High levels of resistance and cross-resistance to B*acillus thuringiensis* Cry1 toxins in *Heliothis virescens* are due to reduced toxin binding and pore formation.

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Heliothis virescens is one of the most important insect pests affecting cotton and other crops. For *H. virescens* control, insecticides based on the *Bacillus thuringiensis* (Bt) parasporal pesticidal proteins have been developed. These proteins posses a unique mode of action (Knowles 1994). After ingestion by the susceptible insect, they are solubilized and activated by midgut enzymes to toxic forms. Activated toxins bind to receptors and insert into the membrane of the columnar cells of the midgut epithelium, leading to cell lysis by osmotic shock. In 1996, transgenic cotton plants producing Cry1Ac toxin (the most active Bt toxin against *H. virescens*) were produced and commercialized in the US to control *H. virescens* populations in the field. One of the

major concerns regarding the use of Bt transgenic plants is the observation that target insects can become resistant. Generation of transgenic cotton lines expressing different Bt toxins and conservation of refuges for susceptible individuals has been suggested as potential methods to prevent resistance. To assure the efficacy of these approaches as well as the usefulness of Bt toxins, information on the mode of action as well as on the potential mechanisms of resistance is needed.

Resistance to Bt insecticides has been reported to occur in wild populations of certain insect species (Tabashnik *et al.* 1990; Shelton *et al.* 1993) as well as in laboratory selected insects (Gould *et al.* 1992; Whalon *et al.* 1993; Oppert *et al.* 1994; Gould *et al.* 1995). To date, no incidences of resistance are reported for field populations of *H. virescens*. The YHD2 strain of *H. virescens* was developed in the laboratory from susceptible individuals (strain YDK) by selection against Cry1Ac (Gould *et al.* 1995). The YHD2 strain developed the highest levels of resistance known in this insect to Cry1Ac, and high levels of cross-resistance to other toxins including Cry1Aa, Cry1Ab and Cry1Fa. At the time, cross-resistance to Cry1Fa was unexpected, since this toxin does not share high homology with the Cry1A family of toxins. These results suggested that cross-resistance can be an important pitfall for strategies of Bt toxin pyramiding or alternation in transgenic plants. Binding competition experiments (Jurat-Fuentes and Adang, submitted) suggest that Cry1A and Cry1Fa cross-resistance is due to shared toxin binding sites.

The resistant strain YHD2 has been continuously selected with Cry1Ac toxin since 1995. Presently, the level of Cry1Ac resistance is 40-fold greater than in 1995. In bioassays, neonate YHD2 larvae were more than 230,000-fold resistant to Cry1Ac, about

2000-fold cross-resistant to Cry1Ab, more than 130-fold to Cry1Fa and more than 20-fold to Cry1Aa. Activity of these toxins against the YDK susceptible strain larvae was high and similar to previous reports (Lee *et al.*, 1995). Cry1Ea was not toxic against either strain.

Although several mechanisms of resistance have been proposed for insect resistance to Bt toxins, the alteration of toxin binding to the specific receptors in the midgut is the best documented (Ferré *et al.* 1991; Lee *et al.* 1995; Van Rie *et al.* 1990).

To study the mechanism of resistance in the YHD2 *H. virescens* strain, binding and pore formation properties of Cry1Aa, Cry1Ab, Cry1Ac and Cry1Fa Bt toxins on brush border membrane vesicles (BBMVs) were measured. BBMV were prepared (Wolfersberger *et al.* 1987) from larval midguts of susceptible (YDK) and resistant (YHD2) *H. virescens* strains.

Purified Cry1A toxins were ^{125}I-labeled (Garczynski *et al.*, 1991) and used in BBMV binding assays. In these assays, quantitative toxin binding values can be obtained. All the ^{125}I-Cry1A toxins tested bound in a specific and saturable manner to BBMV from the susceptible strain, while specific toxin binding to resistant BBMV was not observed. This dramatic decrease in toxin binding was also observed in qualitative binding assays (Western blots) with Cry1A as well as Cry1Fa toxins.

To study if this decrease in binding is important for toxin action, we measured toxin pore formation with a light scattering technique (Carroll and Ellar, 1993). In this technique, the pore forming activity of Cry1 toxins on a BBMV suspension was monitored as changes in the scattered light due to toxin-induced vesicle permeation in a hyperosmotic medium. Neither Cry1Aa, Cry1Ab, Cry1Ac nor Cry1Fa toxins were able

to permeate BBMV from the resistant strain in any of the experiments. These toxins permeated susceptible vesicles to different extents, Cry1Ac being the most active toxin in the light scattering assay. Cry1Ea (not toxic against *H. virescens*) did not affect either susceptible or resistant BBMV, while Nystatin (a pore-forming antibiotic) permeated both BBMV suspensions to the same extent.

These results suggest that the dramatic reduction in toxin binding can account for high levels of resistance and cross-resistance to Cry1A and Cry1Fa toxins observed in the YHD2 strain. Thus, toxin-binding reduction prevents pore formation, which causes larval survival.

To study the transmission of the Cry1Ac resistance trait, binding and pore formation abilities of Cry1Ac to BBMV from larvae of the F1 crosses between susceptible and resistant adults were studied. Similar binding and pore formation values were obtained for reciprocal crosses, suggesting that resistance is not sex-linked. Furthermore, measured values were more similar to the values for susceptible BBMVs, indicating a partially recessive transmission of the resistant trait, as previously suggested from bioassay experiments (Gould *et al.*, 1995).

One of the possible explanations for reduced toxin binding is the absence of specific binding proteins on the resistant BBMV. To study this possibility, ligand-blotting experiments were done (Garczynski *et al.* 1991). The same patterns of binding molecules for all Cry1A and Cry1Fa toxins were detected for blotted susceptible and resistant BBMV proteins. This indicates that toxin-binding molecules are still present in the resistant BBMV, and they are able to bind toxin only under denaturing conditions.

Experiments in our laboratory are presently aimed at elucidating the molecular mechanism by which decreased toxin binding is achieved in the resistant insects. Information obtained from this work will be useful in understanding Bt toxins mode of action and to design resistance prevention strategies.

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