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# Lack of Cry1Fa Binding to the Midgut Brush Border Membrane in a Resistant Colony of *Plutella xylostella* Moths with a Mutation in the *ABCC2* Locus

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**Previous studies reported "mode 1" *Bacillus thuringiensis* resistance in a colony of diamondback moths (NO-QA), and recently, this resistance has been mapped to an ABC transporter (*ABCC2*) locus. We report the lack of binding of Cry1Fa to insects derived from this colony and compare our data with those from other insects with *ABCC2*-associated resistance.**

Although *Bacillus thuringiensis* has been used in foliar sprays for more than 30 years, the commercialization in 1996 of the first genetically modified crops protected against insects (Bt crops) increased the importance of *B. thuringiensis* as a source of insecticidal proteins for the control of insect pests. The extensive use of *B. thuringiensis* proteins in sprays or in Bt crops has led to the evolution of insect resistance in the field (6, 18). Field resistance to *B. thuringiensis* sprays was first described in populations of the diamondback moth, *Plutella xylostella* (5, 6, 12, 19). One of the first resistant colonies (NO-QA) was derived from a Hawaiian population that had evolved resistance to Dipel 2X (Abbott Laboratories, North Chicago, IL) in the field and that was further selected in the laboratory for additional resistance to the same formulated product (20). NO-QA insects showed "mode 1" resistance, which entails strong resistance to at least one Cry1A protein (in this case, Cry1Aa, Cry1Ab and Cry1Ac, all three being found in Dipel), little or nil cross-resistance to Cry1C, recessive inheritance, and reduced binding of at least one Cry1A protein (16). Further characterization showed that resistant insects were cross-resistant to Cry1F and Cry1J (13, 17). Genetic studies indicated that resistance to Cry1A and Cry1F proteins was determined by a single autosomal locus (14) and that in a diet-adapted descendant of NO-QA (NO-QAGE), the Cry1Ac resistance locus was genetically linked to a membrane transporter gene (*ABCC2*) (3).

For susceptible diamondback moths, an integrated model for the binding sites of Cry1A and Cry1Fa proteins was proposed (2). In this model, at least two binding sites are involved: one which is shared by Cry1Aa, Cry1Ab, Cry1Ac, and Cry1Fa and a second which is specific for Cry1Aa and apparently does not contribute to its toxicity. Binding studies with NO-QA resistant larvae showed extremely reduced binding of Cry1Ab and Cry1Ac but not of Cry1Aa (15). Therefore, it was proposed that resistance in NO-QA is due to a mutation that alters the common binding site for Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F.

The aim of the present study was to check whether binding of Cry1Fa was indeed reduced in NO-QAGE insects and in this way to confirm (or reject) the hypothesis that the multiple resistance in this colony is due to an alteration of the shared binding site affecting binding not only of the Cry1A proteins but of Cry1F as well. To achieve this objective, Cry1Fa and Cry1Aa (as a control) were labeled with $^{125}$I and biotin, respectively, and binding to brush border membrane vesicles (BBMV) from susceptible (Lab-V) and resistant (NO-QAGE) larvae was tested.

BBMV were prepared from whole last-instar larvae by the differential magnesium precipitation method (23). For binding studies, Cry1Fa and Cry1Aa proteins were obtained as protein inclusions from recombinant *B. thuringiensis* and *Escherichia coli* strains, respectively. Solubilized toxins were prepared as trypsin-activated and chromatography-purified proteins (10). Bioassays were performed using Cry1Fa protein inclusions. While all susceptible insects died at a dose of 10 μg/ml, NO-QAGE insects all survived a dose of 200 μg/ml. Cry1Fa was labeled with $^{125}$I by the chloramine-T method according to the conditions described by Hernández-Rodríguez et al. (9), and the specific radioactivity obtained was 0.5 μCi/μg. To test total binding, increasing amounts of BBMV from both colonies were incubated with 27 nM labeled Cry1Fa, in a final volume of 0.1 ml of binding buffer (10 mM $Na_2HPO_4$, 1 mM $KH_2PO_4$, 137 mM NaCl, 2.7 mM KCl, pH 7.4, 0.1% bovine serum albumin [BSA]) for 1 h at 25°C. An excess of unlabeled toxin (15-fold) was used to determine the nonspecific binding. After incubation, samples were centrifuged at 16,000 × *g* for 10 min and the pellet was washed with cold binding buffer. The results with $^{125}$I-Cry1Fa showed an increase in the specific binding (obtained after subtracting nonspecific binding from the total binding) when BBMV from susceptible insects were used (Fig. 1A), whereas no specific binding was observed with BBMV from NO-QAGE insects (Fig. 1B).

As a control, binding of biotin-labeled Cry1Aa was performed with 0.2 mg/ml of BBMV proteins. As expected, biotinylated Cry1Aa bound to BBMV from both susceptible and resistant insects (Fig. 2). Addition of an excess of unlabeled Cry1Aa notably reduced binding of biotinylated Cry1Aa in both cases, indicating that most of the binding observed was specific.

Reduced binding has been described as a primary mechanism of insect resistance to Cry1A family proteins in several insect spe-

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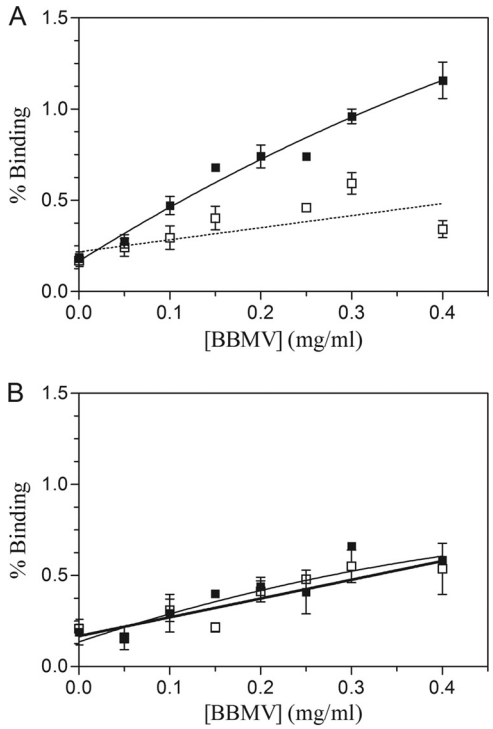


**FIG 1** Binding of $^{125}$I-labeled Cry1Fa at increasing concentrations of BBMV from susceptible (A) and resistant (B) diamondback moths. ■, total binding; □, nonspecific binding.

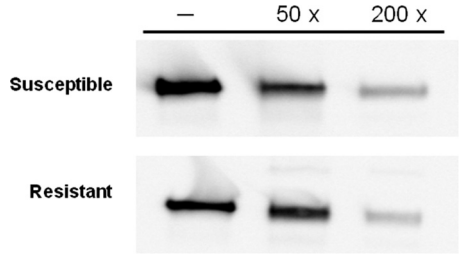


**FIG 2** Binding of biotinylated Cry1Aa to BBMV from susceptible and resistant diamondback moths, in the absence of competitor (lanes labeled −) or in the presence of a 50- or 200-fold excess of unlabeled Cry1Aa.

cies (6), to Cry1Fa in *Heliothis virescens* (11), and, more recently, to Cry2A proteins in two *Helicoverpa* species (4). However, the alteration of the target site for Cry1Fa was only indirectly proposed for resistant colonies of diamondback moths based on the heterologous competition of this protein with labeled Cry1A proteins (2, 8). Mutations in the gene encoding ABCC2 have been implicated in the Cry1Ac resistance of *H. virescens*, *P. xylostella* (NO-QAGE), *Trichoplusia ni*, and *Bombyx mori* (1, 3, 7). A resistant colony of *H. virescens* (YHD2), believed to carry mutations in the genes for both cadherin and ABCC2, was cross-resistant to Cry1Fa and lacked binding of Cry1Fa (11). However, neither toxicity nor binding of this protein has been reported for subsequent colonies in which the two mutations were separated (7). The Cry1Ac-resistant *T. ni* colony showed only weak cross-resistance to Cry1Fa (22). In this colony, binding of Cry1Ab and Cry1Ac was reduced (22), but no binding data for Cry1Fa were reported. Interestingly, in the *B. mori* Cry1Ab-resistant colony, no loss of binding of Cry1Ab was observed; the effect of Cry1Fa was not investigated (1). In the current study, we have looked at *P. xylostella*, the other species in which resistance has been linked to *ABCC2*. We have found a strong correlation between cross-resistance and loss of binding for Cry1Fa, complementing previous findings for Cry1Aa, Cry1Ab, and Cry1Ac. The lack of Cry1Fa binding in this resistant strain supports the common binding site for Cry1Ab, Cry1Ac, and Cry1Fa in the model proposed by Ballester et al. (2). The *T. ni* and *P. xylostella* colonies show a different cross-resistance pattern, despite their resistance phenotypes both being linked to the same *ABCC2* locus. This may represent different mutations in *ABCC2* affecting susceptibility to the toxins in a different manner (for example, in different toxin binding sites), although the lack of current evidence indicating the functionality of ABCC2 as a binding site for *B. thuringiensis* toxins should be noted. Alternatively, it is possible that mutations in *ABCC2* affect susceptibility to the toxins indirectly. It is noteworthy that in the resistant *T. ni* colony, a reduction in the expression of aminopeptidase N (APN1), which is a known receptor for *B. thuringiensis* toxins, was observed via a transregulatory mechanism (21). An unknown, indirect effect may also be able to provide an explanation for the differences in Cry1Ab binding observed with the *T. ni*, *P. xylostella*, and *B. mori* resistant colonies. Thus, while mutations in *ABCC2* can confer resistance in a variety of insects, the effect may be indirect and consequently the resistance phenotype may reflect differences in physiology between the species.

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