

DBT Study Title: Protein Thermal Stability

Dow AgroSciences LLC Study Titles: Thermolability of Cry1F(synpro) Delta-Endotoxin, Unpublished Report of Dow AgroSciences LLC (Study ID 010069). Thermolability of Cry1Ac(synpro) Delta-Endotoxin, Unpublished Report of Dow AgroSciences LLC (Study ID 010085).

Introduction

These studies were conducted to measure the lability of Cry1F protein and Cry1Ac protein when exposed to heat. After incubating aqueous formulations of Cry1F or Cry1Ac at various temperatures for 30 minutes, neonate tobacco budworm (*Heliothis virescens*), a susceptible insect, were exposed to artificial insect diets that had been treated with aliquots of the formulations. Insect mortality was recorded after 6 days. SDS-PAGE and Western analysis were also conducted to verify the presence of Cry1F or Cry1Ac after exposure to heat.

Quality Assurance. The study conduct, data, protocol, protocol changes/revisions, and final report were inspected by the Quality Assurance Unit of Dow AgroSciences LLC in Indianapolis, Indiana.

Archiving. Raw data and the original copy of the final report are archived at Dow AgroSciences' testing facility archives in Indianapolis, Indiana.

Materials and Methods

Test Substances. Separate lyophilized powders containing 15% Cry1F or 14% Cry1Ac, isolated from recombinant *Pseudomonas fluorescens*, were used in these studies.

Heat Treatment and Bioassay. The thermolability of Cry1F and Cry1Ac was determined by incubating aqueous formulations of the individual proteins (in a potassium phosphate buffer) at 60 °C, 75 °C or 90 °C for 30 minutes. Positive controls were maintained in a refrigerator at 4 °C for 30 minutes and samples of the buffer alone were used as negative control. Following incubation, the heated samples were cooled on ice and applied to the surface of artificial insect diet in bioassay trays. Neonate tobacco budworm (TBW), *Heliothis virescens*, were grown in the trays and mortality data were collected after 6 days.

SDS-PAGE and Western Blot Analysis. These were conducted on aliquots of the aqueous formulations to check for protein presence.

Results

Cry1F Protein. Mortality results for Cry1F are shown in Table 1. Cry1F incubated at 60 °C produced 100% mortality of TBW. Incubation at 75 °C and 90 °C resulted in 0% mortality. No mortality resulted from exposure to the negative control (potassium phosphate buffer) and 100%

mortality was noted following exposure to the positive control (Cry1F formulation maintained at 4 °C).

Table 1. Cry1F Mortality Results for TBW

Treatment	% Mortality
Positive Control (Cry1F @ 4 °C)	100%
Cry1F @ 60 °C	100%
Cry1F @ 75 °C	0%
Cry1F @ 90°C	0%
Negative Control (buffer)	0%

The identity of the Cry1F protein under treatment of 4 °C, 60 °C, 75 °C or 90 °C was confirmed by both SDS-PAGE and Western blot analyses.

Cry1Ac Protein. Mortality results for Cry1Ac are shown in Table 2. Cry1Ac incubated at 60 °C produced 100% mortality of TBW. Incubation at 75 °C resulted in 7% mortality and incubation at 90 °C resulted in 0% mortality. No mortality resulted from exposure to the negative control (potassium phosphate buffer) and 100% mortality was noted following exposure to the positive control (Cry1Ac formulation maintained at 4 °C).

Table 2. Cry1Ac Mortality Results for TBW

Treatment	% Mortality
Positive Control (Cry1Ac @ 4 °C)	100%
Cry1Ac @ 60 °C	100%
Cry1Ac @ 75 °C	7%
Cry1Ac @ 90°C	0%
Negative Control (buffer)	0%

The identity of the Cry1Ac protein under treatment of 4 °C, 60 °C or 75 °C was confirmed by both SDS-PAGE and Western blot analyses.

Conclusions

These results confirm that Cry1F and Cry1Ac proteins are deactivated after exposure to 75 °C or 90 °C for 30 minutes. SDS-PAGE and Western blot analysis confirmed that inactivated Cry1F and Cry1Ac proteins were present after exposure to heat.