Evaluation of the susceptibility status of spiny bollworm *Earias biplaga* (Walker) (Lepidoptera: Noctuidae) to Bt cotton in South Africa

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The bollworm complex of cotton in South Africa consists of the African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), red bollworm, *Diparopsis castanea* (Hamps.) (Lepidoptera: Noctuidae), and the spiny bollworms, *Earias biplaga* (Walker) (Lepidoptera: Noctuidae) and *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae) (Green *et al*. 2003). Larvae of these species are regarded as major pests of cotton since they tunnel into the growing tips, flowering buds and cotton bolls, resulting in damage and subsequent yield losses (Bennett 2015). Genetically modified (GM) transgenic Bt cotton plants that express Cry proteins have been cultivated in South Africa since 1998 to control these lepidopteran pests (Thirtle *et al.* 2003). Bollgard®, expressing the Cry1Ac protein, was first commercially produced in South Africa in 1998 and discontinued after the 2010 growing season (ICAC 2007). Bollgard II®, also registered for control of lepidopteran pests on cotton, is a stacked transgenic cotton which expresses two Bt proteins, namely Cry1Ac and Cry2Ab2 (Taverniers *et al*. 2008; Showalter *et al*. 2009).

Since the first deployment of Bt crops there has been concern regarding resistance development of target pests (Tabashnik 1994; Gould 1998). Field-evolved resistance is defined as a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field (Tabashnik 1994). Cases of resistance development to Cry1Ac protein expressed by Bollgard® cotton have been confirmed in Pakistan and northern China for *H. armigera* (Zhang *et al*. 2011; Alvi *et al*. 2012), in India and China for *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (Dhurua & Gujar 2011; Alvi *et al*. 2012; Wan *et al*. 2012) and the United States for *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Luttrell *et al*. 2004; Anikulmar *et al*. 2008; Tabashnik *et al*. 2009; Tabashnik & Carrière 2010).

The high-dose/refuge strategy has been implemented to delay the evolution of resistance in pests of Bt cotton (Cohen *et al*. 2000). Refugia are areas planted with non-Bt plants (Cohen *et al*. 2000), adjacent to an area with Bt plants that express a high dose of the Bt toxin. The aim of this strategy is that the non-Bt planting will sustain large numbers of susceptible individuals of the pest and to prevent the increase of pesticide-resistant insects by providing havens in which the non-resistant insects survive. The hybrid first generation offspring produced by mating between susceptible and resistant adults are killed when they feed on Bt plants. If the mating is random, mating between the rare homozygous resistant adults that emerged from Bt plants will more likely be with the homozygous susceptible adults that emerge from non-Bt plants. Mating between these adults produce hybrid F1 progeny that cannot survive on Bt plants (Liu *et al*. 1999). The offspring that are not resistant to the Bt toxin complete their life cycle if they are in the refuge area and will again produce offspring that are not resistant to Bt. Wild host plants of polyphagous pests such as *E. biplaga* may also contribute to sustaining susceptible populations of this pest (Green *et al.* 2003). These structured refugia contribute to the delay of resistance development in most insect pests (Tabashnik *et al*. 2008, 2009), with the exception of the pink bollworm in India where farmers did not comply with the refuge strategy requirements (Stone 2004; Bagla 2010).

Screening of *E. biplaga* for resistance to Bt cotton has never been done in South Africa. It is important that monitoring of resistance levels of pests to Bt crops is done to evaluate changes occurring in the field. The aim of this study was to determine the response of *E. biplaga* larvae to Bt cotton in South Africa.

Two rearing colonies of *E. biplaga* were established. Larvae were collected from an experimental cotton field at Potchefstroom (26°68′668″S 27°15′801″E), and moths were collected with light traps at Rustenburg (25°72′401″S 27°28′944″E), South Africa during the 2013/14 growing season. Larvae were reared in cotton bolls in plastic tubs

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