Monitoring, characterization and management of diamondback moth resistance to spinosad and indoxacarb

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ABSTRACT

Spinosad and indoxacarb are two different classes of insecticides with novel modes of action introduced in the late 1990s. In some areas such as Hawaii, they were the most important insecticides to control the diamondback moth, Plutella xylostella (L.), and were used in area-wide insecticide resistance management programs. Populations of P. xylostella were collected from fields of crucifer vegetables in the USA and Mexico from 1999 to 2005 for baseline susceptibility tests and resistance monitoring to both new insecticides. Most populations were susceptible to both insecticides, but resistance to spinosad extended from Hawaii (2000) to Georgia (2001) and California (2002) in the USA and then in Mexico (2005). Several populations collected from Hawaii in 2003-2005 showed varying levels of resistance to indoxacarb. Further studies on stability, fitness cost, inheritance, and mechanism of P. xylostella resistance to spinosad and indoxacarb were conducted after the field-derived resistant colonies were obtained. Interstrain complementation tests for allelism showed that there is a major recessive locus for resistance to spinosad shared by three P. xylostella populations from Hawaii, Georgia and California. When spinosad, indoxacarb and Bacillus thuringiensis (Bt) were used simultaneously in a mosaic fashion in a greenhouse cage experiment with P. xylostella, the resistance development to each insecticide was more rapid compared to when these products were used in a rotation every one or three generations of P. xylostella. This study illustrates a useful example of how a proactive monitoring program can be developed prior to the spread of insect resistance to a new insecticide. Regular and proactive monitoring provided industry and extension personnel the necessary information to help growers switch products before a major economic loss of the crop. A more sophisticated resistance management program that employs the thoughtful and cooperative rotation of products on an area-wide basis would enhance the longevity of each product even more.

INTRODUCTION

It is well documented that diamondback moth (DBM), Plutella xylostella, has the potential to develop resistance to most insecticides in the field sooner or later after extensive applications (Talekar and Shelton 1993). Field populations of P. xylostella have developed resistance to all synthetic insecticides used against it as well as biological insecticides like Bacillus thuringiensis (Bt). Proactive resistance monitoring and management are critical for any new insecticide introduced for the control of P. xylostella. Spinosad and indoxacarb are two different classes of insecticides with novel modes of action introduced in the late 1990s. In some areas such as Hawaii, they were very important insecticides for area-wide insecticide resistance
management programs of *P. xylostella* that was resistant to most other insecticides used in the fields (Mau et al. 2001, 2004). Populations of *P. xylostella* were collected from fields of crucifer vegetables in the USA and Mexico from 1999 to 2005 for baseline susceptibility tests and resistance monitoring to spinosad and indoxacarb (Zhao et al. 2002, 2006). Further studies on characterization and management of *P. xylostella* resistance to spinosad and indoxacarb were conducted in recent years after the field-derived resistant colonies were obtained.

Georghiou (1983) suggested that rotation of insecticides is an important measure for resistance management, and experimentally demonstrated in laboratory selections that rotation of three insecticides (temephos, permethrin and propoxur) could delay resistance evolution in the mosquito *Culex quinquefasciatus*, compared with continuous use of a single insecticide. Kanga et al. (2003) tested four different insecticide treatment regimes (continuous use of single insecticide or in rotation) in field experiments for managing resistance in Oriental fruit moth, *Grapholita molesta*. The results indicated that rotation of insecticides by class for each generation of *G. molesta* was successful in managing resistance to both organophosphorus and pyrethroid insecticides. However, there is lack of experimental study to compare the resistance evolution when different insecticides were used simultaneously in mosaic or in a rotation. We evaluated the development of resistance in *P. xylostella* to three insecticides (spinosad, indoxacarb, and Bt) when these products are used in a rotation strategy at two different time periods, compared to when the products are used in a mosaic fashion in which all products are used simultaneously in greenhouse cage experiments.

**BASELINE SUSCEPTIBILITY TESTING, MONITORING AND VALIDATION**

**BASELINE SUSCEPTIBILITY TESTING**

In the 13 populations of *P. xylostella* collected in 1999 and 2000 from the USA and Mexico, most populations were susceptible to spinosad, but one colony from Hawaii in 2000 showed significant resistance compared with our susceptible (S) Geneva 88 strain (Zhao et al. 2002). The geographic variation in susceptibility to spinosad in other populations was 0.9-11.2-fold based on LC₅₀ in a leaf-dip bioassay using second instars of *P. xylostella*. We suggested using 10 mg (AI)/L as the discriminating concentration for a diagnostic assay in routine resistance monitoring, which is equal to 23-fold of the LC₅₀ of the S strain, and caused 100% mortality to most field populations tested, apart from the resistant colony from Hawaii. We confirmed through inheritance studies that spinosad at 10 mg (AI)/liter could kill all susceptible (S) and S × R heterozygous individuals, but caused no mortality to the homozygous resistant strain (Pearl-Sel) (Zhao et al. 2002). Survivors at this concentration were designated homozygous resistant individuals.

Nine populations of *P. xylostella* collected in 2001 from the USA and Mexico were evaluated for baseline susceptibility to indoxacarb (Zhao et al. 2006). Much higher geographic variation in susceptibility in these populations was found (RR = 1.9-140-fold based on LC₅₀ data, but no reduced efficacy or control failure). Based on baseline data in 2001, we suggested using indoxacarb at 50 mg (AI)/liter as the discriminating concentration for diagnostic assays, which caused 100% mortality for the susceptible strain and ≥ 99% mortality for most field populations. Subsequently, we found that this discriminating concentration caused a high mortality (> 90%) of S × R heterozygous (RS) individuals and low mortality (< 10%) of a resistant (R) population. We also found that it was not possible to choose a perfect concentration of indoxacarb that can separate RS and RR genotypes as used for spinosad.

**Resistance Detection and Monitoring**

A state-wide survey in three islands of Hawaii in 2000 and 2001 indicated that 6 of the 12 *P. xylostella* populations were highly resistant to spinosad. Resistance monitoring primarily based on diagnostic assays in
2001-2005 indicated that most populations were susceptible to spinosad and indoxacarb (Zhao et al. 2006). However, resistance to spinosad extended from Hawaii (2000) to Georgia (2001) and California (2002), low frequency of resistance was detected in Mexico in 2005 (6% survival at 10 ng (Al)/liter in Florencia), and several populations collected from Hawaii in 2003-2005 showed varying levels of resistance to indoxacarb.

Resistance Validation

After the first detection of insecticide resistance in a field population by evident survival under a discriminating concentration, two approaches were used to confirm the resistance for validation. The first was to test if the detected resistance in bioassays was associated with reduced efficacy or control failure in the fields, as shown in Hawaii for both spinosad and indoxacarb (Mau and Gusukuma-Minuto 2001, 2004; Zhao et al. 2002, 2006). The second was to do further insect selection in the laboratory to get a homozygous resistant (R) strain (Pearl-Sel resistant to spinosad and Waipio-Sel resistant to indoxacarb), then to compare the insecticide efficacy on the S and R strains in a greenhouse spray test using a field rate. One application of spinosad at the lower rate of 26 g (Al)/ha caused 94.1% mortality to the S strain, while spraying at 26 and 53 g (Al)/ha caused only 15.3% and 17.1% mortality to the Pearl-Sel strain (Zhao et al. 2002). Similar results were observed in the Waipio-Sel strain, i.e. spraying of indoxacarb at the rate of 259 g (Al)/ha resulted in 25.6% mortality after 3 days, significantly lower than that of S strain (96.5% mortality).

- CHARACTERIZATION OF SPINOSAD AND INDOXACARB RESISTANCE -

Resistance Stability and Fitness Cost

The spinosad resistance in the Pearl-Sel colony was stable within 6 generations after the last selection (F9-F14 in Figure 1) as tested by both toxicity ratio (TR) and survival at discriminating dose (DD), but declined significantly after 7 generations (Figure 1). No significant fitness cost was observed at constant room temperature in the BC83-Pearl strain, which is a near-isogenic strain to S but highly resistant to spinosad. The survival at DD of indoxacarb was stable and close to 90% within 4 generations after selection, but decreased to 81% in five generations after selection and to 20% in seven generations after selection.

![Figure 1](image-url) Stability of resistance to spinosad in the Pearl-Sel colony of *P. xylostella* after selection ceased at F6 (TR, toxicity ratio; DD, diagnostic dose)
Inheritance

Reciprocal crosses between the S and R strain of *P. xylostella* were made to test the resistance dominance, followed by backcrosses (BCs) between F₁ (RS genotype) and the parental R (if partially or completely recessive as in spinosad) or S (if partially or completely dominant as in indoxacarb) to test for monogenic inheritance. The results showed that resistance to spinosad was incompletely recessive (D = −0.83), and was controlled autosomal probably by one locus (Zhao *et al.* 2002). Results on inheritance of indoxacarb resistance showed that it was also controlled autosomally, probably by one locus, but was incompletely dominant (D = 0.19, Table 1). Results from repeated backcrosses further confirmed that resistance to either spinosad or indoxacarb was controlled by a major gene. We did three repeated backcrosses (BCs) with the S strain followed by two or three selections (beginning at F₂ of the BC offsprings) using a discriminating concentration of spinosad or indoxacarb. The result was a near-isogenic strain (to S) that was highly resistant to either spinosad (in BCS3-Pearl) or indoxacarb (in BCS3-Waipio).

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>Slope (SE)</th>
<th>LC₅₀, mg/kg AI</th>
<th>95% CI</th>
<th>χ²(df)</th>
<th>Ratio</th>
<th>D*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geneva 88  (S)</td>
<td>250</td>
<td>1.34 (0.21)</td>
<td>0.162</td>
<td>0.100-0.241</td>
<td>1.5 (3)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Waipio (R)</td>
<td>500</td>
<td>2.35 (0.19)</td>
<td>304</td>
<td>262-349</td>
<td>1.9 (3)</td>
<td>1 877</td>
<td></td>
</tr>
<tr>
<td>S/R (F₀)</td>
<td>250</td>
<td>2.28 (0.25)</td>
<td>14.3</td>
<td>9.42-22.8</td>
<td>5.1 (3)</td>
<td>88.3</td>
<td>0.19</td>
</tr>
<tr>
<td>R/S (F₁)</td>
<td>250</td>
<td>2.30 (0.25)</td>
<td>14.8</td>
<td>12.4-18.0</td>
<td>1.5 (3)</td>
<td>91.4</td>
<td>0.20</td>
</tr>
<tr>
<td>Pooled</td>
<td>500</td>
<td>2.29 (0.18)</td>
<td>14.6</td>
<td>10.8-20.2</td>
<td>5.9 (3)</td>
<td>90.1</td>
<td>0.19</td>
</tr>
</tbody>
</table>

*D = degree of dominance.

Allelism for Spinosad Resistance

Interstrain complementation tests for allelism of spinosad resistance in *P. xylostella* were conducted using similar methods as Tabashnik *et al.* (2004). Three field-derived populations from Hawaii (R1), Georgia (R2) and California (R3) resistant to spinosad were used for the crosses and diagnostic assays. All the parental strains and offspring from crosses between S, R1 and R2 or R3 colonies were tested at the diagnostic concentration (10 mg [A1]/liter). As expected, there was no survival for the S and F1 of (S × R) strains, but 100% survival in both R1 and R2 strains. Survival of F1 of (R1 × R2) at 10 mg [A1]/liter was 100% (Table 2), indicating the same locus for the resistance alleles in R1 and R2. Similar results were observed in the crosses using R1 and R3. The results showed that there is a major recessive locus for resistance to spinosad shared by three DBM populations from Hawaii, Georgia and California.

<table>
<thead>
<tr>
<th>Type of strain or cross</th>
<th>Strain or cross</th>
<th>n</th>
<th>Survival at DD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible (S)</td>
<td>Geneva 88</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Resistant (R)</td>
<td>R1 (Hi)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>R2 (GA)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>F₁ of (S × R)</td>
<td>S × R1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S × R2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>F₁ of (R1 × R2)</td>
<td>R1 × R2</td>
<td>15 × 30</td>
<td>97-100</td>
</tr>
<tr>
<td></td>
<td>R2 × R1</td>
<td>15 × 30</td>
<td>97-100</td>
</tr>
</tbody>
</table>

Resistance Mechanism

The synergists DEF and PBO did not have a significant effect on the toxicity of spinosad (Zhao *et al.*
2002) or indoxacarb to the resistant *P. xylostella* colony, indicating that metabolic-mediated detoxification was probably not responsible for the resistance. The indoxacarb-resistant strain of *P. xylostella* showed similar or slightly lower levels of resistance to DCJW, the decarbomethoxylated metabolite of indoxacarb, indicating that the metabolic procedure for activation of indoxacarb to DCJW in *P. xylostella* was not involved in the resistance mechanism.

### EVALUATING RESISTANCE MANAGEMENT STRATEGIES IN GREENHOUSE EXPERIMENTS

Greenhouse cage experiments were conducted using similar methods as testing resistance management programs to Bt plants in *P. xylostella* (Tang et al. 2001, Zhao et al. 2003, 2005). Three treatments, based on possible strategies to use the three insecticides, were included in the experiment: (A) Alternation of each insecticide in every generation; (B) Alternation of each insecticide in every three generations; (C) A mosaic in which each insecticide was used on 4 plants (1/3 of all sprayed plants). The experiment was terminated after 9 generations with one complete cycle of all treatments. Based on the field rate of each insecticide and using 30 GPA spray volume, the concentration for sprays on broccoli plants was 259 mg/kg AI for indoxacarb, 100 ppm AI for spinosad and 205 ppm AI for Bt (Dipel DF). The survival of *P. xylostella* larvae at diagnostic concentrations of the different insecticides, which was related to the frequency of homozygous resistant individuals for each insecticide, were tested before and after selections.

When three insecticides were used simultaneously in a mosaic fashion (Trt. C) for 9 generations, the survival of resistant individuals was significantly higher than that with rotation of each insecticide per generation (Trt. A for spinosad resistance) or per three generations (Trt. B for indoxacarb resistance) (Table 3). The survival of individuals resistant to all three insecticides was also higher in the mosaic treatment than in rotation.

### Table 3  Survival of *P. xylostella* larvae at diagnostic concentrations of three insecticides in different treatments of greenhouse cage experiment

<table>
<thead>
<tr>
<th>G^a</th>
<th>Treatment</th>
<th>Mean % survival on insecticides^b</th>
<th>All three^c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spinosad</td>
<td>Indoxacarb</td>
</tr>
<tr>
<td>0</td>
<td>—</td>
<td>3.1</td>
<td>0.73</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>23.7 (6.1) b</td>
<td>15.3 (0.6) ab</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>72.7 (3.9) a</td>
<td>11.7 (2.0) bc</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>73.5 (0.5) a</td>
<td>22.8 (2.3) a</td>
</tr>
</tbody>
</table>

^a Generations after selection.

^b Values in a column followed by same letter are not significantly different (P > 0.05, HSD).

^c Calculated % survival if treated by all three insecticides simultaneously according to survival on each insecticide.

### SUMMARY AND DISCUSSION

This study illustrates a useful example of how a proactive monitoring (using diagnostic detection) and management (by rotation of different insecticides) program can be developed prior to the spread of insect resistance to a new insecticide. Regular and proactive monitoring provided industry and extension personnel the necessary information to help growers switch products before a major economic loss of the crop. A more sophisticated resistance management program that employs the thoughtful and cooperative rotation of products on an area-wide basis would enhance the longevity of each product even more. Further studies on the cross-resistance and biochemical or molecular mechanisms for resistance to spinosad and indoxacarb will be helpful
for resistance monitoring and management programs in the future.

REFERENCES


