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Rapid selection for resistance to diamide insecticides in *Plutella xylostella* via specific amino acid polymorphisms in the ryanodine receptor

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ABSTRACT

Diamide insecticides, such as flubendiamide and chlorantraniliprole, are a new class of insecticide with a novel mode of action, selectively activating the insect ryanodine receptor (RyR). They are particularly active against lepidopteran pests of cruciferous vegetable crops, including the diamondback moth, *Plutella xylostella*. However, within a relatively short period following their commercialisation, a comparatively large number of control failures have been reported in the field. In this review we summarise the current body of knowledge regarding the molecular mechanisms of diamide resistance in *P. xylostella*. Resistant phenotypes collected from different countries can often be linked to specific target-site mutation(s) in the ryanodine receptors' transmembrane domain. Metabolic mechanisms of resistance have also been proposed. Rapid resistance development is probably a consequence of over-reliance on this one class of chemistry for diamondback moth control.

1. Background

The diamondback moth, *Plutella xylostella* (L.), is one of the most destructive insect pests of cruciferous vegetables, currently accounting for US$2.7 billion worth of annual worldwide crop losses (Zalucki et al., 2012). Most damage is caused by the caterpillars tunneling into the heads and/or foliage of plants such as cabbage, kale, swede, turnip and brussels sprouts. In addition, they can contaminate produce by pupating inside broccoli florets and cauliflower curds. Seedlings of cruciferous forage crops and oilseed rape may well be destroyed by this pest and severe defoliation or pod grazing can also significantly reduce oilseed rape yield. Control costs associated with this pest are in the region of US $1.3 billion to US$2.3 billion annually (Zalucki et al., 2012). Typically, control of this pest depends solely on the use of synthetic insecticides.

Flubendiamide is an extremely effective insecticide against *P. xylostella*, especially when used as a larvicide (Tohnishi et al., 2005; Nauen, 2006; Hirooka et al., 2007). The parent compound structure was discovered by Nihon Nohyaku Co., Ltd during their pyrazine-dicarboxamide herbicide development program conducted in the early 1990s. The discovery of more potent substituents led to the synthesis, in 1998, of a phthalic acid diamide insecticide, later named flubendiamide (Nishimatsu et al., 2005), co-developed by Nihon Nohyaku and Bayer CropScience AG (Nauen, 2006; Tsubata et al., 2007). Flubendiamide has an excellent biological and ecological profile (Hilder and Boulter, 1999; Hall, 2007) and a favourable toxicological profile (Ebbinghaus-Kintscher et al., 2006). The first registration was secured in the Philippines in 2006 and was followed a year later by successful registrations in Japan, Pakistan, Chile, India and Thailand under the trade names Amoli®, Belt®, Fame®, Fenos®, Synapse®, Phoenix® and Takumi® (Hirooka et al., 2007). Flubendiamide was classified as the first member of the new group 28 (ryanodine receptor modulator) insecticides within the IRAC (Insecticide Resistance Action Committee) mode of action classification scheme (Nauen, 2006). This scheme, developed to provide guidance on resistance management strategies, facilitates the alternation of compounds belonging to different groups in order to delay or avoid the rapid development of resistance in treated pest insects.

Chlorantraniliprole or Rynaxypyr® (Dupont, USA), is another insecticide in the IRAC Mode of Action Group 28 family. Chlorantraniliprole was the first member of the anthranilic diamides, and, as with flubendiamide, is particularly effective for control of insects in the order Lepidoptera (Temple et al., 2009). Chlorantraniliprole is relatively harmless to beneficial arthropods and was not found to exhibit cross resistance with existing

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insecticides (Lahm et al., 2009). Products containing this active ingredient were launched on the world market in 2007. This insecticide is currently sold under the trade names Acelepryn®, Altacor®, Coragen®, Dermacor® X-100, Prevathon®, Voliam® Flexi and Voliam® Xpress Durivo® and Virtako®. Cyantraniliprole or Cyazypyr™, a second anthranilic diamide discovered by DuPont and co-developed with Syngenta (Wiles et al., 2011), is chemically similar to chlorantraniliprole, but exhibits a broader spectrum insecticidal activity and provides good control of sucking and piercing insects such as aphids and whiteflies (Foster et al., 2012; Gravalos et al., 2015). The broad spectrum of this anthranilic diamide is thought to be due to its physical properties, i.e a lower log*P* and higher water solubility, in comparison to the other diamide insecticides, making it more suitable for systemic applications (Selby et al., 2013). Products containing cyantraniliprole were launched in selected countries from 2012 under the trade names Exirel®, Verimark®, Ference®, Fortenza Duo®, Benevia® and Spinner®.

2. Baseline susceptibility monitoring for diamide insecticides

The LC_{50} value (lethal concentration that provides 50% mortality) of a particular insecticide can be used to establish a baseline susceptibility for a target population. This can then be used as a baseline reference in future monitoring surveys to determine if the susceptibility of the target population has shifted after the population has been exposed to the insecticide. Actual LC_{50} values can be compared between populations by examining the 95% confidence intervals, whereby if the upper and lower limits do not overlap then it is likely that the population has experienced a significant loss of susceptibility. Such a change could be indicative of a resistance problem and should trigger further investigation. Baseline monitoring for *P. xylostella* susceptibility to chlorantraniliprole was conducted by DuPont in the Philippines from 2006 to 2008, at locations in Benguet Province (Buguias and La Trinidad) and Laguna Province (Calauan and Liliw). The field populations surveyed showed high sensitivity to the diagnostic dose rates of 1 ppm (LC_{95}) and 5 ppm (5X LC_{95}) (Edralin et al., 2011). A future shift to significant survivorship (i.e. >20%) at the higher rate would indicate incipient problems and a greater risk of resistance developing. Similar baseline monitoring for both chlorantraniliprole and flubendiamide were conducted in Thailand from 2008 to 2010 (Sukonthabhirom et al., 2011), with susceptible field populations from Tub Berg, Petchabun Province, displaying approximately similar LC_{50} values to those reported in the Philippines survey (Table 1).

The baseline susceptibility to chlorantraniliprole in China was established using 16 geographically distinct field populations of *P. xylostella* collected during 2008–2009 from the principal vegetable producing areas, and all field populations were susceptible, with a narrow variation in LC_{50} among populations (Wang et al., 2010). Similar data has also been collected for susceptible field strains from Brazil (Ribeiro et al., 2014; da Silva et al., 2012) and Japan (Steinbach et al., 2015).

3. Diamide resistance development

Diamondback moth larvae are historically notorious for the speed at which they can develop resistance to new products. This is probably due to their genetic plasticity, a rapid generation time, high fecundity, and the fact that new chemistry is often heavily used, creating high selection pressure in the field. It has been recorded that *P. xylostella* has developed resistance to 93 insecticides (Whalon et al., 2016) and has become one of the most problematic pests to control in cruciferous vegetables. Flubendiamide (Fenos®) and chlorantraniliprole (Prevathon®) insecticides were registered in the Philippines in 2006 and 2007, respectively and Voliam Flexi®, a premix of chlorantraniliprole and thiamethoxam (a neonicotinoid insecticide) in 2008. Flubendiamide (Takumi® 20WDG) was registered in Thailand in May, 2007. These diamides offered growers excellent control of diamondback moth larvae in cruciferous crops, where few other registered products were adequately effective (Andaloro et al., 2011).

The *P. xylostella* population in Thailand first showed evidence of resistance to flubendiamide (and cross-resistance to chlorantraniliprole) just 18 months after flubendiamide was launched. Field observations in 2009 at Bang Bua Thong, Nonthaburi Province, indicated that Takumi® was not providing adequate control. Resistance factors for flubendiamide and chlorantraniliprole in larvae reared from a field population collected in Sai Noi, Nonthaburi Province (a vegetable growing area near Bangkok), were 66.3 and 35.4 respectively in 2010 (Table 2).

In 2011, the Sai Noi field population showed even higher resistance to flubendiamide (RF = 407.2) and chlorantraniliprole (RF = 152.7). Concomitantly, a Tha Muang population, Kanchanaburi Province, showed a very high increase in resistance to flubendiamide (RF = 4817.4) and high resistance to chlorantraniliprole (RF = 87.7), while a field population collected from Lat Lum Kaew, Pathum Thani Province showed an exceptionally high resistance to flubendiamide (RF = 26,602) and high resistance (RF = 775) to chlorantraniliprole. Since the field recommended dose for flubendiamide treatment of *P. xylostella* is only 60 mg/liter, and the Tha Muang and Lat Lum Laew populations had much higher LC_{50}'s of 771 mg/l and 4256 mg/l respectively, and the Sai Noi population an LC_{50} of 65 mg/l, this provided a strong indication of resistance being present (Table 2). These field control failures were followed up by laboratory testing, which confirmed the lack of control as being due to resistance development. Some of the key factors identified as leading to diamide resistance in Thailand were an over-dependency on a single mode of action, minimal crop rotation (due to continuous plantings of crucifers), under-dosing with insecticide (to save on cost), irrigation practices that led to excessive product wash-off (providing opportunities for insect exposure to sub-lethal levels), and a lack of any coherent insecticide resistance management (IRM) strategies (Sukonthabhirom et al., 2011). It was found that Thai farmers had used flubendiamide more than 4–5 times per crop in tank mixes with other insecticides for the simultaneous control of *P. xylostella* and other pests in order to reduce the labour costs associated with spraying.

In September of 2009, field representatives covering the Cebu area of the Philippines received reports of reduced control of *P. xylostella* using diamides. Subsequently, throughout 2010, further field failures were reported. Susceptibility monitoring from multiple locations in Cebu Province showed low mortality rates for both chlorantraniliprole and flubendiamide at the highest diagnostic dose rate of 5 ppm and cross resistance of *P. xylostella* larvae to both diamide products appeared evident. Additional monitoring at locations in Negros Oriental also showed reduced susceptibility at 1 and 5 ppm compared to earlier assays conducted from the northern islands (Edralin et al., 2011). However, more than 2 years after being introduced, flubendiamide and chlorantraniliprole were still providing good control against *P. xylostella* in the highlands of Benguet (Edralin et al., 2011). This may have been because the climatic conditions of the two locations differ considerably: the highlands of Benguet have a mean temperature range of 18.5–23 °C, whereas for the midlands of Cebu the main crop production areas have warmer mean temperatures of 25–28 °C. Under warmer temperatures the total life cycle of *P. xylostella* tends to be shorter (Talekar and Shelton, 1993), leading to higher selection pressures. In Cebu province an over-dependency of