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EFFECT OF AVIDIN ON THE GROWTH AND MORTALITY OF FIVE LEPIDOPTERAN INSECTS

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Abstract

Artificial diet was supplemented with different concentrations of avidin, 10 ppm and 100 ppm, to determine effects on growth and mortality of five lepidopteran insects: *Helicoverpa zea, Heliothis virescens, Spodoptera frugiperda, Spodoptera exigua,* and *Anticarsia gemmatalis.* All insects were placed on diet immediately after hatching and observed until death or pupation occurred. At a concentration of 10 ppm, avidin had little or no effect on growth and mortality as compared with the control. However, at a concentration of 100 ppm, mortality of all tested insects was approximate 100%.

Introduction

Avidin is a bioactive glycoprotein found naturally in the egg white of bird, reptile, and amphibian eggs. Avidin has a very strong affinity for the vitamin biotin, which is a coenzyme required for enzymes that catalyze carboxylation, decarboxylation, and transcarboxylation reactions in all forms of life (Kramer, 2004). Insecticidal activity of chicken avidin has been known since 1959 (Levinson and Bergmann, 1959). Sequestration of biotin causes vitamin deficiency and in turn leads to stunted growth and mortality of many insect species. To explore potential use of avidin for cotton insect control, we incorporated various concentrations of avidin into artificial diet to study effect on five major cotton insects.

Materials and Methods

Five lepidopteran species were selected for avidin bioassay, including bollworm *Helicoverpa zea* (Boddie), tobacco budworm *Heliothis virescens* (F.), fall armyworm *Spodoptera frugiperda* J.E. Smith, beet armyworm *Spodoptera exigua* (Huebner), and velvetbean caterpillars *Anticarsia gemmatalis* (Huebner). To determine the effect of avidin on larval growth, larvae were reared on artificial diet (Bioserve, Frenchtown, NJ) supplemented with avidin (Sigma A 9275, St. Louis, MO) at different concentrations. Low melting point agarose (Ultrapure L.M.P Agarose, Invitrogen, Carlsbad, CA) was used in place of regular agar in diet. Diet was cooled in a water bath to 33^{o}C before the appropriate avidin concentration was added. All species were reared at a temperature of 26.5^{o}C and 40-60% humidity. Three repetitions of ten larvae were studied for each experiment. Data were statistically analyzed with SAS program (SAS Institute).

Results

Bollworm:

Seven concentrations of avidin were used for bollworm bioassay. Larval body weight was significantly reduced on 15 and 20 ppm avidin diet (Table 1). Ten ppm avidin had no negative effect on larval body weight increasing compared with control. Avidin at concentrations 40 ppm or higher significantly reduced larval body weight on day 7. No measurement was taken for these higher doses avidin treatments because approximately 100% larvae died after day 7. Three treatments with 10, 15, and 20 ppm avidin showed certain reduction of larval body length on day 7 and day 10 (Table 2). However, the body length from low dose avidin treatments reached same level of control on day 12. Body length of the larvae on 40, 60, and 80 ppm avidin diet was

Table 1. Larval Body Weight Changes of the Bollworm on Diet Treated with Seven Concentrations of Avidin

Avidin Concentrations	Larval Body Weight (mg±SE)			
	Day 5	Day 7	Day 10	Day 12
Control	8.4±0.4	16.1±1.1	24.2±1.3	23±1.38
10 ppm	7.9±0.2	15.1±0.2	22.8±1	21±0.8
15 ppm	7.8±0.6	13.6±1.4	16.7±2.7	22±2.1
20 ppm	6.3±0.23	12.11±1	14.7±0.15	20.5±1.2
40 ppm	7.3±0.4	4±0	All dead	All dead
60 ppm	6.9±0.6	2±0	All dead	All dead
80 ppm	6.8±0.4	3±0	All dead	All dead
100 ppm	7±0.36	9.5±0.2	All dead	All dead

reduced to a level below initial length. Bollworm larvae showed different mortality rates at different avidin concentrations (Table 3). Avidin concentrations at or above 40 ppm resulted in 97-100% dead larvae after 10 days. Twenty ppm avidin killed up to 43% larvae. Approximately 23% larvae were killed by 10 and 15 ppm avidin.

Table 2. Larval Body Length Changes of the Bollworm on Diet Treated with Seven Concentrations of Avidin

Avidin Concentrations	Larval Body Length (mm±SE)			
	Day 5	Day 7	Day 10	Day 12
Control	8.9±0.4	53±5.2	146±16.1	235±40.5
10 ppm	25.5±0.46	87.5±3.8	164±8	198±21.4
15 ppm	8.12±0.3	43.84±2.7	64.78±6.4	143.4±13.8
20 ppm	6±2.4	25±7.5	49.4±10.2	93.24±18.5
40 ppm	6.9±1.4	8±0	All dead	All dead
60 ppm	5.8±1	2.4±0	All dead	All dead
80 ppm	4.5±0.5	1.4±0	All dead	All dead
100 ppm	8±0.84	12±0.6	All dead	All dead

Other Four Lepidopteran Insects:

Larvae of the tobacco budworm, fall armyworm, beet armyworm, and velvetbean caterpillars were placed on diet treated with 10 and 100 ppm avidin. Treatment with 100 ppm avidin significantly reduced larval body weight and length of all four insects. Only minor reduction of body weight and length was observed in 10 ppm avidin treatment. Larval mortality reached 100% after 10 day feeding on 100 ppm avidin diet. Ten ppm avidin killed approximately 40% beet armyworm larvae and less that 20% of other three insects.

Discussion

Currently in the Mid-south areas, transgenic Bt cotton has been widely adopted to battle for a few lepidopteran insects, such as bollworm and tobacco budworm. As Bt cotton effectively controlled major lepidopteron insects, the tarnished plant bug and other sucking mouthpart insects have become serious problem because of reduced chemical applications and insecticide resistance development in these insects (Snodgrass, 1996; Snodgrass & Scott, 2000). Therefore, it is highly demanded that transgenic cotton should be equipped with genes to target a wide range of pests. Avidin is a bioactive protein that targets vitamin biotin, which is present in all life forms. Avidin is safe for human because avidin has already been a daily food protein. In this study, we found that growth of all five lepidopteran insects was greatly retarded. Avidin at as much as 40 ppm could kill 100% larvae. We tried avidin bioassay on the tarnished plant bug. The experiment was unsuccessful due to lacking of artificial diet for the bug. Once the artificial diet is available, we shall carry out experiment to explore potential use of avidin to control plant bug and other non-lepidopteran insects. Providing that biotin may be present in the sucking mouthpart insects, avidin can potentially be used on cotton to control important lepidopteran insects and sucking mouthpart insects as well.

Table 3. Larval Mortality of the Bollworm on Diet Treated with Seven Concentrations of Avidin

Avidin Concentrations	Larval Mortality (%±SE)			
	Day 5	Day 7	Day 10	Day 12
Control	0±0	0±0	0±0	0±0
10 ppm	3.3±3.4	10±3.4	20±6.7	23±3.4
15 ppm	0±0	13.3±6.6	13.3±6.6	23±3.3
20 ppm	17±8.8	27±8.8	33.3±11.5	43±12
40 ppm	20±0	33.3±3.3	97±3.3	97±3.3
60 ppm	10±0	47±3.3	97±3.3	97±3.3
80 ppm	13.3±3.3	57±3.3	97±3.3	97±3.3
100 ppm	15±0	60±10	95±3.4	100±0

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