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Pesticide resistance in *Plutella xylostella* (Lepidoptera: Plutellidae) populations from Togo and Benin

L. K. Agboyi[1,2], G. K. Ketoh[2], T. Martin[3,4], I. A. Glitho[2] and M. Tamò[5]*

[1]UR Défense des Cultures et Biotechnologie Végétale, Institut Togolais de Recherche Agronomique (ITRA), Lomé, BP 1163, Togo; [2]Université de Lomé, Laboratoire d'Entomologie Appliquée, Unité de Recherche en Ecotoxicologie, 01 BP 1515 Lomé 01, Togo; [3]Cirad UR Hortsys, Campus de Baillarguet, 34980 Montferrier sur Lez, France; [4]International Centre of Insect Physiology and Ecology (*icipe*), PO Box 30772-00100, Nairobi, Kenya; [5]International Institute of Tropical Agriculture (IITA), Benin Station, 08 BP 0932, Cotonou, Benin

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Abstract. The diamondback moth, *Plutella xylostella* (L.) is the major insect pest of cabbage crops in Togo and Benin. For control, farmers very often resort to spraying chemical insecticides at high dosages with frequent applications. Bioassays were carried out on three populations of *P. xylostella,* two from Togo (Kara and Dapaong) and one from Benin (Cotonou), to assess their level of susceptibility to currently used insecticides. A reference strain of *P. xylostella* from Matuu in Kenya was used as a control. In the laboratory, three insecticide representatives of different chemical families (deltamethrin, chlorpyrifos ethyl and spinosad) were assayed against third instar larvae of *P. xylostella*. Results revealed that *P. xylostella* populations from Dapaong, Kara and Cotonou were more resistant to deltamethrin (13 to 59-fold at LC_{50} level, 149 to 1772-fold at LC_{90} level) and chlorpyrifos ethyl (5 to 15-fold at LC_{50} level, 9 to 885-fold at LC_{90} level) than the reference strain. Spinosad was more toxic to *P. xylostella* populations than the other insecticides with LC_{50} and LC_{90} values less than 1 μg/ml and 15 μg/ml, respectively. However, the population from Cotonou appeared significantly more resistant to spinosad compared to the reference strain. These results are discussed in the light of developing an integrated pest management strategy for reducing the selection pressure of spinosad.

Key words: diamondback moth, integrated pest management, chemical insecticide, resistance

Introduction

In urban, peri-urban and rural areas of Togo and Benin, production of cabbage, *Brassica oleracea* L. (var. *capitata*) (Brassicaceae), is an important income-generating activity for smallholder farmers that provides quick cash income over short periods, and also contributes to food security in West Africa (James *et al.*, 2010). However, production is constrained by the damage caused by a range of insect pests, particularly the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), a cosmopolitan insect considered the most important pest of brassica crops worldwide (Sarfraz *et al.*, 2005; Zhou *et al.*, 2011). Severe attacks have been reported in Africa (Kahuthia-Gathu, 2011; Legwaila *et al.*, 2014). Application of synthetic insecticides remains the most common control strategy (Grzywacz *et al.*,

*E-mail: m.tamo@cgiar.org

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2010). In Togo and Benin, cabbage yield losses have been reported to exceed 30% despite frequent applications of synthetic insecticides (Martin *et al.*, 2006; Agboyi *et al.*, 2013), predominantly organophosphates and pyrethroids (Ahouangninou *et al.*, 2012; Agboyi *et al.*, 2015).

As reported from several tropical countries (Talekar and Shelton, 1993; Baek *et al.*, 2005; Nyambo and Löhr, 2005; Ayalew, 2006; Odhiambo *et al.*, 2010; Santos *et al.*, 2011; Zhou *et al.*, 2011; Legwaila *et al*, 2014), *P. xylostella* populations from Togo and Benin also appear to be resistant to a number of insecticides commonly used in different agroecosystems (Goudegnon *et al.*, 2000; Martin *et al.*, 2006; Godonou *et al.*, 2009; Agboyi *et al.*, 2013). As a reaction to this phenomenon, cabbage producers have entered the vicious circle of increasing both insecticide dosage rates and spraying frequencies (Ahouangninou *et al.*, 2012), which can lead to environmental and health hazards (Pimentel *et al.*, 1997; Gallivan *et al.*, 2001). The intensification of pesticide applications leads to a faster selection of pesticide-resistant populations, and negatively impacts on potential natural enemies that can provide substantial control, e.g. in Kenya (Nyambo *et al.*, 2011). Under this scenario, the bio-insecticide spinosad, which is not commonly used against vegetable pests in Togo and Benin, could be an important new compound for *P. xylostella* management. Spinosad is a metabolite obtained from the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yao, and consists of two major components: the macrocyclic lactones spinosyn A (primary) and spinosyn D (secondary). It is active by contact and/or ingestion, and causes paralysis through the activation of nicotinic acetylcholine receptor and GABA receptor (Thompson *et al.*, 2000). In recent years, spinosad has gained considerable interest in agriculture because of its high activity against insect pests and little ill-effects on beneficial insect species, especially predatory insects (Thompson *et al.*, 2000).

The present study investigated the susceptibility of three *P. xylostella* populations from Togo and Benin to two synthetic insecticides commonly used in cabbage production for the control of *P. xylostella*, as well as the toxicity of spinosad to the same populations.

Materials and methods

Insects

Larvae of *P. xylostella* were collected in major cabbage production areas in the districts of Kara and Dapaong in Togo and Cotonou in Benin between September 2012 and January 2013. A population of *P. xylostella* obtained from Matuu in Kenya was used as a reference strain. This reference strain was collected from a cabbage growing area where the diamondback moth is under biological control by its parasitoid *Cotesia vestalis* (Hymenoptera: Braconidae) (Nyambo *et al.*, 2011). Each population of *P. xylostella* was mass reared separately on 4- to 5-week-old cabbage plants (var. kk-cross) in ventilated cages at 27 ± 2 °C, 75 ± 3% RH and 12:12 (L:D) at the Institut Togolais de Recherche Agronomique (ITRA) station in Dapaong, Togo. Adult moths were fed with a 10% saccharose in water solution. All insecticide toxicity tests were carried out using third instar (L3) larvae.

Bioassays

Insecticide susceptibility was evaluated using a leaf dip bioassay method (Sayyed *et al.*, 2008) with Deltacal 25 EC (deltamethrin 25 g/l) provided by Arysta Life Science (France), and Dursban 480 EC (chlorpyrifos ethyl 480 g/l) and Success Appat 0.24 SC (spinosad 0.24 g/l) provided by Dow AgroSciences (UK). The recommended doses are 1.59 ml/l water for Deltacal 25 EC, 3.17 ml/l water for Dursban 480 EC, and 0.32 ml/l water for Success Appat 0.24 SC. Insecticide solutions were prepared with distilled water to obtain various concentrations. Six concentrations of deltamethrin (0.01, 5, 100, 500, 2000 and 10,000 mg/l water corresponding to 0.00004, 0.02, 0.4, 2, 8 and 40 ml per 100 ml water, respectively), chlorpyrifos ethyl (0.02, 5, 50, 500, 5000 and 50,000 mg/l water corresponding to 0.000004, 0.001, 0.01, 0.1, 1.04 and 10.42 ml per 100 ml water, respectively) and spinosad (0.0001, 0.001, 0.5, 2, 6 and 18 mg/l water corresponding to 0.00004, 0.0004, 0.21, 0.83, 2.5 and 7.5 ml per 100 ml water, respectively) were tested in three replications. Cabbage leaf discs (88 mm diameter) were dipped in the test solution for 20 s and then dried at ambient temperature. Control leaf discs were dipped in distilled water to check the natural mortality (Abbott, 1925). Each dried leaf disc was transferred to plastic Petri dishes (90 mm) containing a single moistened Whatman Grade 1 filter paper. Ten L3 larvae were released in each Petri dish and mortality was observed after 48 h for synthetic insecticides. For spinosad, the assessment of mortality was delayed until 72 h because of its slower activity compared to pyrethroids and organophosphates, which have higher initial rates of penetration through the insect cuticle than spinosad, due to its higher molecular weight (Sayyed *et al.*, 2008; Salgado and Sparks, 2005; Legwaila *et al.*, 2014). Larvae that did not move after prodding with a fine brush were counted as dead. Surviving larvae were reared in their Petri dishes until formation of pupae, and kept until adult emergence.

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Statistical analysis

Mortality data were corrected using Abbott's formula (Abbott, 1925) before submitting to probit analysis (Finney, 1971) using PoloPlus 1.0 (LeOra Software, 2003) for estimating the lethal concentration (LC) values (LC_{10}, LC_{50} and LC_{90}) of each insecticide. The LC values were considered significantly different when their confidence interval (95%) did not overlap. The LCs of the reference population was used for estimating the resistance ratios (RR) of *P. xylostella* populations from Togo and Benin. The RR was considered significant ($P < 0.05$) when the confidence interval did not include the value 1.0 (Robertson *et al.*, 2007).

Results

The three populations of *P. xylostella* showed differences in RRs to deltamethrin and chlorpyrifos ethyl (Table 1). For deltamethrin, the LC_{10} values recorded for *P. xylostella* field populations from Togo and Benin were similar to the reference strain from Kenya. From LC_{10} to LC_{50}, the concentration of deltamethrin increased by 14 times for the reference population, while it increased by 63–407 times for the three other populations (Table 1). The deltamethrin LC_{50} and LC_{90} values for *P. xylostella* populations from Kara, Dapaong and Cotonou were significantly higher as compared to the reference strain. The RR of the three populations at LC_{50} and LC_{90} levels was also significantly higher as compared to the reference strain (Table 1). However, the RR of the Dapaong population was lower than Cotonou population at the LC_{50} level.

For chlorpyrifos ethyl, the LC_{10} values of *P. xylostella* populations from Kara, Dapaong and Cotonou were similar to the reference strain (Table 1). LC_{50} and LC_{90} values of Kara, Dapaong and Cotonou populations and their RRs were significantly higher than for the reference strain. The Cotonou population showed the highest LC_{50} and LC_{90} values for chlorpyrifos ethyl with a RR of 15 and 835, respectively.

Spinosad showed a higher degree of toxicity to *P. xylostella* populations as compared to the two synthetic insecticides (Table 1). When compared to spinosad, the LC_{50} of the Dapaong population was 675 and 8720 times higher for deltamethrin and chlorpyrifos ethyl, respectively; the LC_{50} of Kara population was 7240 and 28,960 times higher for deltamethrin and chlorpyrifos ethyl, respectively; and the LC_{50} of Cotonou population was 2029 and 5353 times higher for deltamethrin and chlorpyrifos ethyl, respectively. There were no significant differences between the spinosad LCs of the Kara and Dapaong populations and the reference strain (Table 1). However, the spinosad LCs of the Cotonou population were significantly higher than for the reference strain. Compared to the reference, the RRs (24- to 27-fold for LC_{10s} and LC_{50s}, respectively) were significantly higher for Cotonou populations.

Discussion

A higher degree of resistance to deltamethrin and chlorpyrifos ethyl was demonstrated for *P. xylostella* populations from Cotonou, Kara and Dapaong, as compared to the reference strain from Matuu, Kenya. The relatively low RRs for the reference strain from Kenya can possibly be explained by the fact that it had been exposed to heavy insecticide regimes for a long time before biological control was introduced, and so still possesses some residual resistance levels (Williamson *et al.*, 2003). In this area, Nyambo *et al.* (2011) carried out releases of the parasitoid *C. vestalis* which resulted in successful establishment, with a 50% reduction in *P. xylostella* damage due to the action of this biological control agent, resulting in more than 75% decrease in the use of synthetic chemicals for diamondback moth control. The population from Cotonou appeared more resistant than either population from Togo, suggesting a higher selection pressure with deltamethrin and chlorpyrifos ethyl currently used by smallholder growers (Ahouangninou *et al.*, 2012). Cotonou is a coastal city with easy access to cheap pesticides imported from neighbouring countries compared to Kara and Dapaong in Northern Togo where insecticides are less accessible. Martin *et al.* (2006) had reported pyrethroid resistance in field populations of *P. xylostella* in Cotonou, but without carrying out any toxicological studies to confirm the resistance. In urban and peri-urban vegetable cultivation areas around Cotonou, cabbage producers apply insecticides, especially pyrethroids and organophosphates, every 3 to 4 days within a 3-month period before harvesting (James *et al.*, 2010), thereby inducing a high selection pressure. The low susceptibility of field populations of *P. xylostella* with regard to chemical insecticides was observed in Ghana (West Africa) where Odhiambo *et al.* (2010) showed high LC_{50} of 386–802 mg/l and 74,617–170,573 mg/l for deltamethrin and chlorpyrifos ethyl, respectively.

The non-significant difference of susceptibility to deltamethrin and chlorpyrifos ethyl at LC_{10} level between populations could be an indication that a small proportion of *P. xylostella* populations from Kara, Dapaong and Cotonou were still susceptible to the two compounds. Field populations might gradually become more susceptible to deltamethrin and chlorpyrifos ethyl if the selection pressure is suppressed, as described by Ninsin *et al.* (2000), where subsequent generations of *P. xylostella* without exposure to acetamiprid decreased their

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Table 1. Toxicity of spinosad and synthetic insecticides to different field populations of *Plutella xylostella* (L3) larvae

Insecticide	*P. xylostella* Locality/Country	n[1]	Slope ± SE	χ^2	LC_{10} (95% CI) (mg [AI]/l)	LC_{50} (95% CI) (mg [AI]/l)	LC_{90} (95% CI) (mg [AI]/l)	RR (95% CI) LC_{10}	RR (95% CI) LC_{50}	RR (95% CI) LC_{90}
Deltamethrin	Matuu/Kenya[2]	180	1.13 ± 0.18	4.87	2.08 (0.05–7.87)	28 (7–59)	382 (164–2656)	–	–	–
	Kara/Togo	180	0.60 ± 0.13	0.70	2.68 (0.03–17.35)	362 (101–950)	56,800 (11,233–2266,329)	1 (0.1–15)	13 (4–34)*	149 (14–1598)*
	Dapaong/Togo	180	0.71 ± 0.13	0.79	2.59 (0.12–11.97)	162 (53–366)	10,404 (3409–84,350)	1 (0.1–11)	6 (2–15)*	27 (5–138)*
	Cotonou/Benin	180	0.49 ± 0.09	8.18	4.09 (0.00–50.53)	1664 (205–2 × 10^5)	676,842 (≥ 21,364)	2 (0.2–20)	59 (16–212)*	1772 (96–32,778)*
Chlorpyrifos ethyl	Matuu/Kenya	180	1.05 ± 0.16	2.74	17.17 (5.39–34.96)	287 (171–524)	4789 (2000–21,248)	–	–	–
	Kara/Togo	180	0.87 ± 0.17	2.66	49.10 (3.81–175.39)	1448 (545–3216)	42,731 (15,121–304,155)	2.9 (0.4–20)	5 (2–14)*	9 (2–51)*
	Dapaong/Togo	180	0.58 ± 0.13	2.86	12.69 (0.18–81.68)	2093 (570–6905)	345,299 (60,493–2 × 10^7)	0.7 (0.5–11)	7 (2–26)*	72 (5–1022)*
	Cotonou/Benin	180	0.43 ± 0.07	3.26	4.55 (0.30–22.08)	4390 (1335–23,059)	4 × 10^6 (4 × 10^5–4 × 10^8)	0.3 (0.03–2)	15 (4–66)*	835 (31–25,324)*
Spinosad	Matuu/Kenya[2]	180	1.10 ± 0.15	4.31	0.002 (0.000–0.007)	0.03 (0.01–0.12)	0.48 (0.17–3.04)	–	–	–
	Kara/Togo	180	0.79 ± 0.11	9.37	0.001 (0.000–0.008)	0.05 (0.002–0.27)	2.13 (0.40–143.28)	0.5 (0.1–3)	2 (0.6–4)	4 (1–16)
	Dapaong/Togo	180	0.78 ± 0.12	6.83	0.006 (0.000–0.034)	0.24 (0.02–1.00)	10.23 (2.27–353.55)	3 (0.4–12)	8 (3–17)*	21 (5–85)*
	Cotonou/Benin	180	1.03 ± 0.13	6.93	0.047 (0.007–0.133)	0.82 (0.25–2.36)	14.53 (4.47–176.03)	24 (5–80)*	27 (11–55)*	30 (9–101)*

CI, confidence interval; χ^2, chi Square; SE, standard error.
[1]Number of larvae of *P. xylostella* tested.
[2]*P. xylostella* population from Matuu, Kenya was used as a reference.
Resistance Ratio (RR): LC value of field population/LC value of the reference population from Matuu. *Resistance ratio significant at $P = 0.05$ (confidence interval does not include the value 1.0).

resistance levels. The relatively higher concentration of chlorpyrifos ethyl required to achieve LC_{10} compared to deltamethrin could be explained by the different modes of action of the two compounds, as described by Tuzmen *et al.* (2008), indicating that, compared to chlorpyrifos, deltamethrin increasingly affects the antioxidant balance as well as the lipid peroxidation level.

Spinosad had a much higher toxicity to *P. xylostella* as compared to the two other chemical pesticides, even at low concentrations. The greater effectiveness of spinosad can be explained by its mode of action, targeting different sites such as nicotinic acetylcholine receptor and GABA receptor (Thompson *et al.*, 2000). Our results suggest the absence of cross resistance with pyrethroid and organophosphate as observed by Oliveira *et al.* (2011) for *P. xylostella* populations, while Sparks *et al.* (1995) showed that spinosad was effective against a *Heliothis virescens* population resistant to cypermethrin (pyrethroid) and profenofos (organophosphate). Legwaila *et al.* (2014) also demonstrated that spinosad can achieve effective control of resistant *P. xylostella* population in Botswana.

Compared to the reference strain, the initial signs of resistance to spinosad in field populations of *P. xylostella* from Cotonou could result from increasing and inappropriate use of this new insecticide since it was first registered in Benin in 2008 and 2009 for cotton and vegetable crops protection, respectively (CNAC, 2012). The discovery of low levels of spinosad resistance in *P. xylostella* populations from Cotonou should be a cause for concern for the use of this recent and still promising biopesticide, as resistance levels will increase if indiscriminate use is unaddressed. Spinosad can be considered as a solution for the control of pesticide-resistant populations of *P. xylostella* in Togo and Benin, but its exclusive and intensive use could lead to resistance in *P. xylostella*, as reported from Pakistan by Sayyed *et al.* (2008).

To maintain effectiveness of spinosad against *P. xylostella*, Zhao *et al.* (2006) developed a proactive management strategy based on rotation between spinosad and other new insecticides, such as indoxacarb or emamectin benzoate in the US and Mexico. In this strategy, a product was withdrawn from the market when resistance was observed, to suppress the selection pressure, and subsequently reintroduced when the susceptibility had recovered. Spinosad is compatible with beneficial insects, including *C. vestalis*, an effective parasitoid against *P. xylostella* in lowlands of East Africa (Nyambo and Löhr, 2005; Kahuthia-Gathu, 2011), South Africa (Kfir, 2005; Manyangarirwa *et al.*, 2009) and West Africa (Goudegnon *et al.*, 2000; Arvanitakis *et al.*, 2014). As described by Rowell *et al.* (2005), the main constraint to the efficacy of this parasitoid in controlling *P. xylostella* in many areas, including Togo and Benin, is the excessive use of broad-spectrum synthetic pesticides. In contrast, it was demonstrated by Liu *et al.* (2007) that *C. vestalis* could develop resistance to spinosad, indicating that this parasitoid could be promoted for wide use in combination with spinosad use without affecting its capacity to control the pest.

Conclusion

Our observations call for a three-way IPM approach based on (i) avoiding the use of pyrethroids and organophosphates, which would otherwise interfere with the activity of *C. vestalis* (Nyambo *et al.* 2011; Sow *et al.*, 2013a); (ii) using insect net covers, a promising technology to reduce pest damage as demonstrated in Benin (Martin *et al.*, 2006; Martin *et al.*, 2015); and (iii) using biopesticides such as neem extracts and the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Sarfraz *et al.*, 2005; Agboyi *et al.*, 2013; Sow *et al.*, 2013b).

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