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INTERACTION OF BT AND PROTEINASE INHIBITORS ON THE GROWTH AND DEVELOPMENT OF THE COTTON BOLLWORM, HEL ICOVERPA ZEA

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Abstract

Interactions of Bt and proteinase inhibitors were investigated by monitoring growth and gut proteinase activities of the bollworm, *Helicoverpa zea*. Three proteinase inhibitors, benzamidine, phenylmethylsulfonyl fluoride (PMSF), and N-α-tosyl-L-lysine chloromethyl ketone (TLCK), were combined with a sub-lethal dose of Bt protoxin, *Bacillus thuringiensis*, in artificial diet and fed to newly molted 3rd-instar bollworm. Synergistic effect of Bt and proteinase inhibitors caused significant decreases in larval body weight and length over time. General, tryptic, and chymotrypsin-like proteinase activities were significantly suppressed by Bt+inhibitor treatments.

Introduction

Currently, Bt toxins used for cotton insect control have a narrow specificity against lepidopteran pests only. Due to large scale adoption of Bt cotton and reduced chemical applications, many originally secondary pests, such as the tarnished plant bug *Lygus lineolaris* and stink bugs, have emerged to cause serious economic loss of cotton production. Another worry lurking behind wide deployment of Bt cotton is adaptation and resistance development in pests. Because proteins are critical nutrients for insect growth and development, proteinases, such as trypsin, play an essential function for protein digestion and absorption in insects. The introduction of proteinase inhibitors into host plants may substantially suppress protein digestion, and subsequently achieve insect control in a broad range through nutrient deficiency. Secondly, introduction of proteinase inhibitors into gut will certainly modify biochemical balance within target insects feeding on Bt cotton. Bt toxins may become stable and more effective against target insects.

Materials and Methods

Helicoverpa zea neonates were reared on regular artificial diet in the laboratory at a temperature of 26.5°C with 40-60% humidity. Newly molted 3rd instar larvae were fed various artificial diets to monitor the effects of added proteinase inhibitors, Bt, and any synergistic effects of proteinase inhibitors with Bt. Larvae were regularly measured for changes in weight and length.

After two, four and six days of feeding on test diets, midguts of larvae were dissected, and gut enzyme solution was prepared. Azocasein was used to measure general proteinase activity. Substrates N-Benzoyl-DL-arginine 4-nitroanilide hydrochloride (BAρNA) and N-Succinyl-Ala-Ala-Pro-Phe p-nitroanilide (SAAPFρNA) were used to determine trypsin and chymotrypsin-like activities. Absorbance at 405 nm was monitored using a micro titer plate reader.

Results

Interaction of Bt and proteinase inhibitors on insect growth:

Three proteinase inhibitors, benzamidine, PMSF, and TLCK, were selected to examine interaction on larval growth with Bt toxin. After 7-day feeding on Bt or benzamidine treated diet, both larval body weight and length were significantly reduced compared to those of control (Table 1. Percentages of reduction were

Table 1. Interaction of Bt Toxin and Proteinase Inhibitors on Growth of the Bollworm, *Helicoverpa zea*

Treatments	Average Growth Reduction (% Relative to Control)	
	Larval Body Weight	Larval Body Length
Bt	-37	-10
Inhibitor	-15	-5
Bt+Inhibitor	**-69**	**-35**

averaged from treatments of Bt with three individual proteinase inhibitors). Bt+benzamidine treatment reduced body weight by 78%, comparing 31% and 11% reduction from treatment of Bt or benzamidine, respectively. Body length was reduced by 44% in treatment of Bt+benzamidine, and only 10% or less decreasing was obtained from individual treatment of Bt or benzamidine. Combination of Bt with PMSF or with TLCK also significantly retarded larval growth than treatment of Bt alone or PMSF alone.

Table 2. Interaction of Bt Toxin and Proteinase Inhibitors on Gut Proteinase Activities of the Bollworm, *Helicoverpa zea*

Treatments	Average Proteinase Activity Reduction (% Relative to Control)		
	General	Trypsin	Chymotrypsin
Bt	-18	-44	-37
Inhibitor	-13	-33	-25
Bt+Inhibitor	**-59**	**-79**	**-65**

Interaction of Bt and proteinase inhibitors on gut proteinase activities:

Bollworm gut proteinase activities were measured after treatment of Bt with three proteinase inhibitors. Results Table 2 (Percentages of reduction were averaged from treatments of Bt with three individual proteinase inhibitors) show that synergistic or additive effect was obtained from Bt+inhibitor treatments. All three Bt+inhibitor treatments showed consistent synergism when determined by using general proteinase substrate azocasein. Trypsin and chymotrypsin activities were also significantly suppressed by Bt+inhibitor treatment compared to treatment of Bt alone or inhibitor alone.

Discussion

Bt-inhibitor interaction is very likely to be achieved by suppression Bt degradation when proteinase inhibitor is present. Although low dose inhibitor treatment did not critically suppress hydrolyzation of dietary proteins for nutritional requirements, it might significantly suppressed gut proteinases, and subsequently suppression of Bt degradation by gut enzymes. More Bt toxins stay actively to bind to targets and create more damage to the gut membrane. Extended gut damage results in further suppression of production of gut proteinases, and subsequently decreasing of proteinase activities showing in Table 2.

In summary, we investigated the interaction between Bt toxins and proteinase inhibitors within gut of target insect. Information from this study may lead to understand how to enhance Bt toxicity and how to slow down tolerance or resistance development in insects. Currently in the Mid-south area, the great concern is the increasing of pest status of plant bugs due to the wide deployment of Bt cotton varieties. Further worry lurking behind wide range use of Bt cotton is potential threat to the Bt cotton by adaptation and resistance development in lepidopteran pests. One of potential solutions is introduction of proteinase inhibitors into ingestion and digestion systems of target pests. Excessive proteinase inhibitors directly suppress proteolytic processes of not only lepidopteran insects, but also non-lepidopteran insects, such as plant bugs with sucking mouth part. Proteinase inhibitors also synergize Bt toxicity by stabilizing Bt toxins within insect guts.