

# Testing insecticide resistance management strategies: mosaic versus rotations

Jian-Zhou Zhao,<sup>a,b\*</sup> Hilda L Collins<sup>a</sup> and Anthony M Shelton<sup>a</sup>

## Abstract

**BACKGROUND:** Developing scientifically valid, economically acceptable insecticide resistance management (IRM) programs is critical for sustainable insect management. The diamondback moth, *Plutella xylostella* (L.), has demonstrated an ability to develop resistance to many different classes of insecticides, including proteins produced by the bacterium *Bacillus thuringiensis* Berliner (*Bt*). Recently it has developed resistance to the novel compounds spinosad and indoxacarb. In greenhouse cage experiments, a laboratory-selected population of *P. xylostella* resistant to spinosad, indoxacarb and *Bt* was used to compare population growth and resistance evolution if these three insecticides were rotated or used in a mosaic fashion.

**RESULTS:** The average population density through nine generations was lowest in the treatment in which the insecticide was rotated every generation (R-1) ( $\bar{x} = 20.7 \pm 3.20$ ) compared with the treatment in which the insecticide was rotated every third generation (R-3) ( $\bar{x} = 41.4 \pm 17.6$ ) or where the insecticides were applied as a mosaic (M) ( $\bar{x} = 41.8 \pm 6.53$ ). After nine generations, the survival of resistant individuals increased for each insecticide (7.2–73.5%) compared with the population without selection (CK) (0.73–3.1%). Survival on spinosad was significantly lower (23.7%) in the single-generation rotation than for the other two treatments, both of which exceeded 72%. The calculated survival on all three insecticides treated simultaneously, according to the survival on each insecticide, was 0.26, 0.81 and 1.6% for R-1, R-3 and M treatments respectively.

**CONCLUSION:** Results of both population density and resistance development indicated that insecticide rotation every generation was better for IRM than if the insecticide was rotated every third generation or if the three insecticides were applied as a mosaic.

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**Keywords:** *Plutella xylostella*; insecticide; resistance; rotation; mosaic

## 1 INTRODUCTION

Resistance to traditional synthetic pesticides has become one of the major driving forces altering the development of integrated pest management (IPM) programs worldwide.<sup>1</sup> Early definitions of resistance focused on the 'development of strains capable of surviving a dose lethal to a majority of individuals in a normal population'.<sup>2</sup> Sawicki<sup>3</sup> expanded this concept to include field performance and suggested the following definition: 'resistance (is) a genetic change in response to selection by toxicants that may impair control in the field'. Georghiou and Lagunes-Tejeda<sup>4</sup> listed over 500 species of arthropods that had developed strains resistant to one or more of the five principal classes of insecticides. Interestingly, the survey did not include insect pathogens as one of the principal classes, although there are cases of insects having evolved resistance to bacteria, viruses, fungi and nematodes.<sup>1</sup> The most current information on arthropod resistance can be found in the Arthropod Pesticide Resistance Database.<sup>5</sup>

One species in particular, the diamondback moth, *Plutella xylostella* (L.), has demonstrated an ability rapidly to develop resistance to many different classes of insecticides, including proteins produced by the bacterium *Bacillus thuringiensis* Berliner.<sup>6–8</sup> More recently, it has developed resistance to the novel active ingredients spinosad and indoxacarb.<sup>9–12</sup> Because of its ability rapidly to develop resistance and its short generation time,<sup>13</sup>

*P. xylostella* is a good candidate for exploring insecticide resistance management (IRM) strategies.

IRM is the scientific approach to managing pests over the long run so that resistance does not interfere with the ability to produce sustainable crops.<sup>14</sup> Because of the increasing costs of developing and registering new classes of insecticides, there is more emphasis on developing scientifically valid, economically acceptable IRM programs. However, to do so requires knowledge of the genetics and mechanism(s) of resistance, and these are generally not known prior to the commercial introduction of the insecticide. The IRM programs for foliar insecticides that have been implemented were employed 'purely on pragmatic grounds and independently of any detailed knowledge of the genetics or mechanisms of resistance'.<sup>15,16</sup> Roush<sup>15</sup> argues that it is usually possible to design reasonable IRM programs based on relatively modest amounts of information about the pesticides

\* Correspondence to: Jian-Zhou Zhao, Department of Entomology, Cornell University, New York State Agricultural Experiment Station, 630 W. North St, Geneva, NY 14456, USA. E-mail: zhaojz@yahoo.com

<sup>a</sup> Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY, USA

<sup>b</sup> Pioneer Hi-Bred, Johnston, IA, USA

used and existing knowledge of population ecology of the pest, and that such first-approximation IRM programs can be modified as more information becomes available. However, it is difficult quantitatively to evaluate the outcome of a particular IRM strategy unless resistance, or at least resistance allele frequency, changes in the 'control'. Such comparisons are difficult, if not impossible, to conduct on a commercial scale with all other factors similar.

IRM strategies are essentially a series of choices (e.g. regulating dose, adding a synergist, changing the insecticide, creating a refuge, targeting a specific stage of insect) that may help lower selection pressure. Chief among such options is the rotation of different insecticides of different classes across generations of the targeted pest. In this strategy, a single insecticide class is used once or multiple times against one or more generations, and then another insecticide class is used in a similar manner, and so on. As discussed by Onstad,<sup>14</sup> in the rotation strategy, treatments are applied to the same space at different times, and insects resistant to one insecticide will be killed by the next insecticide in the rotation. An alternative strategy is a mosaic of treatments in which two or more insecticides are applied to different areas infested by the same insect population, i.e. metapopulation. This situation would occur if different farmers (or the same farmer) used different insecticides on the same pest population infesting different crops or areas in a defined landscape.

If insecticides have different modes of action (Insecticide Resistance Action Committee; <http://www.ircac-online.org/>) and are not in any way cross-resistant to each other, both rotations and mosaics may initially be considered to be reasonable IRM strategies, as both would appear to preserve susceptible alleles in space or time, and the preservation of such alleles is the key to successful IRM. However, in spite of modeling efforts, empirical comparisons of the rotation and mosaic strategies are few.<sup>15</sup> In the present study, use is made of insecticide-resistant populations of *P. xylostella* to compare whether resistance evolution will occur faster if insecticides are rotated (and, if so, to ascertain over how many generations) or if they are used in a mosaic fashion.

## 2 MATERIALS AND METHODS

### 2.1 Insects

A susceptible (S) strain of *P. xylostella*, Geneva 88, has been maintained on a wheat germ-casein artificial diet for over 300 generations.<sup>17</sup> A colony collected from Waipio, Hawaii, in 2003 was resistant to spinosad and indoxacarb<sup>10</sup> and *Bt* (Zhao J-Z, unpublished). The larvae of this colony were selected in F2 and F3 by a mixture of spinosad, indoxacarb and *Bt* (Dipel) at the concentrations of recommended field spray rates for each insecticide using leaf-dip bioassay methods<sup>9,10</sup> to obtain a strain resistant (R) to all three insecticides.

### 2.2 Insecticides

Commercial formulations of three insecticides were used: spinosad 240 g L<sup>-1</sup> SC (SpinTor 2 SC; Dow AgroSciences, Indianapolis, IN); indoxacarb 300 g kg<sup>-1</sup> WG (Avaunt; DuPont Crop Protection, Newark, DE) and *Bt* (*Bacillus thuringiensis* var. *kurstaki*) 105 g kg<sup>-1</sup> WP (Dipel DF; Valent BioSciences, Walnut Creek, CA). Each insecticide belongs to a different class based on its mode of action (Insecticide Resistance Action Committee: <http://www.ircac-online.org/>).

### 2.3 Development and testing of an artificially selected population for greenhouse experiments

The R strain of *P. xylostella* was crossed with the S strain and then backcrossed with the S strain to create an artificially selected population (BCS). The predicted initial resistance allele frequency in the BCS before selection was about 0.25 for resistance to each of the three insecticides. The diagnostic concentration that could separate the R strain from the S strain or the RS heterozygous individuals was 10 mg AI L<sup>-1</sup> for spinosad,<sup>9</sup> 50 mg AI L<sup>-1</sup> for indoxacarb<sup>10</sup> and 10 mg AI L<sup>-1</sup> for *Bt* (Dipel DF) (Zhao J-Z, unpublished). Survival of second instars of the S and R strains, (R × S) F2 and F3 heterozygotes and BCS populations (F4, F5, F10) was tested at the diagnostic concentration of each insecticide using a leaf-dip assay<sup>9,10</sup> in 30 mL plastic cups. For each strain or population, a total of 50–100 larvae were tested against each insecticide (in five replicates) and 50 larvae on a non-insecticide control (<5% mortality in all tests). Survival was determined after 3 days at 27 ± 1 °C.

### 2.4 Plants, cages and foliar sprays

Broccoli, *Brassica oleracea* L. subsp. *italica*, 'Green Comet', was used for all treatments. All cage tests were conducted in greenhouses at the New York State Agricultural Experiment Station in Geneva, New York, using methods similar to those reported in other publications by the present authors.<sup>18–20</sup> Broccoli plants in 15 cm diameter pots were 6–12 weeks old when they were placed into cloth cages. Plants were fertilized with a controlled released fertilizer, Osmocote (N:P:K = 14:14:14; Scotts, Marysville, OH). The dimensions of the base of each cage were 1.83 m long × 0.91 m wide, and its height was 1.83 m. Each cage had three large sleeve openings for routine access and held 14 plants. The broccoli plants were randomly allocated to their positions in each cage. Of the 14 plants per cage, two plants were never sprayed and served as refuge plants<sup>19,20</sup> (such refuge plants would commonly occur in agricultural settings as weeds or untreated or poorly treated plants). Pots were spaced >0.2 m apart, and a nylon netting barrier (0.9 m high) was used to separate the refuge plants from the sprayed plants in each cage. Adults, but not larvae, could easily move between the refuge and sprayed broccoli plants. This arrangement was designed to simulate adjoining fields where there would be frequent interfield movement of adults but negligible movement of larvae. Greenhouse conditions were: temperature 23–27 °C, 16:8 h light:dark photoperiod and uncontrolled relative humidity.

Based on the field rate of each insecticide, and using 281 L ha<sup>-1</sup> spray rate, the concentrations for sprays on broccoli plants were 259 mg AI L<sup>-1</sup> for indoxacarb, 100 mg AI L<sup>-1</sup> for spinosad and 205 mg AI L<sup>-1</sup> for *Bt*. Silwet L-77, at 1% v/v, was added to each insecticide. Plants were removed from the cages and sprayed in a track chamber (Allen Machine Works, Midland, MI), using a single nozzle 50 cm above the plant, and sprayed at 0.276 MPa and 281 L ha<sup>-1</sup>. This allowed more precise spraying of the plants. The choice of spray time for each generation was based on evidence of increased numbers of adults and eggs and the limited number of larvae found on broccoli leaves. Sprayed plants were immediately placed back into the cage from which they came. Each insecticide sprayed on the broccoli plants had 100% control of susceptible larvae but minimal mortality on adults (Zhao J-Z, unpublished). There was one spray for each insect generation. A new and previously unsprayed plant of approximately the same size was used to replace each severely defoliated plant. Plants to

be removed from the cage were cut at the base and placed onto the replacement plants so that larvae on them were not lost.

## 2.5 Treatments in cage experiments

Three treatments, based on possible strategies to use the three insecticides, were included in the experiment: alternation of each insecticide every generation (R-1); alternation of each insecticide every three generations (R-3); a mosaic in which each insecticide was used on four plants (one-third of all sprayed plants) (M). Because greenhouse space was only available for eight cages for the three treatments, there were three replicates (cages) for treatments R-1 and R-3 and two replicates for treatment M. Each cage was considered a replicate. Three hundred BCS F<sub>4</sub> pupae were released into each cage (= G<sub>0</sub> of greenhouse generation). In the rotation experiments (R-1 and R-3), the order of use of insecticides was indoxacarb, followed by spinosad and then *Bt*. The experiment was terminated after nine generations, which resulted in one complete cycle of all treatments.

## 2.6 Data collection and statistical analyses

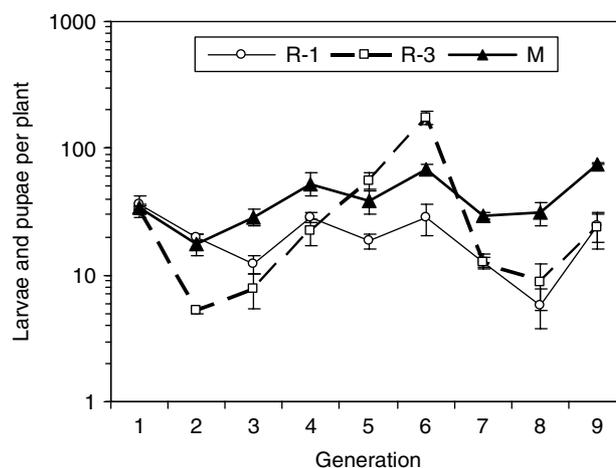
Two types of measurement were made: population abundance and the survivorship of resistant individuals. The numbers of *P. xylostella* larvae and pupae on broccoli plants were counted every generation 7–10 days after the insecticide spray, when the majority of the populations were large larvae (third or fourth instar) or pupae. Four plants each of R-1 and R-3 sprayed with insecticide and six plants of M (two plants sprayed with each insecticide) were randomly selected from each cage, and the numbers of larvae and pupae on each plant were counted. Population abundance was measured by larvae and pupae per plant. To test for resistance, about 30–40 pupae from non-sprayed refuge plants were collected from each cage after nine generations of selection. Survival of second instars derived from each cage was then tested at diagnostic concentrations of each insecticide using the same method as the tests of the synthetic population before the selection experiments.

SAS programs<sup>21</sup> were used for analysis of variance. Data were transformed using the arcsine square-root value for proportion of survival, or the log ( $x + 1$ ) for insect density data, before each analysis of variance was performed. Treatment means were compared and separated by Tukey's HSD at  $P = 0.05$ .

## 3 RESULTS

### 3.1 Tests of the artificially selected population

As the survival of the R strain at diagnostic concentrations was 100% for spinosad and <100% for indoxacarb or *Bt*, the predicted initial resistance allele ( $r$ ) frequency (BCS F<sub>4</sub>) was about 0.25 for spinosad resistance and <0.25 for indoxacarb or *Bt* resistance. The expected number of homozygous resistant individuals ( $rr$ ) that could survive at a diagnostic concentration was about 6% for spinosad because of monogenic inheritance.<sup>9</sup> It should be <6% for indoxacarb or *Bt* because of lower resistance allele frequency, especially with only 40% survival of the R strain ( $rr$ ) at the diagnostic concentration of *Bt* (Dipel) (Table 1). The mean survival rates of BCS F<sub>4</sub>, F<sub>5</sub> and F<sub>10</sub> were used as the initial survival before or without selection, i.e. 3.1, 0.73 and 1.0% for spinosad, indoxacarb and *Bt* respectively (Tables 1 and 2).



**Figure 1.** Density (mean  $\pm$  SEM) of *Plutella xylostella* larvae on broccoli plants sprayed with different insecticides in greenhouse cages. R-1: alternation of each insecticide every generation (indoxacarb, spinosad, then Dipel); R-3: alternation of each insecticide every three generations (generations 1 to 3 indoxacarb, generations 4 to 6 spinosad, generations 7 to 9 Dipel); M: mosaic in which each insecticide was used on one-third of the plants each generation.

### 3.2 *Plutella xylostella* population abundance in cage experiments

The larval population densities in the mosaic treatment (M) were significantly higher than one or both rotation treatments (R-1 and R-3) in four of the nine generations (generation 2:  $F = 52.8$ ;  $df = 2, 5$ ;  $P = 0.0004$ ; generation 3:  $F = 5.96$ ;  $df = 2, 5$ ;  $P = 0.048$ ; generation 7:  $F = 55.4$ ;  $df = 2, 5$ ;  $P = 0.0004$ ; generation 8:  $F = 6.39$ ;  $df = 2, 5$ ;  $P = 0.042$ ) (Fig. 1). In generation 9 the population density in mosaic treatment was more than threefold compared with R-1 and R-3, although not significantly different ( $F = 4.21$ ;  $df = 2, 5$ ;  $P = 0.085$ ). However, after the continuous use of spinosad for two or three generations, the *P. xylostella* population was highest among the three treatments (generation 5:  $F = 12.5$ ;  $df = 2, 5$ ;  $P = 0.011$ ; generation 6:  $F = 21.1$ ;  $df = 2, 5$ ;  $P = 0.004$ ), suggesting rapid development of spinosad resistance if used continuously. The average population density ( $\pm$  SEM) through the nine generations was lowest in the treatment in which the insecticide was rotated every generation (R-1,  $\bar{x} = 20.7 \pm 3.20$ ) compared with the treatment in which the insecticide was rotated every third generation (R-3,  $\bar{x} = 41.4 \pm 17.6$ ) or where the insecticides were applied as a mosaic (M,  $\bar{x} = 41.8 \pm 6.53$ ). Rotating the insecticide every generation also resulted in markedly less variation in the population density compared with rotating the insecticide every third generation.

### 3.3 Resistance development in cage experiments

The survival of *P. xylostella* larvae at diagnostic concentrations of the different insecticides is related to the frequency of homozygous resistant individuals for each insecticide. Prior to the initiation of the treatments, the survival at the diagnostic doses for all three insecticides varied from 1.0 to 4.0% (Table 1, BCS F<sub>4</sub>), suggesting that similarly low resistance levels were able to be achieved for each insecticide prior to beginning the tests. After nine generations of selections, however, survivorship increased markedly for each insecticide (7.2–73.5%) compared with the population without selection (0.73–3.1%) (Table 2). For spinosad, the survival was significantly lower (23.7%) in the single-generation rotation than

**Table 1.** Survival of *Plutella xylostella* (DBM) larvae at diagnostic concentrations of different insecticides

Insecticide	Conc. (mg AI L <sup>-1</sup> )	Survival (%) of DBM strain or cross							
		R	SS or (SS × R) F1	(SS × R) F2	(SS × R) F3	BCS F4 <sup>a</sup>	BCS F5	BCS F10	BCS mean
Spinosad	10	100	0	12.5	12.0	4.0	2.5	2.7	3.1
Indoxacarb	50	93.3	0	7.8	7.0	1.0	0.5	0.7	0.73
<i>Bt</i> (Dipel)	10	40.0	0	4.0	9.0	1.0	1.0	1.0	1.0
CK (water)	–	100	100	98.0	100	100	98.0	98.0	98.7

<sup>a</sup> BCS was an artificially selected population made by crossing the SS and R strains and then backcrossing with the SS strain. Additional rearing without insecticide treatment yielded BCS F4, F5 and F10. BCS F4 was placed into the cages to begin the experiment.

**Table 2.** Survival of *Plutella xylostella* larvae at diagnostic concentrations of three insecticides in different treatments after nine generations in greenhouse cage experiments

Treatment <sup>a</sup>	Mean survival on insecticides (%) (± SEM) <sup>b</sup>			
	Spinosad	Indoxacarb	<i>Bt</i> (Dipel)	All three <sup>c</sup>
BCS-CK <sup>d</sup>	3.1	0.73	1.0	0.002
R-1	23.7 (±6.1) b	15.3 (±0.6) ab	7.2 (±0.9) a	0.26
R-3	72.7 (±3.9) a	11.7 (±2.0) bc	9.5 (±2.6) a	0.81
M	73.5 (±0.5) a	22.8 (±2.3) a	9.5 (±2.3) a	1.6

<sup>a</sup> R-1: insecticide rotated after a single generation; R-3: insecticide rotated every three generations; M: mosaic in which different insecticides were applied to different sets of plants in the same cage.

<sup>b</sup> Values in a column followed by the same letter are not significantly different ( $P > 0.05$ , HSD). Mortality <5% for non-insecticide control for each test.

<sup>c</sup> Calculated % survival if treated with all three insecticides simultaneously, multiplied by % survival of each insecticide for each treatment.

<sup>d</sup> Survival without insecticide sprays in the cage was estimated as the mean survival of BCS F4, F5 and F10 (Table 1).

for the other two treatments, both of which exceeded 72%. The survival was significantly lower (11.7%) in the R-3 than in the M treatment (22.8%) for indoxacarb, and there was no significant difference among the three treatments for *Bt*.

Another measure of the effect of different treatments in suppressing the evolution of resistance was to calculate the percentage survival of any set of individuals of each treatment if treated by all three insecticides simultaneously, according to the survival on each insecticide and assuming independent insecticidal effects for each insecticide. In this case, insects from the R-1 treatment had a 0.26% survival, as opposed to a 0.81% survival for insects from R-3 and 1.6% survival for insects from M, which was more than sixfold higher than the R-1 treatment (Table 2).

## 4 DISCUSSION

A measure of the effectiveness of a particular IRM strategy is how many generations it will take before a population can no longer be controlled economically by an insecticide. Modeling studies are helpful but need to be tested. Only limited field studies have been published to test such strategies.<sup>22,23</sup> It is difficult to conduct such studies because multiple generations are needed, and variables such as climatic conditions, insect movement patterns and natural enemies may interfere with interpreting the results. Compared

with single continuous insecticide selections, insecticide resistance development in horn flies (*Haematobia irritans* L.) was significantly reduced by alternating and mixing insecticides.<sup>22</sup> A strategy based on rotation of insecticides by class for each generation of the oriental fruit moth (*Grapholita molesta* Busck.) was successful in managing resistance to both organophosphorus and pyrethroid insecticides.<sup>23</sup>

In the present study, direct comparisons were made of two commonly used strategies – rotations and mosaics. Based on the data obtained, it is clear that pest populations are best suppressed and resistance is slower to develop when an insecticide of one class is used against a single generation and the next generation is treated with an insecticide with a different mode of action. From a pragmatic standpoint, this strategy can be implemented using a ‘window’ in which only a single insecticide class is allowed to be used at a particular time. The length of that window will be dependent on the generational time of the insect. Coordinated window strategies have proven to be effective in terms of adoption and efficacy of *Helicoverpa armigera* (Hübner) in Australia.<sup>15,24</sup>

Even in the absence of insecticide use, any fitness costs associated with resistance will affect an insecticide rotation strategy. Fitness costs for *Bt* resistance were detected in *P. xylostella* from Hawaii<sup>25</sup> but not in *P. xylostella* from Florida.<sup>26</sup> No evident fitness costs were detected in the spinosad-resistant *P. xylostella* colony used in this study (Zhao J-Z, unpublished) and another strain<sup>27</sup> when tested at constant favorable temperatures; however, the fitness costs increased in scale at unfavorably low and high temperatures.<sup>27</sup>

Ideally, such a window strategy should be initiated at the first introduction of an insecticide, but in reality, for a window strategy to work, multiple insecticides would have to enter the market simultaneously, and this would be a rare event. However, the present data suggest that, even if resistance to one or more of the insecticides already occurs (the present experiment was started with *P. xylostella* already having some level of resistance to each insecticide), their usefulness can be extended if an IRM window strategy is implemented. The data also support the idea that mosaics should be avoided. The use of different insecticides at roughly the same time in neighboring fields where insects move between fields creates a mosaic of treatment patterns and should be avoided, as should very short-term rotations within a generation.<sup>15</sup> The problem with both is that there is simultaneous selection with several insecticides, resulting in much lower insecticide durability.

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