Cry9Ca1 Toxin, a *Bacillus thuringiensis* Insecticidal Crystal Protein with High Activity against the Spruce Budworm (*Choristoneura fumiferana*)

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Insecticides derived from the soil bacterium *Bacillus thuringiensis* are gaining worldwide importance as environmentally desirable alternatives to synthetic chemicals for control of pests in agriculture, forestry, and public health (3). In North America, commercial products based on the HD-1 strain of *B. thuringiensis* subsp. *kurstaki* are widely used to control defoliating forest lepidopterans, in particular the spruce budworm (*Choristoneura fumiferana*) (14). The HD-1 strain produces several insecticidal crystal proteins (ICPs) from the cry1A and cry2A gene families, as per revised nomenclature (1, 1a). Proteins from these families are active against a wide variety of larval lepidopterans, including many species with worldwide agronomic importance. Although these proteins are toxic to spruce budworms (16, 17), the ongoing discovery of new toxin genes (4) offers a prospect of identifying more effective ICPs.

A novel crystal protein designated Cry9Ca1 was recently reported (10). This ICP, previously named CryH1, showed particular promise for insect control because of its broad activity spectrum against larval lepidopterans, including several members of the Noctuidae family, and its specific receptor-binding characteristics. Here, we report its toxicity to spruce budworm and two other economically important forest pests, gypsy moth (*Lymantria dispar*) and black army cutworm (*Acetabia fennica*), and to a species of general interest, the Chinese silkworm (*Bombyx mori*).

The toxicity of Cry9Ca1 was compared with the toxicities of Cry1Ab6, -1Ba1, -1Ca2, -1Da1, and -1Ea1, ICPs that were tested previously against the same species (16, 17). We also tested Cry1Fa2 (11), formerly CryIF, which was not included in the earlier assays. The cry1Ab6 toxin gene was isolated from *B. thuringiensis* subsp. *kurstaki* HD-110 (Biotechnology Research Institute, Montreal, Canada) (7), cry1Ba1 from *Bacillus thuringiensis* subsp. *entomocidus* HD-110 (Plant Genetic Systems, Ghent, Belgium) (8), cry1Ca2 from *B. thuringiensis* subsp. *aizawai* HD-133 (Biotechnology Research Institute) (10a), cry1Da1 from *B. thuringiensis* subsp. *aizawai* HD-68 (Plant Genetic Systems) (9), cry1Ea1 from *B. thuringiensis* subsp. *darmstadiensis* (Plant Genetic Systems) (19), and cry1Fa2 from *B. thuringiensis* subsp. *aizawai* PS811 (Mycogen, San Diego, Calif.) (11). The cry1Ca1 gene had been modified to eliminate the trypsin cleavage site at position 164 (Arg→Lys substitution) (10). The Cry1Fa2 toxin protein was produced in *Pseudomonas fluorescens* (Mycogen formulation MYX837-936) by the CelIcap encapsulation process (5). All other toxins were obtained by expression of cloned genes in *Escherichia coli* as described previously (17).

Insect assays were conducted with activated toxins obtained by digestion of the ICPs with gut juice from silkworm according to the procedure of Gringorten et al. (6). The rationale for using silkworm gut juice was discussed previously (16). The concentration of activated toxin in the final solutions was estimated by electrophoresis and scanning densitometry of Coomasie blue-stained sodium dodecyl sulfate–12% polyacrylamide gels (17) and was checked periodically for stability during storage. The relative amounts of activated toxin as a proportion of the total protein content were as follows: 0.775 for Cry1Ab6, 0.478 for Cry1Ba1, 0.599 for Cry1Ca2, 0.659 for Cry1Da1, 0.246 for Cry1Ea1, 0.529 for Cry1Fa2, and 0.234 for Cry9Ca1.

The insecticidal activity of the toxin preparations was determined by force-feeding larvae of spruce budworm (day 1 sixth instars), gypsy moth (early fourth instars), black army cutworm (early fifth instars), and silkworm (day 1 fourth instars) obtained from the Canadian Forest Service rearing facility in Sault Ste. Marie. The force-feeding technique and general test conditions were described previously (16). We also compared the toxicities of Cry1Ab and Cry9Ca1 to fifth-instar spruce budworm, for which a droplet-imbing technique (18) was used because fifth instars are too small to force-feed. Both techniques yield the same results (18). Six twofold dilutions of each toxin solution were tested in three to five replicates of 10 to 20 larvae per dilution. Effective dose estimates were obtained by probit analysis (13) of percent mortality observed after 48 h in the silkworm assays (50% lethal dose) or of percent failure to produce frass after 72 h, a measure of feeding inhibition, in all other assays (50% frass failure dose [FFD₅₀]) (15). Control response (larvae dosed with the gut juice-buffer solution, n = 10 to 20 per replicate) was <2%.

The Cry9Ca1 toxin was highly active against the spruce budworm, with an FFD₅₀ of <10 ng per sixth instar in the force-feeding assays (Table 1). Cry9Ca1 was three- to fourfold more toxic at both the 50 and 95% response levels than Cry1Ab6,
which until now was the most effective toxin known (16, 17). Similar results were obtained for fifth instars with the droplet-imbing method (Table 2). Fifth instars were two to three times more susceptible than sixth instars (Tables 1 and 2), which is in agreement with earlier observations on stadial susceptibility to crystal-spore suspensions of commercial products (18). The Cry1Fa2 toxin was approximately ninefold less active than Cry1Ab6 at the FFD95 level (Table 1), which makes it the least effective budworm toxin of the Cry1 proteins tested so far. The Cry9Ca1 toxin was not active against gypsy moth, as it failed to elicit a feeding-inhibitory response at the highest concentration tested (Table 3). The Cry1Fa2 toxin also exhibited very low toxicity to this species (Table 3). Both proteins were highly toxic to silkworm, with 50% lethal dose estimates in the range of 10 to 20 ng per larva. Neither toxin elicited a feeding-inhibitory response in larvae of black army cutworm at the highest concentrations tested.

It must be noted that in our previous assays we observed a higher potency of the Cry1Ca2 and Cry1Ea1 toxins against the highest concentrations tested.

The Cry9Ca1 toxin was not active against gypsy moth, as it failed to elicit a feeding-inhibitory response at the highest concentration tested.

The Cry9Ca1 toxin is unique for its activity against several members of the Noctuidae, including Spodoptera exigua, Spodoptera littoralis, Mamestra brassicae, Heliothis virescens, and Agrotis segetum (10). Other noctuids, such as Helicoverpa armigera and Spodoptera frugiperda, were not susceptible. The black army cutworm is another noctuid that does not respond to Cry9Ca1. Results of this study confirm earlier observations that the black army cutworm is not susceptible to any of the Cry1 toxins tested to date, which makes it one of the most refractory members of this family.

Since Cry9Ca1 binds to different receptors than do other ICPs that are currently used in conventional sprays or transgenic plants (10) and resistance to ICPs often involves a binding-site modification (20), this toxin is expected to become a valuable tool for management of insect resistance. The cry9Ca1 toxin gene has already been used to engineer corn that is resistant to European cornborer and other pests (10). Considering its high toxicity to spruce budworm, it might also be a good candidate for engineering budworm resistance in conifers in place of or in combination with Cry1Ab, which is currently being used (2).

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REFERENCES


10u. Masson, L. Unpublished data.


18. van Frankenhuyzen, K., J. L. Gringorten, J. Dedes, and D. Gauthier. 1997. Susceptibility of different instars of the spruce budworm (Lepidoptera: Tortricidae) to *Bacillus thuringiensis* var. *kurstaki* estimated with a droplet-feeding method. J. Econ. Entomol. 90:560–565.
