

Avidin and streptavidin as insecticidal and growth inhibiting dietary proteins *

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Accepted: March 9, 1993

Key words: avidin, streptavidin, biotin, insecticide, stored product insects, corn borer, beetles, moths, plant resistance

Abstract

Avidin was found to be an insecticidal and growth inhibiting dietary protein for five species of Coleoptera (red flour beetle, *Tribolium castaneum*, confused flour beetle, *T. confusum*, sawtoothed grain beetle, *Oryzophilus surinamensis*, rice weevil, *Sitophilus oryzae*, and lesser grain borer, *Rhyzopertha dominica*) and two species of Lepidoptera (European corn borer, *Ostrinia nubilalis*, and Indianmeal moth, *Plodia interpunctella*). At levels ranging from 10 to 1000 ppm in the diet depending on the species, avidin retarded the growth and caused mortality of all seven species. Addition of biotin to the avidin-treated diets for *T. castaneum*, *T. confusum*, *R. dominica*, and *O. nubilalis* prevented the growth inhibition and mortality caused by avidin. Streptavidin exhibited similar insecticidal and growth inhibiting activity towards *T. castaneum* and *O. nubilalis*. The results support the hypothesis that feeding avidin or streptavidin to insects causes a biotin deficiency which in turn leads to stunted growth and mortality. Avidin and streptavidin are insect growth inhibiting proteins whose genes potentially could be manipulated into plants and provide host plant resistance to insect pests.

Introduction

Avidin is a water-soluble tetrameric glycoprotein (MW \approx 60 kDa) found in chicken egg white where

* This research was conducted by the Agricultural Research Service, U.S. Department of Agriculture in cooperation with the Department of Biotechnology Research, Pioneer Hi-Bred International. Mention of an insecticide or proprietary product does not constitute a recommendation or endorsement by the USDA.

it accounts for approximately 0.05% of the total protein (Green, 1975; Stevens, 1991). It is composed of four identical subunits with each having a high affinity ($K_D = 10^{-15}$ M) for the vitamin, biotin. A nonglycosylated homologous protein, streptavidin, is present in the culture supernatant of *Streptomyces avidinii* (Bayer *et al.*, 1990) and it also binds very tightly to the vitamin. Because of their high affinity for an essential growth factor, avidin and streptavidin have the potential to act as antinutritional proteins towards organisms that

require the vitamin for normal growth and development. In chicken egg white, avidin may function as an antibiotic and protect the chick embryo from pathogenic organisms that require biotin (Stevens, 1991).

Biotin is an essential nutrient for many species of insects (Dadd, 1985). For example, Baker (1975) demonstrated that the rice weevil, *Sitophilus oryzae*, requires biotin in the diet for normal larval development. Avidin or streptavidin could therefore inhibit insect growth by causing a biotin deficiency if the binding protein sequestered the vitamin in the gut and prevented uptake by tissues. Previous studies have shown that dietary avidin caused mortality of the house fly, *Musca domestica* (Levinson & Bergmann, 1959), the hide beetle, *Dermestes maculatus* (Levinson *et al.*, 1967), the fruit fly, *Drosophila melanogaster* (Bruins *et al.*, 1991), and the flour mite, *Acarus siro* (Levinson *et al.*, 1992). The antivitamin, desthiobiotin, reduced egg production in the olive fruit fly, *Dacus oleae* (Tsiropoulos, 1985).

Vitamin binding proteins such as avidin and streptavidin are examples of antinutritional proteins that have potential to act as growth inhibiting or toxic proteins towards insects. The genes for these proteins could be transferred to and manipulated in plants to enhance host resistance to insect pests. In this study we determined the effects of avidin or streptavidin added to wheat and semi-artificial agar-based diets on the growth of seven species of Coleoptera and Lepidoptera, including the red flour beetle, *Tribolium castaneum*, confused flour beetle, *T. confusum*, saw-toothed grain beetle, *Oryzaephilus surinamensis*, rice weevil, *S. oryzae*, lesser grain borer, *Rhyzopertha dominica*, European corn borer, *Ostrinia nubilalis*, and Indianmeal moth, *Plodia interpunctella*. At ppm levels avidin or streptavidin substantially retarded larval growth of or was toxic to seven insect pests. Addition of biotin to the avidin-treated diets prevented the detrimental effect of the vitamin binding protein and allowed normal growth to occur in four of the species. The results suggest that feeding avidin or streptavidin to insects causes a biotin deficiency that leads to stunted growth and mortality, and that plants

which express genes encoding avidin or streptavidin may exhibit increased resistance to insect pests.

Materials and methods

Chemicals. Avidin, streptavidin, d-biotin, sorbic acid, methyl-p-hydroxybenzoate and glycerol were obtained from Sigma Chemical Co. (St. Louis, MO). One mg of avidin or streptavidin binds 10–15 μ g of d-biotin.

Insects. Stored product insects were obtained from U.S. Grain Marketing Research Laboratory stock cultures. *T. castaneum*, *T. confusum*, and *O. surinamensis* were maintained on a mixture of 95% hard red winter wheat flour mixed with 5% torula yeast. *S. oryzae* and *R. dominica* were reared on whole wheat, and *P. interpunctella* on a standard laboratory diet (McGaughey, 1985). The stock culture of *O. nubilalis* was maintained on a semi-artificial diet at the Pioneer Entomology Laboratory (King & Hartley, 1985).

Bioassays. For *T. castaneum*, *T. confusum*, and *O. surinamensis*, the bioassay diet consisted of ground wheat germ from Quaker Oats Co., Manhattan, Kansas. The germ was ground through a 20-mesh screen using a Wiley micromill. For *P. interpunctella*, the diet contained 89.7% ground wheat germ, 10% glycerol, 0.15% sorbic acid, and 0.15% methyl- ρ -hydroxybenzoate. Avidin, streptavidin and/or biotin were dissolved in water (1.5 mL) and mixed with the wheat germ or flour based diet (1 g) at room temperature. The cracked wheat diet was prepared by grinding whole wheat briefly in a coffee grinder and soaking 1 g with 0.5 mL water containing additives. The diets were frozen, lyophilized and then equilibrated at 29 °C and 60–80% relative humidity. Flour-based diets were either broken into small pellets of about 5 mm diam. or ground into a powder in a mortar with a pestle prior to equilibration. Wheat germ diets were also ground in a mortar prior to equilibration.

Bioassays of stored product insects were usu-

ally conducted at 29 °C with a 16L:8D photoperiod at 60–70% relative humidity, except for *P. interpunctella* which was reared at 70–80% RH. Fifteen eggs of either *T. castaneum*, *T. confusum*, or *O. surinamensis* were used to infest 0.3 g of wheat germ or 5 g of cracked wheat. Newly hatched larvae were weighed on a Cahn C-31 microbalance. Depending on the species and its development rate, larvae in the infested diets were not disturbed until 6 to 11 days post hatching when 10 larvae were selected at random, weighed individually, and then isolated in vials containing approximately equal amounts of the original diet. The insects were weighed at several time intervals until they either pupated, died or the experiment was terminated after approximately 60 days. Mortality and adult eclosion were also recorded.

For *P. interpunctella* 15 eggs were added to 1.65 g of wheat germ or 3 g of cracked wheat. For *S. oryzae* five replicates of 10 g each of flour pellets were infested with 20 adults for one week, after which the insects were removed. For *R. dominica* three replicates of 1 g each of cracked wheat were infested with 15 eggs, or three replicates of 5 g each of flour pellets were infested with 60 eggs. Rice weevil and lesser grain borer larvae were not observed directly in these diets because they feed internally, but evidence of their activity was obtained using an automated acoustic detection system (Hagstrum *et al.*, 1991). The infestation rate of these two species is strongly correlated to the number of sounds made by the larvae as counted by a frequency counter (Vick *et al.*, 1988). Sound was monitored for 20 sec intervals over a period of 10 min for a total of 30 intervals. Background sound of uninfested diets was subtracted from that of infested samples. The number of adults that emerged was recorded, and the time interval between infestation and adult emergence from the pellets or grain was noted. In some of the experiments, adults that developed were placed on a new diet and numbers of their progeny determined.

Bioassays of the European corn borer were conducted at 25 °C with a photoperiod of 14L:10D essentially as described by Czaplá and

Lang (1990) with plastic tray modifications described by Balasubramaniam *et al.* (1991). Avidin or streptavidin was incorporated into the semi-artificial diet at 10–300 ppm levels based on the wet weight of the diet which contained approximately 84% water. Sixteen neonate larvae were used per treatment. They were reared individually on 1.2–1.3 g of semi-artificial diet, and the larval weight and mortality were recorded seven days after infestation.

Statistics. Statistical tests of significant differences between diet treatments were performed by using the Tukey HSD multiple comparison (Tukey, 1951), independent t-test, chi-square or linear regression analysis available in SYSTAT software (Wilkinson, 1989).

Results

Red flour beetle. Avidin or its nonglycosylated homolog, streptavidin, was added to wheat germ at 10, 100 or 1000 ppm, and the mixture was fed to *T. castaneum* (Fig. 1). In the 100 and 1000 ppm avidin- and 1000 ppm streptavidin-treated diets, larval growth rates were substantially slower than those of the control and 10 ppm avidin-treated groups of larvae. After several weeks, larvae fed 1000 ppm avidin- or streptavidin-treated diets were also significantly smaller than those fed 100 ppm avidin diet. Mortality in the larval or pupal stages was 100% with 1000 ppm avidin or streptavidin, whereas no mortality occurred with 0 and 100 ppm avidin ($n = 10$ larvae). Two subsequent experiments testing 100 ppm avidin under similar environmental conditions resulted in $65 \pm 5\%$ mortality (mean ± 0.5 range). These results demonstrate that avidin or streptavidin cause severe growth inhibition and mortality of *T. castaneum* when present at 100–1000 ppm in wheat germ.

The effect of a protein such as avidin in an insect bioassay may be influenced by the environmental conditions, such as intensity of lighting, under which an experiment is conducted. Bruins *et al.* (1991) demonstrated that avidin increased

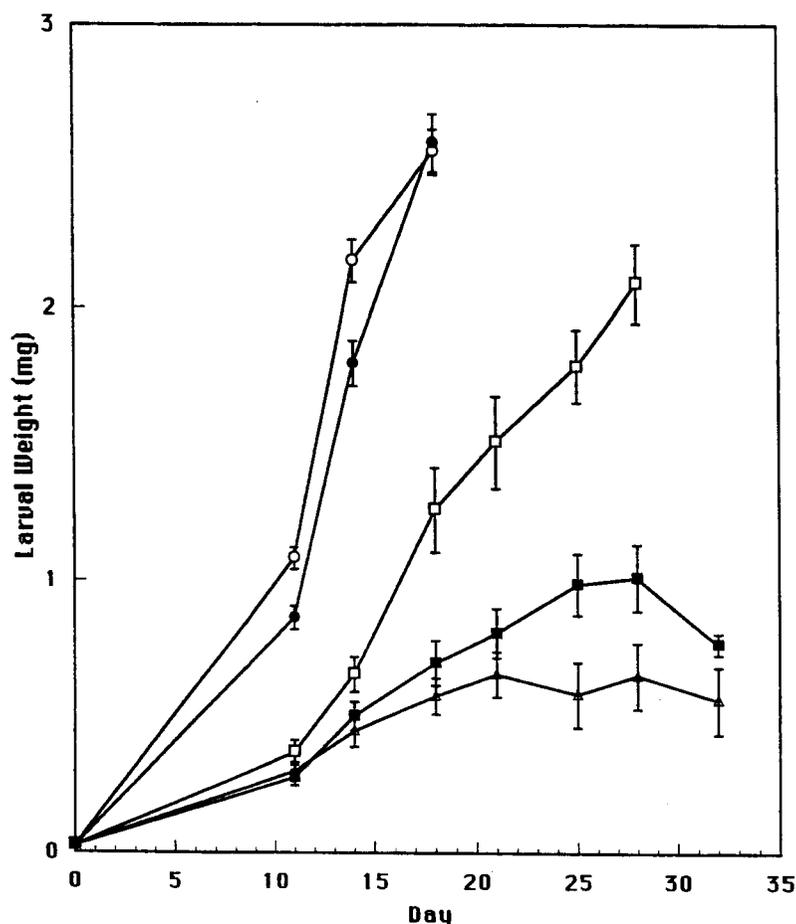


Fig. 1. The effect of avidin and streptavidin added to wheat germ on the growth of the red flour beetle, *Tribolium castaneum*. Avidin levels in ppm: 0 (—○—) (a); 10 (—●—) (a); 100 (—□—) (b); 1000 (—■—) (c); and streptavidin: 1000 (—△—) (c). Mean values \pm s.e. ($n = 4-10$). Data with the same letter (in parentheses following legend identification) were not significantly different ($\alpha = 0.05$) as determined by Tukey statistical analysis of individual regression coefficients of larval weights between days 11 and 28.

the developmental time of *D. melanogaster* and that developmental time was further increased when bioassays were conducted under high relative to low light intensities. Therefore, we tested the effect of the fluorescent lighting in our rearing chamber on the growth of *T. castaneum* using diets with 0 or 1000 ppm avidin and with or without exposure to light. In our experiments there was no significant effect of light on the larval weight on day 15 in the two groups of diets (Tukey HSD multiple comparison, $n = 9-10$, $\alpha = 0.05$), and the final mortalities were 100% in the avidin treatment and 0% in the control. The mortality

was 80% on day 33 in the avidin treatment with a 16L:8D photoperiod, which was significantly higher than the mortality in the corresponding treatment with continuous darkness (10% mortality, chi-square test, $n = 10$, $\alpha = 0.05$). The mortality in the latter treatment reached 80% on day 51, a result suggesting that mortality is delayed by lower light intensity.

Humidity may also be a factor which influences the mortality caused by avidin. At 65% RH in the dark, 0 and 60% mortality occurred at 10 and 100 ppm avidin, respectively, whereas 0 and 100% mortality occurred at 45% RH in the dark.

However, the mortalities at the two different humidities were not significantly different (chi-square test, $n = 6-10$, $\alpha = 0.05$). Although additional work is necessary to determine the subtle effects of light and humidity on avidin toxicity, our results demonstrate that 100 ppm avidin is toxic to flour beetles under environmental conditions similar to those under which grain and grain products are stored.

Approximately one mg of commercial preparations of avidin or streptavidin that we used bind approximately $13 \mu\text{g}$ of d-biotin. Biotin, added to avidin-treated wheat germ at a level two-fold higher (26 ppm) than was calculated to saturate the binding protein, rescued *T. castaneum* from

the effects of avidin (Fig. 2). Larvae in the 1000 ppm avidin-treated diet were again substantially smaller than larvae in the control group. However, larvae fed a diet containing either 26 ppm biotin or 26 ppm biotin plus 1000 ppm avidin grew at rates comparable to that of control larvae. In addition, there was no mortality, compared to 90% mortality in the 1000 ppm avidin-treated diet group. Table 1 compares the weights of *T. castaneum* larvae on the day of the test that control larvae were at or very near their maximal size. The highest dose of avidin reduced larval weight by approximately 75% and biotin supplementation prevented the antinutritional effect of avidin. This result suggests that the mode of ac-

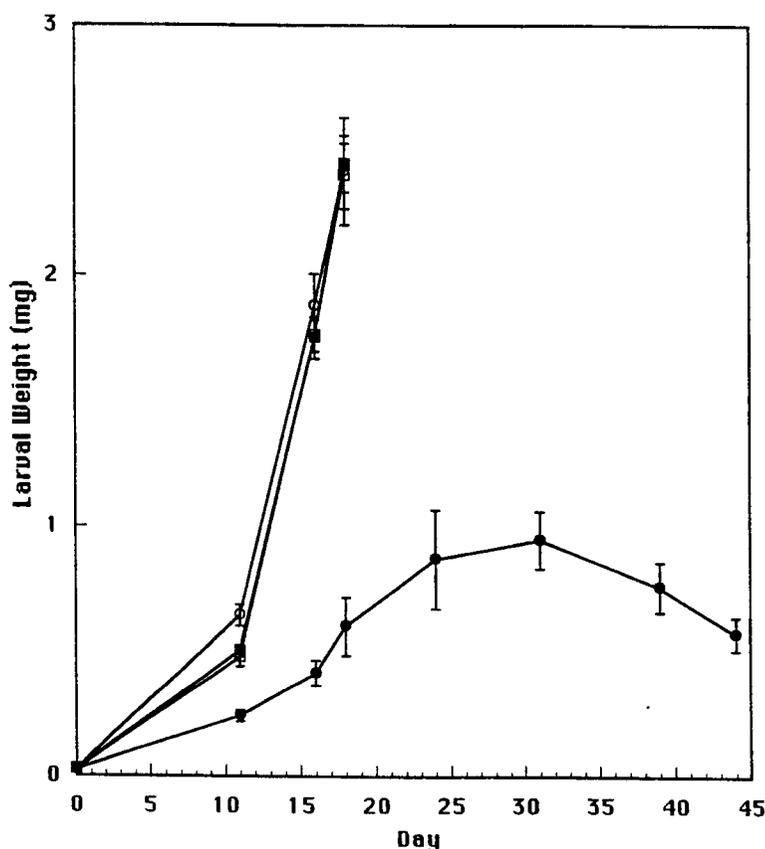


Fig. 2. The effect of avidin and/or biotin added to wheat germ on the growth of the red flour beetle, *Tribolium castaneum*. Avidin levels in ppm: 0 (-O-) (a); 1000 (-●-) (b); 1000 ppm avidin + 26 ppm biotin (-□-) (a); and 26 ppm biotin (-■-) (a). Mean values \pm s.e. ($n = 5-10$). Data with the same letter (in parentheses following legend identification) were not significantly different ($\alpha = 0.05$) as determined by Tukey statistical analysis of individual regression coefficients of larval weights between days 11 and 18.

Table 1. The effect of avidin, streptavidin, and biotin on the larval weight of *Tribolium castaneum*

Treatment	Level (ppm)	Weight (mg) ¹
Control	-	2.55 ± 0.08 (a)
Avidin	10	2.58 ± 0.11 (a)
	100	1.26 ± 0.15 (b)
	1000	0.70 ± 0.08 (c)
Streptavidin	1000	0.57 ± 0.07 (c)
Biotin	26	2.60 ± 0.09 (a)
Avidin + Biotin	1000 26	2.46 ± 0.30 (a)

¹ Weights were determined at day 18 of treatment. Mean ± s.e. (n = 9-10). Data with the same letter were not significantly different ($P < 0.05$) as determined by Tukey statistical analysis.

tion of avidin in insect diets is a sequestration of biotin, preventing absorption by tissues.

The effect of an additive in an insect diet can be influenced by the size of particles present and the spatial distribution of the additive in the particles. We compared the mortalities for *T. castaneum* in both coarsely and finely ground diets that were soaked in solutions of avidin prior to lyophilization. As in the case with finely ground wheat germ, avidin was toxic at 100 and 1000 ppm in cracked wheat (70 and 90% mortality, respectively, which was significantly higher than the 10% mortality in the control, chi-square analysis, $n = 10$, $\alpha = 0.05$). Therefore, in both coarsely and finely ground diets, avidin caused severe growth retardation and mortality of the red flour beetle larvae.

Confused flour beetle. Avidin also retarded the growth of *T. confusum* when added to wheat germ at 1000 ppm (Fig. 3). As demonstrated with *T. castaneum*, 26 ppm biotin was sufficient to prevent the effects of avidin on both growth rate and mortality. Mortality for the avidin-treated group after several weeks was 44%, which was significantly greater than the 0% mortality for the control group (chi-square test, $n = 9$, $\alpha = 0.05$).

Sawtoothed grain beetle. The weights of sawtoothed grain beetle larvae that were fed

1000 ppm avidin in wheat germ for 9 and 13 days after the eggs hatched were significantly lower than the weights of larvae fed 100, 10 and 0 ppm (Fig. 4). For example, 13 days after hatching larvae fed 1000 ppm avidin were about two- to three-fold smaller than larvae fed none or lesser amounts of avidin. After four weeks, mortalities were 30, 30, 80 and 100% in the 0, 10, 100 and 1000 ppm avidin-treated groups, respectively. Some of the mortality was apparently caused by injuries that occurred when larvae were handled during their weighing. The mortalities in the 100 and 1000 ppm treatments were significantly higher than those from the 0 and 10 ppm treatments (chi-square test, $n = 10$, $\alpha = 0.05$). Most of the mortality occurred in the larval stage. However, two pupae were found in the 100 ppm treatment and one in the 1000 ppm treatment, but all three died before adult eclosion. The results demonstrate that avidin causes mortality of *O. surinamensis* at concentrations of 100 ppm or higher in a wheat germ diet.

Rice weevil. Acoustic detection was used to monitor activity of internally feeding species such as *S. oryzae* reared in pellets of whole wheat flour that contained 10 and 100 ppm avidin (Table 2). Whereas the number of insect sounds was very high in control (0 ppm) and 10 ppm avidin-treated pellets, sound counts were about 50-fold lower in 100 ppm avidin-treated pellets. No adults emerged from the 100 ppm avidin pellets. These results demonstrate that the rice weevil is susceptible to 100 ppm avidin when the protein is homogeneously distributed in the pelleted diet.

Lesser grain borer. Acoustic detection was also used to determine the effect of avidin on growth of *R. dominica* in pellets of whole wheat flour (Table 3). The number of insect sounds in the 100 ppm avidin-wheat pellet diet was negligible in comparison to those detected in control (0 ppm) and 10 ppm avidin-treated diets. No adults emerged in the 100 ppm avidin-treated pellets and there was about a 60% reduction in emergence in the 10 ppm avidin wheat pellet diet. In another

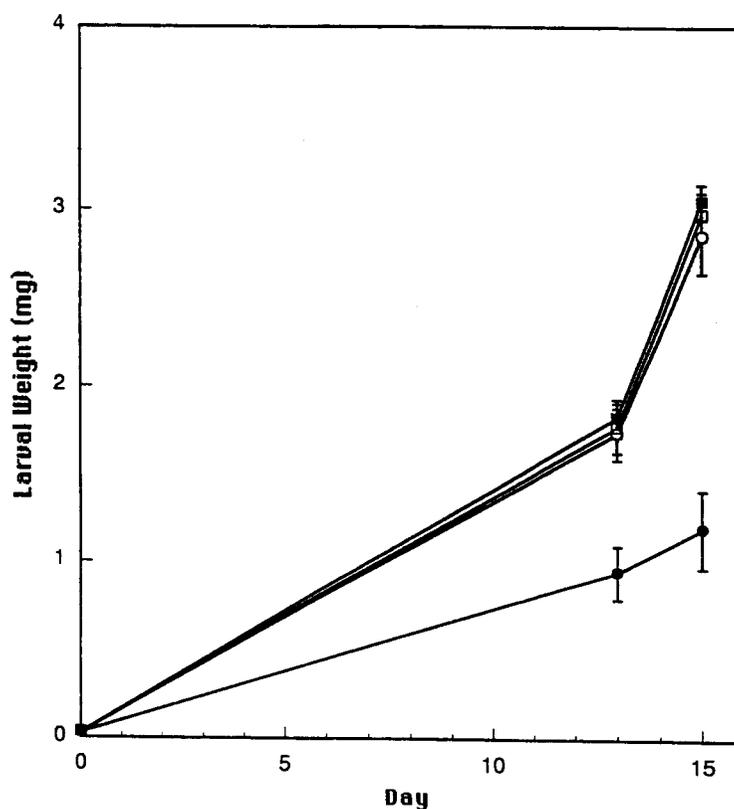


Fig. 3. The effect of avidin and/or biotin added to wheat germ on the growth of the confused flour beetle, *Tribolium confusum*. Avidin levels in ppm: 0 (—○—) (a); 1000 (—●—) (b); 1000 ppm avidin + 26 ppm biotin (—□—) (a); and 26 ppm biotin (—■—) (a). Mean values \pm s.e. (n = 9–10). Data with the same letter (in parentheses following legend identification) were not significantly different ($\alpha = 0.05$) as determined by Tukey statistical analysis of the means of larval weights at day 15.

experiment with whole wheat flour, 100 ppm avidin caused 100% mortality of lesser grain borer larvae, but addition of 2.6 ppm biotin allowed normal growth and development (data not shown).

In cracked wheat, a higher level of avidin (1000 ppm) reduced adult emergence by about 80% (data not shown). Avidin was less uniformly distributed in cracked wheat than in the pellets so that the endosperm, where the larvae primarily feed, probably contained relatively low amounts of the vitamin binding protein. These results demonstrate that development of the lesser grain borer is inhibited by 10 ppm avidin when it is mixed homogeneously in the diet, but that higher amounts may be needed in diets where heterogeneous distribution of the protein occurs.

As a negative control we also tested whether another vitamin, riboflavin, had the capacity to alleviate avidin toxicity. In contrast to the rescue observed when 2.6 ppm biotin was added to the avidin-treated diet, addition of 200 ppm riboflavin had no effect on avidin toxicity.

European corn borer. Avidin and streptavidin were toxic to the European corn borer with lethal effects observed at a concentration as low as 25 ppm (Table 4). Plateau values of approximately 50% reduction in larval weight and 50% mortality were obtained at 7 days with diets containing 25 to 300 ppm of either protein. Larvae reared on 25 ppm avidin-treated diet failed to pupate, whereas those on 10 ppm developed normally. The effect of 100 ppm avidin on borer

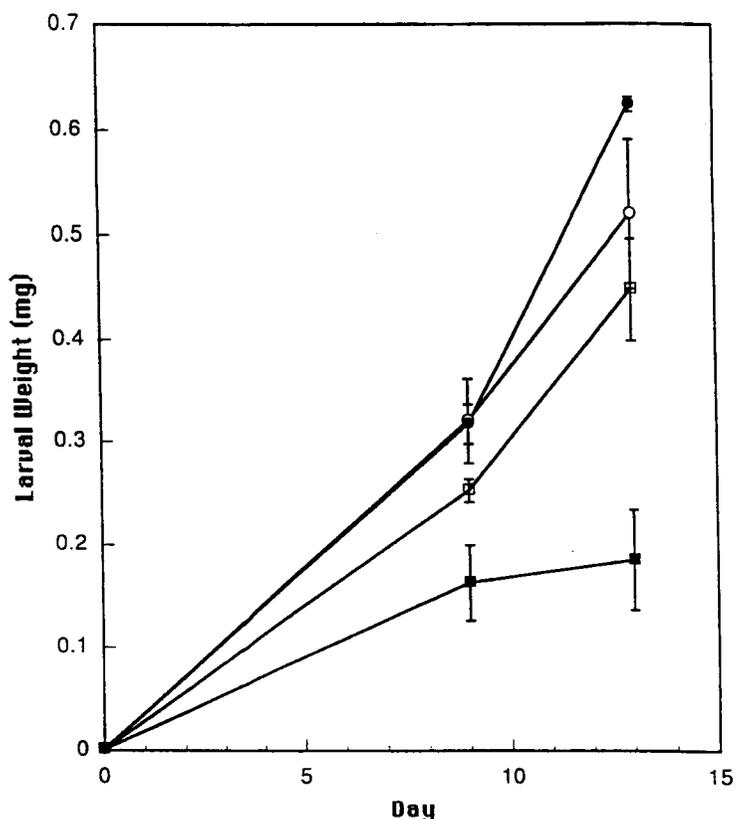


Fig. 4. The effect of avidin added to wheat germ on the growth of the sawtoothed grain beetle, *Oryzaephilus surinamensis*. Avidin levels in ppm: 0 (—○—) (a); 10 (—●—) (a); 100 (—□—) (a); 1000 (—■—) (b). Mean values \pm s.e. ($n = 5-10$). Data with the same letter (in parentheses following legend identification) were not significantly different ($\alpha = 0.05$) as determined by Tukey statistical analysis of the means of larval weights at day 13.

growth was prevented by the addition of 100 ppm biotin (Table 5).

Indianmeal moth. When using a wheat germ diet, the mortality for *P. interpunctella* was $29 \pm 8\%$, 100% and 100% for 0, 100 and 1000 ppm avidin, respectively ($n = 3$). The insects were reared in groups of 15 and some of the mortality was due to cannibalism of the pupae by the larvae. Development was also completely inhibited in cracked wheat treated with 1000 ppm avidin. To preserve the microenvironment around the insect, the diet was disturbed little and monitored for insect activity acoustically at weekly intervals. At 20 and 27 days after hatching, a more than 10-fold higher number of sounds was recorded in the control

group compared to the 1000 ppm avidin-treated group (data not shown). Direct observation on day 23 revealed that 13.7 ± 2.4 larvae ($n = 3$) were present in the control treatment (larval weight = 2.5 ± 0.2 mg), whereas only 1.7 ± 0.3 larvae were present in the avidin treatment (larval weight = 0.5 ± 0.2 mg), demonstrating that 89% mortality had already occurred in the avidin treatment at that time. Final mortality to the adult stage was $33 \pm 10\%$ and 100% for the control and avidin treated groups, respectively. These results demonstrated that avidin applied at 1000 ppm to coarsely cracked wheat or incorporated homogeneously at 100 ppm in wheat germ prevents the development of the Indianmeal moth.

Table 2. The effect of avidin added to a ground wheat pellet diet on the development of the rice weevil, *Sitophilus oryzae*

Avidin level (ppm)	No. of insect sounds ¹	No. of adults emerged ²	No. of progeny ³
100	173 ± 80 a	0	0
10	12968 ± 2620 b	48 ± 5 a	185 ± 46 a
0	9463 ± 2692 b	57 ± 3 a	287 ± 45 a

¹ Sound monitored for 10 min per sample at approximately three weeks post hatching. Mean values ± s.e. (n = 5). Data with same letter were not significantly different ($\alpha = 0.05$) as determined by Tukey statistical analysis.

² Monitored for seven weeks. Mean values ± s.e. (n = 5). Data with same letter were not significantly different ($\alpha = 0.05$) as determined by the independent t-test.

³ Adults from the original test were placed on whole wheat for 13 d and progeny counted for 61 d post infestation. Mean ± s.e. (n = 7 for 10 ppm avidin, n = 9 for 0 ppm avidin). Data with same letter were not significantly different ($\alpha = 0.05$) as determined by the independent t-test.

Discussion

We investigated the efficacy of avidin or streptavidin as a growth inhibiting or insecticidal protein for several insect pests. The proteins were mixed with wheat-based diets, such as wheat germ or flour, and fed to the red and confused flour beetles,

Table 3. The effect of avidin added to a ground wheat pellet diet on the development of the lesser grain borer, *Rhyzopertha dominica*

Avidin level (ppm)	No. of insect sounds ¹	No. adults emerged ²	No. 1st generation progeny ³
100	27 ± 8 a	0	..0
10	3367 ± 549 b	17 ± 1 a	199 ± 48
0	19596 ± 5732 c	53 ± 6 b	199 ± 54

¹ Sound was monitored for 10 min at approximately 19 d post hatching. Mean values ± s.e. (n = 3). Data with same letter were not significantly different ($\alpha = 0.05$) as determined by Tukey statistical analysis of the logarithmic values of the data.

² Examined 10 wk post hatching. Mean ± s.e. (n = 3). Data with same letter were not significantly different ($\alpha = 0.05$) as determined by the independent t-test.

³ Twenty adults from the original test were placed on whole wheat for 12 d and progeny counted for 56 d post hatching. Mean ± 0.5 range (n = 2).

Table 4. The effect of avidin and streptavidin on the growth and mortality of neonate larvae of the European corn borer, *Ostrinia nubilalis*¹

Level (ppm)	Avidin		Streptavidin	
	Weight (mg)	Mortality (%)	Weight (mg)	Mortality (%)
0	7.1 ± 0.3 a	0	9.6 ± 0.5 a	0
10	7.0 ± 0.3 a	0	9.2 ± 0.5 a	5
25	3.1 ± 0.4 b	30	4.5 ± 0.7 b	43
50	2.6 ± 0.5 b	46	3.3 ± 0.8 b	65
100	2.5 ± 0.5 b	55	4.3 ± 0.8 b	61
200	3.6 ± 0.6 b	64	3.6 ± 0.7 b	51
300	3.2 ± 0.5 b	49	3.0 ± 0.9 b	69

¹ Avidin or streptavidin was added to the diet as indicated, and larvae were weighed after seven days. Mean ± s.e. (n = 16). Data with the same letter were not significantly different ($\alpha = 0.05$) as determined by Tukey statistical analysis.

sawtoothed grain beetle, lesser grain borer, rice weevil and Indianmeal moth. All six species of stored product insects were adversely affected by avidin or streptavidin. In a semi-artificial diet, the biotin binding proteins were also toxic to a field crop pest, the European corn borer. Addition of biotin prevented avidin from inhibiting growth of both species of flour beetles, the lesser grain borer and the corn borer. Biotin rescues were not attempted with the other species, but it is probable that excess vitamin would overcome the effect of the binding protein for those insects as well.

Depending on the species and the type of diet used, avidin or streptavidin inhibited larval

Table 5. The effect of avidin and biotin on the growth and mortality of neonate larvae of the European corn borer, *Ostrinia nubilalis*¹

Treatment	Weight (mg)	Mortality (%)
Control	9.1 ± 2.2 a	0
100 ppm avidin	5.1 ± 1.4 b	70
100 ppm avidin + 100 ppm biotin	9.7 ± 3.0 a	5
100 ppm biotin	9.5 ± 2.7 a	0

¹ Weights were taken at day 7 of treatment. Mean ± s.e. (n = 16). Data with the same letter were not significantly different ($\alpha = 0.05$) as determined by Tukey statistical analysis.

growth and caused mortality at levels ranging between 10 and 100 ppm. Avidin was toxic to the lesser grain borer at 10 ppm in whole wheat flour pellets. For the rice weevil, however, 100 ppm avidin was required to cause mortality with this diet. The same level of avidin was toxic when administered with wheat germ to the Indianmeal moth, sawtoothed grain beetle and red flour beetle. For the confused flour beetle, avidin was toxic at 1000 ppm, but it was not tested at lower levels. A preliminary assay with the flat grain beetle, *Cryptolestes pusillus*, also resulted in high mortality (> 100%, n = 15) with 1000 ppm avidin (Morgan *et al.*, unpublished data). Streptavidin was toxic to the red flour beetle at 1000 ppm, and additional experiments are being conducted to examine the relative toxicity of this protein at lower levels for other stored product insects. These results suggest that all or nearly all of the biotin must be sequestered by avidin or streptavidin for toxicity to occur. Since whole wheat and wheat germ contain about 60 and 170 ppb biotin, respectively (Souci *et al.*, 1989), we estimate that the concentration of avidin or streptavidin would need to be at least 5 pm in whole wheat and 13 ppm in wheat germ to bind all of the endogenous vitamin in those materials.

Similar levels of biotin binding proteins (10 to 100 ppm) may be required in other types of insect diets as well. In a semi-artificial agar-based diet, 25 ppm avidin or streptavidin, based on wet weight, or about 150 ppm, based on dry weight, was toxic to the European corn borer. As avidin was increased from 25 to 300 ppm (wet weight), both the growth rate during the first seven days of larval life and the cumulative mortality remained essentially unchanged. The significance of this plateau response is unclear, but the moderate toxicity (30–69% mortality) was due to the short duration of the test. The growth response may be due to substantial amounts of maternally-derived biotin in neonate larvae or, alternately, the larvae may be able to obtain some of the vitamin from the diet regardless of the avidin level. The plant products that were incorporated in this diet had been autoclaved, and this treatment probably denatured the endogenous biotin-con-

taining proteins. Avidin may bind biotin that is covalently linked to protein (Green, 1975), and it is possible that more of the vitamin in denatured proteins remains unbound by avidin until proteolysis of the denatured proteins, allowing some degree of absorption by the gut. However, in preliminary bioassays using semi-artificial diets, $\geq 75\%$ mortality was obtained at 1000 ppm avidin with other lepidopterans such as the tobacco budworm, black cutworm, sunflower moth, beet armyworm and bollworm (Czapla *et al.*, unpublished data).

The threshold level for biotin that allowed development of the confused flour beetle was estimated to be about 5 ppb by Fraenkel and Blewett (1943). Little other information about threshold levels for other arthropod species is available due to the difficulty in preparing basal diets which are free of trace levels of biotin (Dadd, 1985). Mortality of the hide beetle occurred when approximately a two-fold excess of avidin relative to biotin was added to a semi-synthetic diet (Levinson *et al.*, 1967), but a larger excess of the binding protein was required for mortality of the fruit fly (Bruins *et al.*, 1991). For the flour mite, a 10 to 100 fold excess of avidin was toxic (Levinson *et al.*, 1992). Since biotin is present at trace levels in plants, the proteins probably need only be present in the diets at ppm levels to have growth inhibiting and toxic effects on most species of arthropods.

Avidin/streptavidin biotechnology has wide applications, including uses in chromatography, cytochemistry, gene probing and drug delivery (Wilchek & Bayer, 1990). We are now proposing to use these proteins as biopesticides for insect pest management. They are excellent candidate proteins whose genes might be manipulated to provide host plant resistance.

For avidin or streptavidin to become a useful as an insect growth inhibiting or insecticidal resistance protein, an important problem to address is the method of incorporating them into plant material. The most promising approach would be via genetic engineering. The genes for avidin and streptavidin have been cloned (Argarana *et al.*, 1986; Gope *et al.*, 1987; Chandra & Gray, 1990)

and it should be possible to generate transgenic plants that would express the gene for avidin or streptavidin synthesis in subcellular or extracellular compartments separate from those containing biotin that may be required by the plant for growth. During feeding by an insect pest, the plant's compartments would be mixed together, allowing sequestration of biotin by the binding protein, and the insect would thereby be deprived of a substantial portion of the vitamin.

Some vitamin binding proteins are known to be toxic to vertebrates (Jaffe, 1973). For such substances to be introduced into food or feed, testing will be required to ensure safety to consumers (Kessler *et al.*, 1992). Although there is no recommended dietary allowance for biotin in the human diet, consumption of only 0.1 to 0.2 mg is considered an adequate daily intake (Committee on Diet Allowances, 1980). The requirement of dietary biotin is probably supplemented through endogenous production of the vitamin in the intestine by symbiotic bacteria (Davidson *et al.*, 1979; Scholtissek *et al.*, 1990). Consumption of plant material containing avidin or streptavidin would be comparable to consumption of egg white proteins, one of which is avidin. Ingestion of large amounts of raw egg white can lead to a biotin deficiency in animals (Sydenstricker *et al.*, 1942; Jaffe, 1973). However, processing may reduce or eliminate the toxic effect of avidin. When eggs are well cooked, most of the avidin becomes denatured and unable to bind biotin (Durance, 1991). Heat treatment of plant foodstuffs containing avidin or streptavidin or addition of supplementary biotin may prevent harmful effects of the vitamin binding proteins on consumers.

Acknowledgements

We are grateful to Leon Hendricks, Christina Culbertson, Brian Barnett, David Hagstrum and Paul Flinn, U.S. Grain Marketing Research Laboratory, George Milliken, Kansas State University, and David Isenhour, Bruce Lang, Brain Sabus and Brenda Cummings, Pioneer Hi-Bred International, for assistance with this study.

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