in DBM populations, could be attributed to the gradual development of acetamiprid-resistance in the field populations of DBM. Since 1996, most growers from the DBM collection areas in Hyogo Prefecture have been using the granular formulation of acetamiprid. The insecticide is used twice a year at transplanting, once per growing season, and this protects cabbage plants against DBM damage through maturity. In the Toyohashi area where the most tolerant DBM were collected, some growers also rely on the granular formulation of acetamiprid for DBM control. The lasting protection given by acetamiprid in granular formulation is by virtue of its
Table 2. Responses of field populations of DBM to acetamiprid at 72 h after treatment

<table>
<thead>
<tr>
<th>DBM Gen.</th>
<th>LC&lt;sub&gt;30&lt;/sub&gt; (mg L&lt;sup&gt;-1&lt;/sup&gt;) (95% CI)</th>
<th>LC&lt;sub&gt;95&lt;/sub&gt; (mg L&lt;sup&gt;-1&lt;/sup&gt;) (95% CI)</th>
<th>Slope (± SE)</th>
<th>LC&lt;sub&gt;30&lt;/sub&gt; (mg L&lt;sup&gt;-1&lt;/sup&gt;) (95% CI)</th>
<th>LC&lt;sub&gt;95&lt;/sub&gt; (mg L&lt;sup&gt;-1&lt;/sup&gt;) (95% CI)</th>
<th>Slope (± SE)</th>
<th>RR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>LC&lt;sub&gt;30&lt;/sub&gt;</th>
<th>LC&lt;sub&gt;95&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSS</td>
<td>46.4 (42.1–51.6)</td>
<td>99.1 (78.9–114)</td>
<td>4.99 (0.78)</td>
<td>35.1 (28.7–45.3)</td>
<td>137 (83.8–177)</td>
<td>2.78 (0.39)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HYG</td>
<td>251 (213–297)</td>
<td>809 (628–1,160)</td>
<td>3.24 (0.55)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.4</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>KAG</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>—</td>
<td>185 (150–236)</td>
<td>762 (475–1,000)</td>
<td>2.67 (0.66)</td>
<td>5.3</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>KOB</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>—</td>
<td>157 (122–223)</td>
<td>1,210 (681–1,720)</td>
<td>1.85 (0.27)</td>
<td>4.5</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>TYH</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>—</td>
<td>315 (251–400)</td>
<td>2,020 (1,070–2,920)</td>
<td>2.04 (0.45)</td>
<td>9.0</td>
<td>14.8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Generation at which bioassay was conducted after field collection, in order to obtain adequate number of larvae per population.
<sup>b</sup> Resistance ratio (RR) = LC of field population/LC of OSS strain.

controlled release formulation (Takahashi et al., 1998a, 1999), which allows the toxicity of the insecticide to be present in cabbage plants for an extended time. Under such conditions, all larval stages of the DBM will receive a prolonged exposure to acetamiprid thus generating a higher selection for resistance (Taylor and Georgiou, 1982) than will foliar spray (Prabhaker et al., 1997).

Alternatively, the tolerance to acetamiprid observed in the field populations could be the result of cross-resistance, the effect of previously used insecticides. This may be especially true in the case of the KAG population, because in Nishiwaki acetamiprid is used as a minor insecticide and applied as foliar spray. Wen and Scott (1997) observed >4-fold cross resistance to imidacloprid in two strains of the German cockroach (Blatella germanica L.) resistant to permethrin and propoxur, and two strains of housefly (Musca domestica L.), one resistant to abamectin and the other a multi-resistant strain having high levels of resistance to pyrethroids.

Even though there is no previously documented case of DBM resistance to acetamiprid, Prabhaker et al. (1997) reported 3.5 to 15-fold tolerance to imidacloprid, a neonicotinoid insecticide, in field populations of the silverleaf whitefly collected from the Imperial Valley, California. This report suggested that the neonicotinoid insecticides are vulnerable to resistance development, and our results confirm this.

The slope values observed for KOB and TYH suggest that the populations are heterogeneous in their response to acetamiprid. The heterogeneities suggest that KOB- and TYH-populations have the potential to develop higher levels of resistance to acetamiprid if they are continuously exposed to the compound, as evidenced by their LC<sub>95</sub> values. According to Hoskins and Gordon (1956), regression lines become shallower during selection, and steeper as resistance genotypes increase in the population.

When TYH was maintained in the laboratory with no exposure to acetamiprid, the population became more susceptible to the insecticide (Table 3). The resistance ratios of both LC values declined over seven generations reflecting an increase in the susceptibilities of TYH to acetamiprid. The increase in the susceptibility of this population is in accordance with the observation that insecticide resistance in insects, in either the field or laboratory, does not persist in the absence of the insecticide pressure (Brown, 1958). A decrease in resistance levels following several generations of laboratory rearing in the absence of the insecticide pressure is usually expected (Georgiou, 1963). This is because the mechanisms of resistance are not essential to the insect in the absence of insecticides, and so the proportion of resistant individuals usually declines progressively (Georgiou, 1971). The rate at which susceptibility has increased in the TYH-nonselected strain could be attributed to few resistant individuals in the population and their inability to compete effectively with the susceptible ones in terms of reproductive potential, longevity, and other biotic factors (Georgiou, 1963). The RR values for the TYH-population, from F<sub>5</sub>–F<sub>8</sub>, suggest that the observed acetamiprid-tolerance in the TYH field population is not stable.

The results of the bioassay conducted on INA, KAG, KOB and TYH at F<sub>5</sub>, F<sub>3</sub>, F<sub>2</sub> and F<sub>1</sub> respectively, suggested that the LC values observed could have been higher if the susceptibility levels had been determined just after field collection.
### Table 3. Responses of OSS and TYH population of DBM to acetamiprid at 72 h after treatment

<table>
<thead>
<tr>
<th>Gen.</th>
<th>LC$_{50}$ (mg l$^{-1}$) (95% CI)</th>
<th>LC$_{90}$ (mg l$^{-1}$) (95% CI)</th>
<th>Slope (±SE)</th>
<th>LC$_{50}$ (mg l$^{-1}$) (95% CI)</th>
<th>LC$_{90}$ (mg l$^{-1}$) (95% CI)</th>
<th>Slope (±SE)</th>
<th>LC$_{50}$</th>
<th>LC$_{90}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_1$</td>
<td>35.1 (28.7–45.3)</td>
<td>137 (83.8–177)</td>
<td>2.78 (0.39)</td>
<td>315 (251–400)</td>
<td>2,020 (1,070–2,920)</td>
<td>2.04 (0.45)</td>
<td>9.0</td>
<td>14.8</td>
</tr>
<tr>
<td>F$_2$</td>
<td>72.9 (63.8–86.2)</td>
<td>153 (111–181)</td>
<td>5.07 (0.36)</td>
<td>167 (135–222)</td>
<td>798 (484–1,060)</td>
<td>2.43 (0.43)</td>
<td>2.3</td>
<td>5.2</td>
</tr>
<tr>
<td>F$_3$</td>
<td>67.5 (54.0–82.1)</td>
<td>335 (173–466)</td>
<td>2.37 (0.38)</td>
<td>224 (191–290)</td>
<td>579 (376–692)</td>
<td>3.98 (0.35)</td>
<td>3.5</td>
<td>1.7</td>
</tr>
<tr>
<td>F$_4$</td>
<td>53.2 (45.7–64.2)</td>
<td>138 (95.6–168)</td>
<td>3.98 (0.25)</td>
<td>86.1 (71.4–104)</td>
<td>348 (255–551)</td>
<td>2.72 (0.14)</td>
<td>1.6</td>
<td>2.5</td>
</tr>
<tr>
<td>F$_5$</td>
<td>63.1 (52.5–76.4)</td>
<td>219 (135–282)</td>
<td>3.05 (0.25)</td>
<td>78.9 (64.7–96.7)</td>
<td>375 (228–500)</td>
<td>2.43 (0.45)</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>F$_6$</td>
<td>41.6 (33.5–52.0)</td>
<td>252 (136–361)</td>
<td>2.10 (0.32)</td>
<td>72.4 (58.0–91.4)</td>
<td>500 (330–929)</td>
<td>1.96 (0.23)</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>F$_7$</td>
<td>40.6 (33.7–49.1)</td>
<td>173 (109–229)</td>
<td>2.61 (0.30)</td>
<td>63.7 (52.0–78.7)</td>
<td>277 (189–528)</td>
<td>2.58 (0.20)</td>
<td>1.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

a Generation at which bioassay was conducted after field collection.
b Resistance ratio (RR) = LC of TYH population/LC of OSS.

In conclusion, our results have shown that although acetamiprid is still effective against field populations of DBM, there is a gradual development of resistance. To continue benefiting from the significant role that acetamiprid plays in DBM management programs, it is important to maintain a routine monitoring of field populations of DBM aimed at detecting changes in their susceptibilities to acetamiprid. There is also the need for effective strategies that would delay the progress of acetamiprid-resistance in the DBM.

**AKNOWLEDGEMENTS**

We are grateful to Drs. Toshiharu Tanaka and Takao Itohaki of our laboratory for their support and encouragement. Our sincere thanks go to Nippon Soda Company Limited for providing us with acetamiprid, and Dr. Kimitoshi Umeda of Sumitomo Chemical Company Limited for his assistance. Thanks to Dr. Satoshi Kono (Hyogo Prefecture Agricultural Institute), Dr. Akira Tanaka (Osumi Branch of Kagoshima Agricultural Research Station) and Mr. Tadami Hirano (JA Aichi) for their help in the collection of the field populations of DBM. This work was supported in part by a Grant-in-Aid for Scientific Research (A) No. 11356002 and Grant-in-Aid for the Encouragement of Young Scientists No. 98466 from the Ministry of Education, Science, Sports and Culture, Japan.

**REFERENCES**


Susceptibilities of Field DBM to Acetamiprid


