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Characterization of indoxacarb resistance in *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae): Cross-resistance, stability and fitness cost

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ABSTRACT

The cotton mealybug, *Phenacoccus solenopsis* Tinsley is an important agricultural insect pest of cotton in Pakistan. Some populations of *P. solenopsis* are reported to have significant levels of insecticide resistance, resulting in its control failure. In our study, we selected a population of *P. solenopsis* with indoxacarb, which resulted in a 2223-fold resistance level after five generations compared with the unselected strain. This selected-population also developed a moderate level of cross-resistance to spinosad (36-fold), but only very low cross-resistance to chlorpyrifos (2.3-fold) and bifenthrin (4.5-fold) compared with the unselected population. Indoxacarb resistance in this population was associated with several negative fitness attributes which would slow the selection for resistance in field popualtions. A significant decline in indoxacarb resistance was observed when the selected-population was left unexposed for eight generations. These results suggest that field populations of *P. solenopsis* should be surveyed for indoxcarb resistance and associated cross-resistances, and that alternative chemistries should be adopted and rotated to improve the control and resistance management tactics.

Introduction

Cotton, *Gossypium hirsutum* L. is the key crop in Pakistan's economy as a major source of foreign exchange earnings (Ali et al., 2014). Many chewing and sucking insect pests attack cotton in Pakistan during the season and can significantly reduce yield (Saeed et al., 2007). The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) is a serious pest in Pakistan since 2005 (Abbas et al., 2007), and has reduced the average cotton yield by 50% (Muhammad, 2007). *P. solenopsis* has a broad host range and feeds on numerous vegetable, ornamental, medicinal, and weedy plant species besides cotton (Arif et al., 2009). *P. solenopsis* is an invasive pest in many other countries, including the USA (Williams and Willink, 1992), Argentina (Granara de Willink, 2003), Nigeria (Akintola and Ande, 2008), India (Hodgson et al., 2008), China (Wang et al., 2009), Sri Lanka (Prishanthini and Laxmi, 2009), Australia (Charleston et al., 2010), Iran (Moghaddam and Bagheri, 2010), Brazil (Culik and Gullan, 2005; Silva, 2012), and Turkey (Kaydan et al., 2013).

The long-term and broad-scale reliance on a few classes of insecticides to control cotton pests imposes strong selection pressure for the evolution of resistance (Tian et al., 2014). Control failure of insect pests in the field is due to insecticide resistance (Basit et al., 2011). Switching to alternative classes of insecticides is possible if cross-resistance does not occur, but is often associated with higher management costs for the growers (Ishtiaq et al., 2012). Recently, *P. solenopsis* has been reported to show resistance to new chemical insecticides (Afzal et al., 2015; Saddiq et al., 2015), as well as, to some conventional insecticides (Saddiq et al., 2014).

Several insecticides have been used by growers to control *P. solenopsis* in Pakistan, but significant crop losses have often occurred (Saddiq et al., 2014; Afzal et al., 2015;). Our hypothesis is that general loss of cotton is associated with low efficacy of insecticide and some other management practices, such as spray rates, timing, and coverage. The oxadiazine, indoxacarb, is pyrazoline-type sodium channel blocker and was introduced to control different pests of cotton in the late 1990s using a new mode-of-action (McCann et al., 2001; IRAC, 2012). Indoxacarb is an effective foliar insecticide against various lepidopteran, hemipteran, and homopteran pests (Wing et al., 2000; McCann et al., 2007). Resistance to indoxcarb has been reported in several important insect pests including *Musca domestica* Linnaeus (Shono et al., 2004), *Spodoptera litura* Fabricius (Sayyed et al., 2008a), *Heliothis virescens* Fabricius (Sayyed et al., 2008b), *Helicoverpa armigera* Hubner (Ghodki et al., 2009), *Plutella xylostella* Linnaeus (Sayyed and Wright, 2006),

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Helicoverpa assulta Guenee (Pang et al., 2012) and *Spodoptera exigua* Hubner (Gao et al., 2014).

Fitness costs and life history traits are important factors affecting the evolution of insecticide resistance (Carriere et al., 2004). The resistance to insecticides is often unstable due to correlated fitness costs in the absence of selection (Sayyed et al., 2008b; Basit et al., 2011). Fitness costs due to indoxacarb resistance have been previously studied in detail in *Heliothis virescens*, in which it was found that selected population exhibited significantly lower fecundity, egg hatchability, net reproductive rate, and intrinsic rate of population natural increase (Sayyed et al., 2008b).

Herein, we report our study to assess the current response of population of *P. solenopsis* collected from Multan Punjab province, Pakistan to indoxcarb. The population was exposed to laboratroy selection with indoxcarb for five generationas and then selection was removed for eight generations. The toxicity of indoxcarb and other insecticides was tracked over different populations of *P. solenopsis*. Moreover, fitness factors, such as survival rate, fecundity, developmental times, net reproductive rates, and intrinsic rate of population natural increase were measured for different populations.

Materials and methods

Insects

The insect population of *P. solenopsis* consisting of 200–400 individulas (nymphs and adults) were collected from a cotton field located in Central Cotton Research Institute, Multan (30.1978° N, 71.4697° E), Pakistan. The population was brought and reared in the laboratory on leaves and twigs of China rose, *Hibiscus rosasinensis* L. in transparent plastic jars (12 cm × 24 cm) and designated as Field pop. The culture was refreshed after every 2–3 days and maintained at standard laboratory conditions at 27 ± 2 °C, 60 ± 5% RH and 14:10 h (L/D photoperiod) (Afzal et al., 2015).

Insecticides

Several formulations of insecticides were tested in bioassays: indoxacarb (Steward®, 150 SL; DuPont, Pakistan), spinosad (Tracer®, 24 SC; Arysta Life Sciences, Pakistan), chlorpyrifos (Lorsban®, 40 EC; Dow Agro Sciences, Pakistan) and bifenthrin (Talstar®, 10 EC; FMC, Philadelphia, PA).

Selection and bioassays

The Field pop at G_2 was divided into two sub populations in the laboratory. One sub population was reared without any exposure to indoxacarb and designated as Unsel pop. Second sub population was continuously selected from G_3-G_7 by exposing indoxacarb with different values of LCs of indoxacarb bioassay performed at G_2. Three-day old 2nd nymphal instar was used for selection by leaf-dip protocol. This population was named as Indoxa-SEL. Number of nymphs selected per generation ranged from 100 to 200.

Bioassays were conducted on Field pop, Unsel pop and Indoxa-SEL strain of *P. solenopsis* on three-day old 2nd instar nymphs by leaf dip protocol (Ahmad et al., 2007). Leaves of China rose were dipped for approximately 10 s in freshly synthesized solution. Treated leaves were air dried for 1–2 h at room temperature before putting them in the Petri dishes. Five concentrations of each insecticide were tested. Five replicates of each concentration were made. The range of concentrations of indoxacarb was 70.3–1125 mg/mL for Field pop (G_2), 18.8–300 mg/mL for Unsel pop, 11,402.9–182,446 mg/mL for Indoxa-SEL (G_8), and 3125–50,000 mg/mL for Indoxa-SEL (G_{15}). Spinosad concentrations were used in the range 12.5–200 mg/mL for Field pop (G_2), and Unsel pop, 250–4000 mg/mL for Indoxa-SEL (G_8) and 93.8–1500 mg/mL on Indoxa-SEL (G_{15}). Chlorpyrifos concentrations were used in the range 71.9–1150 mg/mL, 9–143.8 mg/mL, 25–400 mg/mL, and 3.1–50 mg/mL for Field pop (G_2), Unsel pop, Indoxa-SEL (G_8), and Indoxa-SEL (G_{15}), respectively. The range of bifenthrin concentrations was 9.4–150 mg/mL for Field pop (G2), 6.3–100 mg/mL for Unsel pop and Indoxa-SEL (G_8), and 3.1–50 mg/mL for Indoxa-SEL (G_{15}). In a separate control, leaves were only treated with water (five replicates of five nymphs). A total of 150 nymphs were tested in each bioassay including control. The bioassays were conducted under the standardized laboratory conditions described above in section "Insect". Nymphal response of bioassay and/or selection was assessed after 48 h for conventional insecticides and 72 h for new chemistry insecticides.

Test for stability

Indoxa-SEL strain was further reared for eight additional generations without any insecticide exposure to measure stablility of resistance. A decline in resistance (DR) to insecticides was measured by following formula:

$$R = [\log(\text{final } LC_{50}) - \log(\text{initial } LC_{50})]/n,$$

where n is the number of generations reared without insecticide selection pressure.

Reciprocal crosses

For the fitness study, two reciprocal crosses $Cross_1$ and $Cross_2$ were made. $Cross_1$ was made by mass mating of males (3) and females (15) from the Indoxa-SEL pop (G_8) and Unsel pop, respectively. Another reciprocal cross $Cross_2$ was made as a result of the mass mating of females (15) and males (3) from the Indoxa-SEL pop (G_8) and Unsel pop, respectively (Afzal et al., 2015).

Biological parameters

Seventy five newly hatched crawlers were taken randomly from Unsel pop, $Cross_1$, $Cross_2$ and Indoxa-SEL pop (G_8) to study the different biological traits affected by indoxacarb resistance. These crawlers from each population were divided into three replicates. After weighing, they were kept inside plastic jars (11 × 8 cm) and allowed to feed on fresh *H. rosasinensis* leaves. Survival rate from crawler hatching to 2nd instar, male nymphal duration from crawler to 2nd instar, female nymphal duration from crawler to 3rd instar, crawler weight, pupal weight, weight of adult male and female and pupal duration were recorded. Moreover, emergence rate of healthy male adults, fecundity, egg viability, developmental time (DT) from egg to adult, longevity and generation time of both sexes were also determined.

In order to determine net replacement rate (R_o), the following formula was used according to Jia et al. (2009).

$$R_o = N_{n+1} \div N_n$$

where N_n represents the neonate numbers of the parental generation, and N_{n+1} is that of the next generation.

Relative fitness (R_f) was estimated according to Cao and Han (2006) as following formula:

$$R_f = R_o \text{ of tested population} \div R_o \text{ of Unsel pop}$$

The formula of Birch (1948) was used to calculate intrinsic rate of natural increase (r_m)

$$r_m = \text{In}R_o \div DT$$

where DT is developmental time from egg to adult for male or female.