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Protection of stored maize from insect pests using a two-component biological control method consisting of a hymenopteran parasitoid, *Theocolax elegans*, and transgenic avidin maize powder ☆

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Abstract

The hymenopteran parasitoid, *Theocolax elegans* (Westwood), and transgenic avidin maize powder were tested to determine if their individual or combined use would protect stored grain from infestation by both internal and external insect pests. Small-scale tests were conducted in plastic jars containing 3 kg of non-transgenic maize. We tested treatments of 0.3% powdered avidin maize, the parasitoid wasp, and the combination of the parasitoid plus 0.3% powdered avidin maize. One pair each of *Sitophilus zeamais* Motschulsky, *Tribolium castaneum* (Herbst), and *Cryptolestes ferrugineus* (Stephens) was added to each jar. After 8 weeks, the entire contents of each jar were examined for adult insects. Control and avidin maize powders had no detrimental effects on the beneficial insect parasitoid *T. elegans*. The parasitoid suppressed populations of the internal feeder *S. zeamais*. The avidin maize powder treatment had no effect on *S. zeamais* because these larvae developed inside the maize kernels where no avidin maize powder was present. For *S. zeamais*, the combination treatment was not significantly different from the parasitoid treatment. In contrast, populations of the external feeder *T. castaneum* were not suppressed by the parasitoid but were suppressed by the avidin maize powder treatment. The parasitoid-avidin combination treatment produced the greatest percentage reduction for all three insect species and resulted in 78%, 94%, and 70% reductions in populations of *S. zeamais*, *T. castaneum*, and *C. ferrugineus*, respectively, when

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compared to the control treatment. The percentage reductions for the parasitoid treatment were 70%, 8%, and 20% for *S. zeamais*, *T. castaneum*, and *C. ferrugineus*, respectively. For the avidin maize powder treatment, populations of *S. zeamais*, *T. castaneum*, and *C. ferrugineus* were reduced by 10%, 85%, and 40%, respectively. The combination treatment of avidin maize powder plus the release of parasitoid wasps was superior to either treatment alone when applied to mixed populations of internal and external feeders.
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Keywords: Stored grain; Biopesticide; *Tribolium castaneum*; *Sitophilus zeamais*; *Cryptolestes ferrugineus*

1. Introduction

Alternatives to traditional chemical insecticides such as predators, parasitoids, microbes and natural products have been gaining interest among researchers concerned with developing integrated pest management (IPM) approaches for insect control (Copping and Menn, 2000). However, only a few of these alternative methods, such as pyrethrum- and *Bacillus thuringiensis* (Bt)-based products, have been commercially successful in the pesticide market. For several years, we have been investigating a variety of alternatives to conventional chemical pesticides in stored-product IPM. Controlling insect pests in stored grain and grain products can be very difficult because of the variety of species that can infest grain. Insect parasitoids have been shown to be effective in suppressing a limited number of pest species both in bulk grain storages and in food-processing facilities and warehouses (Schöller and Flinn, 2000). One of the more effective parasitoids is *Theocolax elegans* (Westwood), a small pteromalid wasp (1–2 mm) that attacks primary grain pests, whose immature stages develop inside the grain kernels, including the weevils, *Sitophilus* spp., lesser grain borer, *Rhyzopertha dominica* (F.), drugstore beetle, *Stegobium paniceum* (L.), cowpea weevil, *Callosobruchus* spp., and Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Burks, 1979; Flinn et al., 1996; Flinn, 1998; Flinn and Hagstrum, 2001). However, *T. elegans* does not parasitize species that are secondary grain pests, including the flour beetles, *Tribolium* spp., and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), whose immature stages develop outside of the grain kernel. Therefore, we wished to identify a biopesticide that might be used to supplement the species-specific activity of the parasitoid, which, when used in combination with the parasitoid, would protect grain from both internal and external pest species, and at the same time be non-toxic to the beneficial parasitoid species.

There are many biocidal proteins that occur in nature, which are potential biopesticides for stored-grain protection. One of these that could be used in combination with a parasitoid to control grain pests is transgenic avidin maize powder (Kramer, 2004). The insecticidal activity of chicken avidin has been known since 1959 when it was first reported that this protein was toxic to the housefly, *Musca domestica* (L.), when administered in the diet to larvae (Levinson and Bergmann, 1959). Avidin causes mortality in many species of stored-product insects by preventing the absorption of dietary biotin (Morgan et al., 1993). The avidin gene has been incorporated into maize plants and avidin maize kernels are resistant to insects, especially after the kernels are ground into a meal or powder (Kramer et al., 2000). When avidin was present in transgenic maize kernels at levels of approximately 100 ppm or higher, it prevented development of almost all insect pests that damage grain during storage, including the maize weevil, *Sitophilus zeamais*

Motschulsky, *R. dominica*, *S. cerealella*, warehouse beetle, *Trogoderma variabile* Ballion, sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), flat grain beetle, *Cryptolestes pusillus* (Schönherr), red and confused flour beetles, *Tribolium castaneum* (Herbst) and *T. confusum* du Val, Indian meal moth, *Plodia interpunctella* (Hübner), and Mediterranean flour moth, *Anagasta kuehniella* (Zeller) (Kramer et al., 2000). Unfortunately, only about half of the individual kernels from harvested lots of transgenic avidin maize possess insecticidal levels of recombinant avidin because transgenic plants are male sterile, which results in only about 50% of the kernels containing avidin (Hood et al., 1997). On the other hand, meals or powders derived from those lots of avidin maize kernels have a more homogeneous distribution. These homogeneous powders are thought to be more toxic than the commercial lots of avidin maize kernels, especially to external insect pests, although we have not made a direct comparison of toxicities with any insect species (Kramer et al., 2000).

Based on the results of our previous studies, it was apparent that a formulation like transgenic avidin maize powder might have a potential application in stored-product protection as a supplement to biological control agents that are often limited to a specific host range. Therefore, we tested the hypothesis that maize treated with a combination of the hymenopteran parasitoid, *T. elegans*, and transgenic avidin maize powder would be protected from infestation by both primary and secondary insect pests. We also determined whether the avidin maize powder had any detrimental effect on the parasitoid.

2. Materials and methods

Avidin maize powder was provided by ProdiGene, Inc., College Station, TX. It contained approximately 850 ppm avidin according to an ELISA assay (Hood et al., 1997). When the avidin maize powder was added at an application rate of 0.3% to the test maize samples (Asgrow, RX877), the final overall concentration of the avidin in the test maize sample was only about 2.5 ppm. The Asgrow variety of maize kernels was used for a control sample either as is, ground in a blender, or ground to a fine powder in a falling number mill.

Insects from laboratory colonies of *S. zeamais*, *T. castaneum*, *C. ferrugineus*, and *T. elegans* maintained at the Grain Marketing and Production Research Center were used for the experiments.

Plastic jars (3.8-L) were filled with maize (3 kg) that was previously cleaned. Thirty grams of ground maize (prepared in a Waring blender) was added to each jar and the contents were thoroughly mixed. The ground maize was added to ensure that the secondary feeders, *T. castaneum* and *C. ferrugineus*, would have enough food during the experiment. Plastic lids with a 3 cm diameter opening covered with silkscreen were used to allow air movement and prevent insects from leaving the jars. A factorial design was used consisting of six treatments: maize, maize plus 0.3% powdered maize, parasitoid, parasitoid plus 0.3% powdered maize, 0.3% powdered avidin maize, and parasitoid plus 0.3% powdered avidin maize. Each treatment was replicated four times. Three insect species were combined in each test. One pair each of sexed 2- to 3-week-old *S. zeamais*, *T. castaneum*, and *C. ferrugineus* was added to each jar. The jars were held in an environmental chamber maintained at $27\pm1\,^{\circ}$C and 65 ± 5% r.h. Twenty days after beetle release, two pairs of sexed 1- to 4-day-old *T. elegans* were added to the parasitoid treatment jars.

A second introduction of *T. elegans* was also made 27 days after beetle release. We waited 20 days because this is the amount of time required at this temperature for *S. zeamais* to develop to the fourth instar, the stage *T. elegans* prefers to parasitize (Sharifi, 1972). After 8 weeks from initial beetle release, the insects were removed from the maize using an Insectomat®, a motorized inclined sieve (Samplex LTD, Willow Park, UK). Each grain sample was processed with the Insectomat® twice, and then sieved by hand (3.35 mm opening) to remove any remaining weevils. The numbers of live and dead adults of each species of insect were recorded.

Data on live insect counts were analyzed by two-way ANOVA using the PROC GLM procedure (SAS Institute, 2000) to estimate differences in treatments. Data were transformed using $\log(x+1)$ to stabilize heteroscedastic variances. Multiple comparisons were made between treatments using the PROC MEANS Tukey's studentized range test (HSD) (SAS Institute, 2000) on the transformed data. Means were considered to be significantly different at the 5% level ($P \leqslant 0.05$).

3. Results

First, we examined whether the maize powders had any detrimental effects on the parasitoid's efficacy in controlling the maize weevil. There were no significant differences in the numbers of *T. elegans* wasps that emerged from maize treated with parasitoid alone, avidin maize powder plus parasitoid and powdered maize plus parasitoid treatments ($F = 0.52$, df $= 2, 9$, $P = 0.61$). The mean numbers of live adult wasps in maize treated with parasitoid alone, avidin maize powder plus parasitoid, and powdered maize plus parasitoid were 5.5 ± 3.0, 7.2 ± 7.3, and 3.8 ± 2.9, respectively. Thus, neither the avidin maize powder nor the control with 0.3% powdered maize had any effect on either the wasp's host finding ability or its mortality.

To simplify the statistical analysis, we used the maize plus powdered maize treatment as the control sample. Treatment, species, and treatment-species interactions were found to be significant factors in the test (Table 1). Both the parasitoid and combination (parasitoid plus avidin maize powder) treatments significantly suppressed *S. zeamais* populations when compared to the control (Fig. 1). As expected, the avidin maize powder treatment alone had no effect on *S. zeamais* because those larvae developed inside the maize kernels where no avidin maize powder was present. The pair of *S. zeamais* adults initially placed in each jar may have been affected by the avidin powder. However, these females probably would have been ovipositing at a normal rate for several weeks before their biotin levels were diminished to a level where oviposition was

Table 1
Outcome of the analysis of variance for three species of stored-product insects on untreated maize, maize treated with avidin maize powder, parasitoid wasps, or the combination of parasitoid wasps and avidin maize powder[a]

Source	df	ms	*F*	*P*
Species	2	8.63	26.22	<0.0001
Treatment	3	8.07	24.50	<0.0001
Species × treatment	6	2.91	8.83	<0.0001

[a]Data were transformed using $\log(x+1)$ to stabilize heteroscedastic variances.

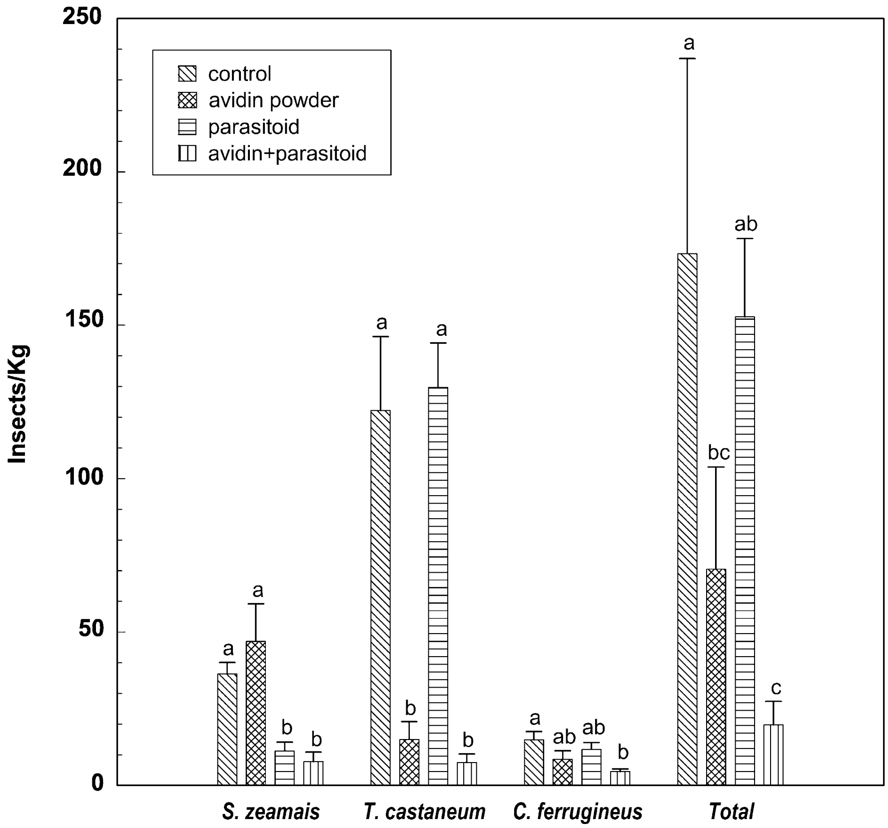

Fig. 1. Effect of 0.3% avidin maize powder, parasitoids, or the combination of 0.3% avidin maize powder and parasitoids on live adult insect densities of *T. castaneum*, *S. zeamais*, and *C. ferrugineus*. Vertical bars indicate standard error of the mean ($n = 4$). Bars designated by the same letter, within a species, were not significantly different ($P > 0.05$; Tukey's Studentized Range (HSD) Test.

adversely affected. The combination treatment was not significantly different from the parasitoid treatment. In contrast, *T. castaneum* populations were not suppressed by the parasitoid but were suppressed by the avidin maize powder treatment. The combination treatment was not significantly different from the avidin maize powder treatment.

There were no significant differences in *C. ferrugineus* density in the control, avidin maize powder, or parasitoid treatments. Control populations of *C. ferrugineus* did not increase very rapidly in the maize samples used in this study; thus, there was not as large a difference between the effects of treatment with avidin maize powder and the control experiment when compared to the other species. This result may have occurred because *C. ferrugineus* does not reproduce as rapidly on maize as compared to other grains (Rilett, 1949). Nonetheless, populations of this species were significantly less in the combination treatment when compared to the control experiment.

The total number of insects of all three species in the avidin maize powder treatment was significantly less than that in the control treatment (right-hand-side of Fig. 1). The parasitoid treatment, on the other hand, was not significantly different from the control treatment for the total number of insects. However, the total number of insects was significantly less in the combination treatment than in the control or in the parasitoid treatment.

The combination treatment had the greatest percentage reduction for all of the insect species. It resulted in 78%, 94%, and 70% reductions of *S. zeamais*, *T. castaneum*, and *C. ferrugineus*, respectively, when compared to the control treatment. The percentage reductions for the parasitoid treatment alone resulted in 70%, 8%, and 20% reductions of *S. zeamais*, *T. castaneum*, and *C. ferrugineus*, respectively. For the avidin maize powder treatment, populations of *S. zeamais*, *T. castaneum*, and *C. ferrugineus* were reduced by 10%, 85%, and 40%, respectively.

4. Discussion

The avidin maize powder mixed with whole kernel maize was very effective in controlling the secondary pest insects at an application rate of only 2.5 ppm in the test samples. When used alone, the avidin maize powder was an effective control method for the external feeder, *T. castaneum*, causing an 85% reduction. Based on the average concentration of avidin in the grain mass ($\approx$3 ppm), this efficacy is greater than previously reported for *T. castaneum* (Kramer et al., 2000). However, it is likely that this species fed primarily on the fine material that consisted of avidin maize powder and the Asgrow maize meal. The latter would dilute the concentration of avidin in the transgenic maize powder from approximately 850 to about 200 ppm, which is four-fold higher than the LC_{95} of nearly 50 ppm avidin determined for the red flour beetle in Kramer et al. (2000). The concentration of avidin in the fine material is important from the viewpoint of the secondary pest insects. The concentration of avidin in the entire maize sample is also important, because this affects the risk to non-target organisms. As a powder, less total avidin maize needs to be added to grain as a protectant because the level of avidin in the powder is much greater than the LC_{95}. This strategy would result in a lower effective application rate of avidin relative to the entire grain mass. An avidin concentration of approximately 2.5 ppm would be expected to bind approximately 38 ppb biotin, which is about half of the biotin concentration (70 ppb) of whole-kernel maize (Wright, 1987).

The avidin maize powder treatment was not significantly different from the control for *C. ferrugineus*. The minute larvae of this species prefer to feed on the germ and will tunnel into this location if there are tiny cracks in the grain pericarp (Rilett, 1949). If some of the *C. ferrugineus* larvae fed on the maize germ, they would not have been exposed to the avidin powder. *Cryptolestes ferrugineus* larvae may also have chosen to feed in areas of the kernels that had been previously fed upon by the primary pest, and thus could have avoided feeding on the avidin powder. However, the avidin maize powder-parasitoid combination treatment was significantly different from the control treatment. The combined treatment may have been more effective than the avidin or parasitoid treatment alone because some of the *C. ferrugineus* larvae may have been stung by the parasitoids, while others may have died from eating the avidin powder.

The avidin maize powder treatment combined with the release of parasitoid wasps worked very well together to suppress both the external and internal insect pests of maize. Other researchers have also tried to use combinations of insecticides with parasitoid wasps, but frequently the insecticides were more lethal to the parasitoid than to the pest. For example, Perez-Mendoza et al.

(1999) found that diatomaceous earth was more toxic to the parasitoid than the host. In our study, we found that the powdered avidin maize had no effect on the parasitoid. We did not expect the avidin maize powder to cause significant mortality of *T. elegans* because this species does not feed on the grain. However, we were unsure whether the presence of 0.3% fine material in the grain would interfere with host searching. Our results showed that there was no significant difference in parasitoid efficacy in treatments with and without 0.3% powdered maize or powdered avidin maize.

Another combination treatment tested was an insecticidal protein-rich pea flour extract combined with two different species of parasitoid wasps, which has been used to control some insects in stored wheat (Hou et al., 2004). Pea flour had no significant effect on the parasitoid wasp species used in that study, *Anisopteromalus calandrae* (Howard) or *Cephalonomia waterstoni* (Gahan). The combination of protein-rich pea flour added to stored wheat at a level of 0.1% and parasitoid wasps reduced populations of the rice weevil, *Sitophilus oryzae* (L.), and *C. ferrugineus* by 98% and 75%, respectively.

The combination treatment of avidin maize powder plus the release of parasitoid wasps was superior to either treatment alone when tested against mixed populations of the internal feeder, *S. zeamais*, and the external feeders, *T. castaneum*, and *C. ferrugineus*. Normally, multiple insect species that are both external and internal feeders are found in stored grain. While avidin maize powder is fairly effective as an insecticide against the external feeders, it is not very effective against the internal feeders. By using the combination treatment, stored grain managers would be assured of protection from both internal and external feeders. Releasing the correct parasitoid species would not be difficult, because most of the species of parasitoid wasps that attack the internal feeders in stored grain, such as *R. dominica* and *Sitophilus* species, are generalists among these species. The advantage of using this particular beneficial species is that by releasing only *T. elegans*, it would control most of the insect species that are internal feeders in stored grain. Avidin maize powder could then be used in a combination treatment to suppress most of the insects that are external feeders, such as *C. ferrugineus*, *T. castaneum* and *O. surinamensis*.

Several other species of insect pests have been or are being screened in other laboratories for susceptibility to avidin toxicity including various pests of rice, cowpea, potato, and cotton (Kramer, 2004). In addition, some of those host plants have been or are being genetically engineered to produce avidin for host plant resistance to pest insects. For example, it was recently demonstrated that avidin expressed in rice confers protection against the confused flour beetle, *T. confusum* (Yoza et al., 2003). Insecticidal powders made from other materials besides maize expressing avidin, such as rice, could also be used in combination with parasitoids to protect the corresponding non-transgenic commodity against both primary and secondary insect pests.

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