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Transgenic avidin maize is resistant to storage insect pests

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Avidin is a glycoprotein found in chicken egg white, that sequesters the vitamin biotin. Here we show that when present in maize at levels of ≥100 p.p.m., avidin is toxic to and prevents development of insects that damage grains during storage. Insect toxicity is caused by a biotin deficiency, as shown by prevention of toxicity with biotin supplementation. The avidin maize is not, however, toxic to mice when administered as the sole component of their diet for 21 days. These dates suggest that avidin expression in food or feed grain crops can be used as a biopesticide against a spectrum of stored-produce insect pests.

Keywords: genetic engineering, insect biology, stored grain, insect resistance, corn, host plant resistance, biotin, transgenic plants

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After harvest, maize and commodities produced from maize are frequently hosts to a great variety of insect and fungal pests that cause severe economic damage[1-6]. Although *Bt* formulations have been evaluated extensively for controlling insect pests of stored grain, commercially acceptable levels of protection against coleopteran (beetle) pests have not been achieved [7-10]. In addition, the development of insect resistance to these naturally occurring toxins is a concern[11], prompting efforts to discover other insecticidal proteins.

Previously we reported that avidin, a chicken (*Gallus gallus*) glycoprotein composed of four glycosylated subunits[12-14], is toxic to seven species of stored-product beetles (Coleoptera) and moths (Lepidoptera)[15]. Avidin and a related protein, streptavidin, produced by the bacterium *Streptomyces avidinii*, exhibit one of the highest known affinities in nature between a protein and their ligand, biotin ($K_a = 10^{15}\ M^{-1}$). Biotin is a coenzyme required for all forms of life, so feeding avidin or streptavidin to many insects causes a biotin deficiency that leads to stunted growth and mortality. Avidin also is toxic to the house fly (*Musca domestica*[16]), hide beetle (*Dermestes maculatus*[17]), olive fruit fly (*Dacus oleae*[18]), fruit fly (*Drosophila melanogaster*[19]), flour mite (*Acarus siro*[20]), European corn borer (*Ostrinia nubilalis*[15]), and tobacco hornworm (*Manduca sexta*[21]).

Recently, the first commercial production of avidin in transgenic maize was described[22]. Avidin is used in a variety of biochemical and diagnostic procedures, and its cost is greatly reduced by production in transgenic plants. Based on the retail price of the purified protein, the transgenic maize has an estimated worth of about $5 million per hectare[23]. The susceptibility of this crop to stored-product insects has not been examined until now. Here we report that avidin maize is highly resistant toward many species of stored-product insect pests[15].

Results

ELISA and near-infrared spectroscopy (NIRS) analysis of avidin concentration in maize. ELISA of extracts of individual kernels of avidin maize revealed that the avidin concentration was highly variable, with levels ranging from 0 to ~2,500 p.p.m. In one sample of 92 individual kernels, the mean concentration of avidin was 164 ± 34 p.p.m. In theory, 50% of the kernels should not contain avidin, since the avidin-expressing plants were male sterile[22].

We investigated whether NIRS of single kernels could be used to identify transgenic kernels containing high levels of avidin, using a nondestructive method of analysis. By comparing NIR spectra of 200 single kernels with quantitation of avidin by ELISA, we developed a calibration model (7 factors) to predict avidin concentrations. The analysis showed that 92% of kernels with ≥60 p.p.m. avidin were correctly classified by the NIRS prediction model as containing significant levels of avidin, whereas 77% of kernels with <60 p.p.m. were correctly identified as containing low levels of avidin. Although the prediction of actual avidin levels in individual kernels was poor ($r^2 = 0.29$, standard error of cross validation (SECV) = 526 p.p.m.), the average avidin level in those kernels predicted as containing high or low levels of avidin was about 800 and 65 p.p.m., respectively.

Bioassays using whole kernels. The maize weevil, *Sitophilus zeamais*, was added to transgenic avidin and nontransgenic maize kernels. Overall, mortality was significantly higher on transgenic maize, compared with nontransgenic maize (21 ± 2% vs. 5 ± 1%, respectively, $p < 0.01$). Visual inspection and x-ray analysis were used to identify infested kernels, which had a mean avidin concentration of 163 ± 27 p.p.m. (n = 3 replications, total number of kernels = 149). There was approximately 7% mortality in kernels with ≤10 p.p.m. avidin (78% of infested kernels), 53% mortality in kernels with 11–20 p.p.m. (7% of infested kernels), and 96% mortality in kernels with 21–2,500 p.p.m. (15% of infested kernels). There was no mortality in kernels with low avidin (0–2 p.p.m.) and 100% mortality in those with >300 p.p.m. These data indicated that overall mortality was greatly influenced by the high proportion of kernels with ≤10 p.p.m. avidin. Probit analysis of the mortality for the weevil as a function of avidin concentration in individual kernels yielded LC_{50} and LC_{95} values of 30 and 307 p.p.m., respectively (Table 1).

Newly hatched larvae of the Angoumois grain moth, *Sitotroga cerealella*, were introduced into maize kernels (Table 1). We found 51 kernels with <20 p.p.m. avidin with emergence holes for adults, whereas 3 contained larvae that failed to pupate. Of kernels with 96–1,145 p.p.m. avidin, 13 had larvae inside, but none of these developed into pupae. Dissection of kernels from a similar experiment revealed that approximately 30% of the infested kernels without emergence holes contained live larvae, and thir growth was probably suspended. Probit analysis yielded EC_{50} and EC_{95} val-

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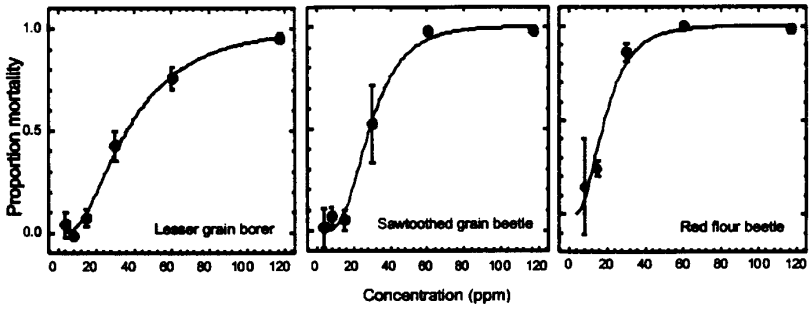

Figure 1. Mortality of three species of Coleoptera as a function of avidin concentration in maize meal: lesser grain borer (*Rhyzopertha dominica*), sawtoothed grain borer (*Oryzaephilus surinamensis*), and red flour beetle (*Tribolium castaneum*). Data are mean values ± s.e. from three replicates. Lines are from fitted probit equations. Details are included in the Experimental Protocol.

ues of 36 and 274 p.p.m., respectively, for preventing the development of *S. cerealella*.

Bioassays in ground maize. When reared in groups on cakes of transgenic maize meal with an avidin concentration of 123 p.p.m., the lesser grain borer *Rhyzopertha dominica*, sawtoothed grain beetle *Oryzaephilus surinamensis*, and red flour beetle, *Tribolium castaneum*, had mortalities of 100 ± 0, 99 ± 1, and 100 ± 0%, respectively. When reared on loose meal in groups, the red flour beetle, confused flour beetle *Tribolium confusum*, and flat grain beetle *Cryptolestes pusillus*, had 100 ± 0, 98 ± 2, and 100 ± 0% mortalities, respectively.

Control mortalities for the group bioassays were relatively high (13–31%), which was caused in part by cannibalism and the failure of some of the eggs to hatch. These variables were eliminated in bioassays of isolated newly hatched larvae of two lepidopteran species. The mortalities of the Indianmeal moth *Plodia interpunctella*, and Mediterranean flour moth *Anagasta kuehniella*, were 95 ± 5 and 100 ± 0%, respectively, on the transgenic meal, whereas the control mortalities on nontransgenic meal were 0 ± 0 and 8 ± 3%, respectively. With the latter species, 20 ± 5% of the larvae grew at a normal rate on the control diet but failed to pupate after three months, indicating that they were in diapause.

With the warehouse beetle *Trogoderma variabile*, we performed bioassays with ground meal from transgenic maize containing either 123 or 265 p.p.m. avidin. Development to the adult stage reached a maximum on meal from the nontransgenic maize by 7 weeks. On the meal from transgenic maize, none of the larvae had pupated by 16 weeks. Our data showed that avidin was highly toxic to the warehouse beetle at 265 p.p.m. and that larval development was greatly suppressed at 123 p.p.m., perhaps as a result of suboptimal nutrition and diapause induction.

The only species examined to date that exhibited a tolerance for avidin was the larger grain borer *Prostephanus truncatus*. Mortalities were 0 ± 0% on transgenic maize, 17 ± 10% on control maize, 27 ± 7% on control maize supplemented with 100 p.p.m. avidin, and 17 ± 10% on control maize supplemented with 1,000 p.p.m. avidin (not significantly different at $p = 0.05$, n = 2 replicates of 14–15 individuals each). We also recorded the developmental period from egg hatch to adult eclosion, and found that avidin at levels up to 1,000 p.p.m. in ground maize was not toxic to and did not influence the developmental time of this bostrichid beetle.

Concentration–mortality relationships were determined for the lesser grain borer, sawtoothed grain beetle, and red flour beetle using cakes of control maize meal with and without supplementation with avidin (Fig. 1). The groups that were reared on cakes with ~120 p.p.m. avidin had 96–99% mortality (Table 1).

In addition to recording the mortality of groups that were reared on cakes of meal, we recorded the mortality of isolated *T. castaneum* individuals that were reared on the loose meal and also weighed some representative individuals (n = 15–17). The weight of the larvae fed a control diet increased from ~0.03 mg to 1.58 ± 0.09 mg during 18 days following egg hatch. In contrast, a diet supplemented with 60 p.p.m. avidin produced larvae that weighed only 0.51 ± 0.06 mg at 18 days. The mortality of larvae fed this diet for 18 days was 13%, but this increased to 100% after 14 weeks. There was no mortality of larvae fed the control diet. The LC_{50} was similar for individuals tested on loose meal and for groups of larvae reared on cakes of maize meal. The potency of avidin in these two concentration/response experiments was not significantly different (relative potency was 0.90 with 95% confidence limits of 0.79–1.03). These data showed that even though avidin was highly toxic to *T. castaneum* in a diet of maize meal, larval feeding and growth may occur for an extended period of time before death occurs.

Biotin rescue. To determine whether exogenous biotin can prevent the effects of avidin, exogenous biotin was added to the transgenic avidin maize meal and fed to the sawtoothed grain beetle (Table 2). Whereas the transgenic maize diet caused 100% mortality, the addition of only 5 p.p.m. biotin rescued nearly all of the insects. The time from egg hatch to adult eclosion and the weight of adults for insects reared on avidin corn plus biotin were the same as those for insects fed nontransgenic corn.

Mice bioassay. Maize was fed as the sole diet to young adult mice for up to 21 days, during which time the mice were examined for adverse symptoms. Whereas the maize diet was not nutritionally balanced, it nonetheless allowed for a maximum ingestion of 0.5–1.0 mg of avidin per day. Despite the fact that no source of biotin other than corn was given to the mice, the subjects appeared completely normal. The average weight gain per mouse fed 123 p.p.m.-avidin cornmeal for 14 days (0.86 ± 1.35 g) was not significantly different from that of mice fed control cornmeal (0.15 ± 1.03 g, Mann–Whitney U-test). Mice fed the more nutritious food in the absence of cornmeal had a higher weight gain (2.85 ± 0.72 g/mouse). All three of the experimental groups of mice appeared healthy throughout the duration of the experiment. Thus, avidin at ~120 p.p.m. in maize exhibited no acute oral toxicity to mice that had consumed the transgenic corn for 21 days.

Discussion

Maize is an important staple crop in many countries[24]. Depending on the climatic ands storage conditions, it can become infested by a wide variety of stored-product insect pests[25–29]. Postharvest grain losses caused by pests because of poor storage practices total more than than $1 billion per year in the US. A recent survey of stored-product insects in the US found that weevils, Indianmeal moths, lesser grain borers, and saw-toothed grain beetles caused most the of the problems in processed foods[31]. Avidin-containing maize and its processed products would be resistant to infestations caused by all of the species.

We found that avidin maize has excellent resistance to storage insect pests when the kernels contain a concentration of >100 p.p.m. avidin. Many of the kernels used in this study, however, had poor resistance because expression of the protein was not uniform and the majority of the kernels had little or no avidin. Because levels of avidin in individual kernels were widely variable, NIRS was used to rapidly sort kernels into groups containing either high or low levels of avidin. Absorption of NIR radiation at wavelengths

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Table 1. Probit analyses of mortality of five species of stored product insects on transgenic avidin maize kernels and meal[a]

Species	n	LC_{50}[b]	LC_{95}[b]	Slope[c]	Intercept[c]	χ^2
Sitophilus zeamais (maize weevil)	150	30 (17–88)	307 (99–4,129)	1.63 ± 0.34	-2.39 ± 0.34	–
Sitotroga cerealella (Angoumois grain moth)	67	36 (19–160)[d]	274 (83–9,580)[d]	1.88 ± 0.51	-2.93 ± 0.62	–
Rhyzopertha dominica (lesser grain borer)	1,200	36 (32–40)	112 (95–138)	3.37 ± 0.28	-5.25 ± 0.46	3.0[f]
Oryzaephilus surinamensis (sawtoothed grain beetle)	1,200	29[e]	63[e]	4.79 ± 0.51	-6.98 ± 0.79	23.8[g]
Tribolium castaneum (red flour beetle)	630	19[e]	47[e]	4.08 ± 0.55	-5.17 ± 0.77	32.5[g]

[a]Lesser grain borer, sawtoothed grain beetle, and red flour beetle were reared on maize meal; maize weevil and Angoumois grain moth were reared in maize kernels. For the latter two species, SAS PROC LOGISTIC was used to fit an equation to the data because concentration of avidin was measured in each kernel, that is, there were no concentration groups. Thus, goodness of fit was assessed by significance of the equation parameters and examination of residuals.
[b]Lethal concentration in parts per million, with 95% confidence limit in parentheses.
[c]Slope and intercept are ± s.e.; data were transformed using probit transformation and $\log_{10}$ (concentration).
[d]Effective concentrations for preventing development.
[e]Confidence limits on lethal concentration values were not calculated when χ^2 was significant. Examination of observed and predicted proportion mortality (Fig. 1) indicated that lack of fit was not a concern.
[f]Not significant.
[g]Significant at the $p = 0.01$ level.

associated with protein and/or lipid functional groups was different between kernels with high and low levels of avidin. Based on this observation, use of an NIRS system could be used to select high-avidin kernels for commercial production.

Avidin may interfere with enzymes that depend on enzyme-bound biotin, such as those involved in carboxylation, decarboxylation, and transcarboxylation reactions[30,32]. Biotin deficiency in the blowfly, *Aldrichina grahami*, caused decreases in several fatty acids[33]. Presumably, a similar biochemical effect led to the stunted growth and mortality of the stored-product insects studied here.

The public acceptability of avidin maize as a food or feed is difficult to predict. Currently, the safety of avidin maize is of little concern because it is grown only on limited acreage for commercial protein production. Careful examination of its safety, however, is needed before consumption by humans and livestock. This study reports that at least there is no acute toxicity for mice. Long-term ingestion of high levels of avidin maize may be a problem, because a biotin deficiency can decrease the growth rate of mice and affect reproduction[34,35]. However, avidin is a food protein that is consumed in the form of egg whites at a concentration of >400 p.p.m. by dry weight, which is four times higher than its concentration in most of the maize used in the present study. Therefore, avidin maize is comparable to a mixture of three parts of conventional maize meal plus one part of egg white. Moreover, avidin has an antidote (biotin), which can be used to prevent toxicity or to rescue potential victims from adverse effects. Food and feed uses of avidin maize might involve processing that includes supplementation with the vitamin. Another method that would help to prevent potential toxicity of the avidin maize is heat treatment, which would denature most of the avidin as well as the avidin–biotin complex and release most of the vitamin[36–39].

Currently, avidin maize is grown as a specialty crop for commercial production of avidin[22,23]. Because it is a pest-resistant product, additional commercialization as a background germplasm for production of other valuable proteins is possible, and its development as a food or feed grain could be considered following a thorough risk assessment. In addition to its efficacy against postharvest insect pests, avidin also is effective against preharvest pests such as the beet armyworm, black cutworm, bollworm, and other species for which biotin is an essential growth factor[15]. Transferring the avidin gene to other crops will be important in determining its potential usefulness in a variety of other commercial protein production and pest control situations.

Experimental protocol

Avidin maize. The construction of plasmids used for transformation of maize with the chicken *avidin* gene, transformation, tissue culture, and generation of avidin-expressing transgenic plants are described[22].

ELISA. Rabbit antibodies to avidin were raised by sending avidin purchased from Sigma (St. Louis, MO) to Bethyl Laboratories (Montgomery, TX) for injection into rabbits using their standard protocols. Single seeds were powdered and extracted in 10 mM PBS pH 7.0 containing 0.05% Tween-20. ELISAs were performed as described by Hood et al.[22]

NIRS analysis. Corn kernels were individually placed on the quartz window of a interactance fiber-optic bundle attached to a diode-array spectrometer (DA7000, Perten Instruments, Springfield, IL). The spectrometer measures radiation (400–1700 nm) at a rate of 30 spectra/s. Eight spectra from each corn kernel were collected and averaged. Because no color difference related to avidin presence was expected, only the NIR region (700–1700 nm) was used in the analysis. Data were analyzed using partial least squares (PLS) regression of the first derivative of the absorbance spectra. Correlation plots (r^2), SECV, and regression coefficients indicating the importance of specific wavelengths to calibrations were obtained from the PLS analysis[40]. More detail on the instrumentation and statistical analysis is provided in refs 41 and 42.

Insects. Depending on the species, bioassays were infested with eggs from a 24 h period of oviposition, neonate larvae that were 0–24 h old, or adults that were two to four weeks old. These were obtained from stock cultures maintained at the Grain Marketing and Production Research Center. *Sitophilus zeamais* Motschulsky and *Prostephanus truncatus* (Horn) were maintained on whole maize, whereas *Rhyzopertha dominica* (Fabricius) and *Sitotroga cerealella* (Olivier) were maintained on hard red winter wheat. *Tribolium castaneum* Jacquelin du Val and *Tribolium confusum* (Herbst) were maintained on 95% wheat flour and 5% brewer's yeast. *Oryzaephilus surinamensis* (Linnaeus) and *Cryptolestes pusillus* (Schönherr) were maintained on 90% rolled oats, 5% brewer's yeast, and 5% wheat germ. *Trogoderma variabile* Ballion was maintained on 50% rolled oats and 50% powdered dog food. *Plodia interpunctella* (Hübner) and *Anagasta kuehniella* (Zeller) were maintained on a standard laboratory diet[43].

Bioassays of primary pests using whole kernels. Kernels were examined radiographically and damaged kernels were discarded. The intact kernels

Table 2. Effect of avidin and biotin on *Oryzaephilus surinamensis* reared on transgenic avidin cornmeal[a]

	Mortality (%)	Developmental period (days)	Adult weight (μg)
Avidin cornmeal	100[b]	–	–
Avidin cornmeal + 5 p.p.m. biotin	9	19.8 ± 0.2	465 ± 24
Control cornmeal	12	19.3 ± 0.1	455 ± 10
Control cornmeal + 5 p.p.m. biotin	0	19.6 ± 0.2	481 ± 27

[a]Mean ± s.e. or 0.5 range, n = 2 replicates (a total of 17–24 insects). Insects were reared individually on 20 mg of diet. The avidin corn contained ~123 p.p.m. avidin. According to the Tukey test, there was no significant difference between means for developmental period or adult weight ($p < 0.05$). The developmental period was the time from egg hatch to adult eclosion. Mortality was recorded for the combined replicates because of the small number of insects.
[b]Significantly different from the other mortalities according to Fisher exact test ($p < 0.01$). There was no significant difference between other mortalities ($p < 0.05$).

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were used for assays with two species of primary pests: *S. zeamais*, which penetrates kernels during oviposition, and *S. cerealell*, which penetrates kernels during initial larval feeding. For *S. zeamais*, the kernels were equilibrated at 30°C, 12 h light/12 h dark photoperiod, and ~75% relative humidity (RH) over a saturated solution of NaCl. Three replicates of 236 g of kernels of transgenic maize (~810 kernels) or nontransgenic maize (~750 kernels) were infested with five female adults for 48 h[44]. The progeny were removed on the day that they emerged as adults. Two weeks after the last adult had emerged, the kernels were examined radiographically to detect any dead larvae and pupae within the kernels. For *S. cerealella*, the kernels were equilibrated at 28°C, 16 h light/8 h dark, and ~75% RH over a saturated solution of NaCl. Three replicates of 30 neonate larvae were placed on 20 g of transgenic maize. Emerged moths were removed daily. Two weeks after the last moth had emerged, the kernels were examined radiographically to detect any dead or living larvae inside. The concentration of avidin in each infested kernel was estimated by ELISA.

Bioassays of primary pests using ground maize. Two species of bostrichid beetles, *R. dominica* and *P. truncatus*, are primary pests that can penetrate intact kernels, but they have behavioral characteristics that make them less suitable for bioassays on those kernels. The females of *R. dominica* lay the majority of their eggs outside the kernels, and therefore, the larval diet is often derived from more than one kernel, until each larva finally bores into a kernel to complete its development. Each female of *P. truncatus* bores into a kernel before laying several eggs in a side tunnel. Because it was difficult to obtain reliable information about the growth and development of these pests on kernels that contained a wide range of avidin concentrations, we used ground maize for bioassay of these species.

Maize kernels were ground through a screen (20 mesh/in.) in a Wiley mill. *Rhyzopertha dominica* was bioassayed on cakes of this ground maize, because small particles of uncompacted maize were less suitable for the development of the larvae. Ground transgenic or nontransgenic maize (95%) and wheat gluten (5%) were mixed with water in a 1:1.05 ratio (wt/vol) in a 1 oz cup (Solo Cup Co., Urbana, IL). It was compacted into a cake and then the cake was lyophilized. Three cakes of each type of maize were prepared. After equilibration at 28°C, 16 h light/8 h dark, and ~75% RH over a saturated solution of NaCl, 50 *R. dominica* eggs were added to each of the 2.1 g cakes. Mortalities of the immature stages were recorded from the time of infestation until all of the first generation had either died or developed into adults. For estimation of lethal concentrations of avidin, six control cakes were prepared using water and three cakes of each concentration of avidin were prepared using a solution of homogeneous avidin.

In a preliminary experiment, the mortality of *P. truncatus* on cakes of nontransgenic meal was more than 50% from the egg to the adult stage. Therefore, we used a second method to bioassay this species. The ground maize meal was compacted to a density of ~0.7 g ml^{-1} in 0.2 ml^{-1} microcentrifuge tubes. A newly hatched larva was placed in a small depression in the meal and then loosely covered with a portion of the meal. Each larva was placed on 25 mg of either transgenic or nontransgenic meal that had been slurry mixed with either water or a solution of avidin followed by lyophilization and grinding in a mortar with a pestle (two replicates for a total of 29–30 insects).

Bioassays of secondary pests. Maize cakes were prepared in an identical fashion and tested with two species of secondary pests, *O. surinamensis* and *T. castaneum*, that do not attack whole kernels. Either 50 eggs of *O. surinamensis* or 30 eggs of *T. castaneum* were placed on the cakes, and the egg-to-adult mortality was used to estimate lethal concentrations. We also estimated the lethal concentrations for isolated individuals of *T. castaneum*. Individual eggs of *T. castaneum* were placed on 50 mg of maize meal in 1.5 ml microcentrifuge tubes (two replicates of ~20 eggs).

Several species of secondary pests were also assayed on loose meal, because such pests normally feed on broken kernels and their larval development may be completed more quickly on loose media. Bioassays were performed with three replicates of 25 eggs of *C. pusillus* or 10 eggs of *T. confusum* or *T. castaneum* on 0.5 g of loose meal in 2 ml tubes. To minimize the incidence of diapause in *T. variabile*, which occurs more frequently when larvae are reared in isolation[45,46], two replicates of 40 eggs were placed on 8 g of meal. The pyralid moths, *P. interpunctella* and *A. kuehniella*, were assayed as isolated individuals to avoid cannibalism. Each newly hatched larva was placed on 200 mg of diet in 2.0 ml microcentrifuge tubes (two replicates for a total of 40 larvae per treatment per species), and the lids of the tubes were pierced with a #00 insect pin to provide holes for air exchange.

Biotin rescue. Ground maize was mixed with solutions of biotin (Sigma Chem. Co.) in a 1:1.2 ratio (wt/vol). After lyophilization, the diet was ground using a mortar with a pestle. Individual eggs of *O. surinamensis* were placed on 20 mg of diet in 0.2 ml tubes (two replicates for a total of 17–24 eggs that hatched), and the lids of the tubes were pierced with a pin to provide air exchange. Adult emergence was recorded daily.

Statistical analyses. Means were compared with the MANOVA procedure using the Tukey test of significance[47]. For insect bioassays using whole kernels, PROC LOGISTIC[48] was used to determine the relationship between mortality and concentration of avidin in kernels because the concentration was measured in each kernel, that is, there were no concentration groups. Confidence limits on LC values were calculated using Fieller's theorem[49]. For insect bioassays in ground maize, POLO PC was used for probit analyses[50]. The Fisher exact test was used to test for significant differences between mortalities of small groups of individuals.

Mice bioassay. Groups of seven ND4 Swiss Webster outbred mice in early adulthood (20–24 g; Harlan Laboratories, Indianapolis, IN) were fed diets of coarsely ground maize meal (5–7 g/day), either transgenic maize seed containing on average 123 p.p.m. avidin or a commercially available nontransgenic maize, continuously for up to 21 days. A third group was fed mouse chow (Teklad Rodent Diet, Harlan Laboratories, Indianapolis, IN).

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