

ECOTOXICOLOGY

Evaluation of a Resistant Parasitoid for Biological Control of Weevils in Insecticide-Treated Wheat

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ABSTRACT Interaction of a resistant strain of *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) and a host, the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), on wheat treated with malathion was studied in the laboratory. Based on dose response and serial time-response bioassays, malathion concentration had no significant effect on longevity, fecundity, or effectiveness of the Bamberg strain of *A. calandrae* parasitizing *S. oryzae* in wheat. Suppression of the immature weevil population exceeded 90% on malathion-treated wheat. Bamberg *A. calandrae* were more successful parasitizing the Savannah laboratory strain of *S. oryzae* compared with the Bamberg strain of *S. oryzae*, possibly because of the larger size of the Savannah weevils. The Bamberg strain of *S. oryzae* (12 times resistant at the LD₉₉ based on vial bioassays) was more tolerant of malathion applied to wheat than was the susceptible Savannah strain of *S. oryzae*. However, malathion concentration had no significant effect on emergence of adults of either weevil strain when wheat containing immatures was treated. Although oviposition was significantly reduced, both weevil strains oviposited on wheat treated with malathion. These results indicate that the ecology of host development (for example, protected weevil larvae feeding within grain kernels) may be primarily responsible for development of the resistance in Bamberg *A. calandrae* by providing a food source when the parasitoid is under selection pressure. Use of the resistant strain of *A. calandrae* in a management system for insect pests in stored grain is discussed.

KEY WORDS resistance, biological control, insecticide, parasitoid

STORED-PRODUCT ECOSYSTEMS OFFER many advantages for application of biological control technologies. These include numerous beneficial insects, both predators and parasitoids, associated with the major insect pests attacking stored grain and other commodities (Arbogast 1984, Brower et al. 1994); a broad knowledge base on population dynamics of the major insect pests that are hosts for these species (Throne 1995); and target insect hosts that are confined to commodities stored in discrete and often isolated storage systems that are protected from rapid changes in the environment (Brower 1990, Baker and Weaver 1993). This last advantage of these agricultural ecosystems is similar to advantages of biological control efforts in greenhouses. In both ecosystems, host insect populations can be estimated with precision and the effect of various release strategies of different beneficials into the essentially closed systems can be closely monitored. Despite the effectiveness of beneficial insects in the storage ecosystems, however, the strict requirements for allowable pest in-

sect infestation levels in most commodities may dictate that the best overall use of biological control efforts would be in an integrated program with other cultural, physical, or chemical control technologies.

In stored-grain ecosystems, a group of highly specific hymenopterous parasitoids attack the immature stages of the major primary pests of stored corn and wheat (Brower et al. 1995). Adults of most of these pest species are not attacked by the parasitoids but are long-lived and cause extensive feeding damage and weight loss of grain over their lifetime (Campbell et al. 1976). For these pest species, the integration or combination of biological control for immatures feeding within grain kernels and applications of grain protectants for control of exposed adults would significantly reduce their subsequent population development. However, the integration of biological control and chemical control in agricultural systems has been difficult and slow, primarily because of the increased sensitivity of most beneficials to pesticides (Croft 1990) and the general lack of significant resistance development in field strains of biological control organisms (Croft 1990, Hoy 1990, Tabashnik and Johnson 1995). Genetically improved strains of predaceous mites and hymenopterous parasitoids obtained

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through laboratory selection with insecticides have been successfully released into vineyards, greenhouses, strawberries, and orchard ecosystems (see Hoy 1993). Thus, in the absence of field strains of a particular parasitoid or predator with significant naturally occurring resistance, strains with laboratory-selected resistances can be effective. Nevertheless, because laboratory selected resistances tend to be polygenic in nature (Roush and McKenzie 1987), selection methods and techniques used to develop these strains must be carefully controlled (Rosenheim and Hoy 1988).

Field strains of 3 beneficial insects that prey on major stored-product insect pest species have recently been shown to have significant naturally occurring resistance to malathion, an organophosphate insecticide commonly used as a grain protectant in the United States since 1958 (Baker and Weaver 1993, Baker 1995, Baker and Arbogast 1995, Baker et al. 1995). The 2,800-fold resistance for a field strain of *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae), an effective parasitoid of grain weevils and other pest insects whose immature stages feed within grain kernels, is the most notable (Baker and Weaver 1993, Baker 1995).

The malathion resistance documented in the *A. calandrae* strain collected from a farm storage near Bamberg, SC, in September 1992 is unique among hymenopterous parasitoids and, for the first time, allows us to test the feasibility of integrating a resistant parasitoid simultaneously with commercial application rates of a grain protectant for control of immatures and adults of an economically important pest of stored grain, the rice weevil, *Sitophilus oryzae* (L.). In tests described below, we examine the efficacy of the Bamberg strain of *A. calandrae* as a parasitoid of *S. oryzae* in an environment of application rates of malathion newly applied to wheat. Responses of both susceptible (Savannah) laboratory strains and resistant (Bamberg) field strains of both parasitoid and host weevil, and their interactions, were compared. Tests were also designed to evaluate several of the hypotheses outlined in Baker and Weaver (1993) for evolution of resistance in this pteromalid wasp.

Materials and Methods

Insect Strains. Laboratory (Savannah) and Bamberg strains of *S. oryzae* were maintained on soft red winter wheat (a mixture of varieties, but primarily Florida 302) with a moisture content of 13.7% at $25 \pm 2^\circ\text{C}$ and 50–60% RH with a photoperiod of 12:12 (L:D) h. The laboratory strain has been maintained in culture for >20 yr and is considered malathion susceptible. The Bamberg strain of *S. oryzae* was collected from a corn bin in September 1992 and was 1.6 times resistant to malathion at the LD_{50} compared with the laboratory strain (Baker and Weaver 1993). F_{13} to F_{14} progeny were used in these studies.

Laboratory (Savannah) and Bamberg strains of *A. calandrae* were cultured on laboratory *S. oryzae* at $25 \pm 2^\circ\text{C}$ and 50–60% RH with a photoperiod of 12:12 (L:D) h. Emergent adult *A. calandrae* were transferred onto 21-d-old weevil cultures for each succeeding generation. Laboratory *A. calandrae* have been maintained in culture for >15 yr. Bamberg *A. calandrae* were collected near a corn bin in September 1992 and F_{23} to F_{27} progeny were used in these studies.

Insecticide. A commercial preparation of malathion (Drexel Malathion 5 emulsifiable concentrate [EC], 57% , Drexel, Memphis, TN) was obtained locally. Samples were weighed into volumetric flasks and dilutions were prepared to give the desired amount of active ingredient. The label rate for malathion on wheat is 8 ppm. For these laboratory tests, our highest test concentrations exceeded the label rate for malathion on wheat.

Spray Treatments. Samples of wheat (generally 200 or 250 g) were evenly distributed on a stainless steel tray (21.5 by 23 by 1.5 cm) and the tray placed in a fume hood. To obtain thorough coverage on all kernels, a 5 ml aqueous solution containing the appropriate amount of malathion EC was sprayed onto the wheat with a Crown Sprayer Tool (Hebron, IL) atomizer from a distance of ≈ 20 cm. Wheat sprayed with distilled water was used for controls. After spraying, the wheat was placed on aluminum foil and allowed to air dry for 2 h at room temperature. Samples were thoroughly mixed before bioassay and malathion analysis.

Analysis of Malathion. Samples of wheat from each experiment were ground in a blender and 20-g aliquots were extracted with 50 ml acetone for 4 h on a shaker. Filtrate (4 μl) was analyzed with a gas chromatograph equipped with a flame photometric detector on a glass column (1.8 m by 2 mm i.d.) packed with 5% OV-101 on GasChrom Q (60–80 mesh). Column oven temperature was 210°C and flow rate of nitrogen carrier gas was 30 ml/min. Gas flows into the detector were adjusted for optimum detector sensitivity for phosphorus. Peak areas were compared with those of an analytical malathion standard (American Cyanamid, Princeton, NJ).

Experimental. The experiments described below were designed to determine the effect of malathion on Bamberg and Savannah strains of the host *S. oryzae* and Bamberg and Savannah strains of *A. calandrae* and on the interaction of the 2 species. Specific questions asked included (1) Does malathion applied to wheat affect the mortality (or longevity) of adults of field and laboratory strains of either the host weevil or parasitoid. (2) Are survival and subsequent development of immature weevils within wheat kernels, as measured by emergence of adult weevils, affected by malathion applications to the wheat. (3) Does malathion affect the fecundity or effectiveness of *A. calandrae* parasitizing immature weevils in treated wheat.

Does host strain influence parasitoid success. (4) Will weevils oviposit on malathion-treated wheat and thus provide a constant source of hosts in the field. (5) What is the rate of dissipation of malathion under our experimental conditions. (6) Are results of bioassays with malathion-treated wheat consistent with those previously obtained with glass vial bioassays.

Mortality of *A. calandreae* on Wheat Treated with Malathion. Mortality of Bamberg and Savannah strains of *A. calandreae* was determined with 2 types of bioassays. In the 1st bioassay, 25 g samples of wheat treated with 0, 5, or 10 ppm malathion were placed in plastic vials (3.2 by 8 cm) with 40-mesh screen lids. *A. calandreae* were anesthetized with CO₂ and 15–20 adults were placed in each vial. Mortality was determined after 72 h at 25°C and 75% RH. Results are based on 4 replicates per concentration per strain. Treatment mortality was corrected for control mortality with Abbott's formula (Abbott 1925).

In the 2nd bioassay, 10-g samples of treated wheat (0, 5, and 10 ppm) were placed in plastic petri dishes (15 by 150 mm). Ten *A. calandreae* were placed in each dish and the dishes were held at 25°C and 75% RH. Mortality in this serial time-response bioassay was determined at 24-h intervals. Results are based on three tests with 5 replicates per concentration per strain for each test. Data were pooled and analyzed with the matrix-based probit procedure of Throne et al. (1995a). Slopes and intercepts were compared with a *z* test (Snedecor and Cochran 1967). Transformed bioassay data were back-transformed to original units (proportion insects killed) with a program written in Mathematica language (Throne et al. 1995b).

Mortality of *S. oryzae* on Wheat Treated with Malathion. A serial time-response bioassay was used to determine mortality rates of Bamberg and laboratory strains of *S. oryzae* on wheat treated with 0, 2.5, 5, and 10 ppm malathion. Samples of 10 g of treated wheat were placed in petri dishes as described above. Ten adult weevils were placed in each dish and the dishes were held at 25°C and 75% RH. Mortality was assessed at 24-h intervals. Results are based on 3 tests, with 5 replicates per concentration per strain for each test. Data were analyzed as above except time was transformed to $t + 12$ h before analysis (Tukey et al. 1985).

Emergence of *S. oryzae* from Wheat Treated with Malathion. Survival of immatures and subsequent emergence of the Bamberg strain of *S. oryzae* on wheat treated with malathion was determined with 2 separate tests. First, 200-g samples of wheat containing 16- to 23-d-old larvae were treated with 0, 1, 2, 5, or 10 ppm malathion. Four replicates from each sample were taken. Adult emergence at 28°C and 50–60% RH was determined. In the 2nd test, wheat containing 0- to 7-d-old weevil larvae was treated with 0, 5, or 10 ppm malathion. Three 200-g samples of wheat were treated with each concentration. Three 25-g

replicates were removed from each sample and placed in vials for a total of 9 replicates per concentration. Adult emergence at 28°C and 50–60% RH was determined as described previously.

Emergence of the Savannah strain of *S. oryzae* from malathion-treated wheat was determined by treating 200-g samples of wheat containing 20-d-old larvae with 0, 2, 5, and 10 ppm malathion. Adult emergence from five replicates of each sample was determined. Data from these experiments were analyzed by using the general linear models (GLM) procedure (SAS Institute 1987) to test for effects of concentration.

Interaction of Bamberg *A. calandreae* and Bamberg *S. oryzae* on Wheat Treated with Malathion. Immature weevil hosts were obtained by allowing ≈500 adult weevils (2–3 wk old) to oviposit in 1.5 kg of wheat for 7 d at 28°C and 50–60% RH. Adults were removed by sieving. Within a given experiment host densities were comparable, but densities were not specifically controlled between experiments.

Samples of wheat (250 g) containing 15- to 22-d-old larvae of Bamberg *S. oryzae* were treated with 0, 5, or 10 ppm malathion. Each wheat sample was divided into 10 25-g subsamples and placed into plastic vials with screen lids. Five female Bamberg *A. calandreae* were added to 5 subsamples from each treatment. Adult weevil emergence and production of *A. calandreae* was determined after 49 d at 28° and 50–60% RH. A 2nd identical test was performed on wheat containing 14- to 21-d-old Bamberg *S. oryzae* larvae.

The GLM procedure (SAS Institute 1987) was used to test for differences among doses of malathion, between parasitoid infestation levels, and for interaction between main effects. When pesticide effect was significant, linear contrasts were used to determine whether the number of weevils emerging was linearly or quadratically related to concentration of malathion.

Interaction of Bamberg *A. calandreae* and Savannah *S. oryzae* on Wheat Treated with Malathion. Three tests were done to evaluate the effect of malathion on effectiveness of Bamberg *A. calandreae* on wheat containing larvae of the Savannah strain of *S. oryzae*.

Test 1. Wheat containing 15- to 29-d-old larvae of *S. oryzae* was divided into 3 samples and treated with 0, 5, or 10 ppm malathion. Wheat in each of these treatments was subdivided into 10 samples (25 g). Five female Bamberg *A. calandreae* were placed into 5 of the subsamples. Weevil emergence, number of adult *A. calandreae*, and amount of frass was determined in each vial after 55 d at 28°C and 50–60% RH.

Test 2. Wheat containing 14- to 21-d-old weevil larvae was treated with 0 or 10 ppm malathion. Each of the 2 treatments was subdivided into 20 samples (25 g) and placed into vials. Bamberg *A. calandreae* were added to the subsamples within each treatment as follows: 5 vials received 5 *A.*

calandreae, 5 vials received 10 *A. calandreae*, 5 vials received 5 *A. calandreae* plus an additional release of 5 *A. calandreae* 5 d later, and 5 control vials received no *A. calandreae*. Weevil emergence, number of adult *A. calandreae* present, and amount of frass was determined in each vial after 49 d at 28°C and 50–60% RH.

Test 3. Test 3 was identical to test 2 except that the weevil larvae used for hosts were 16- to 23-d-old.

Data were analyzed with the GLM procedure as above, after Box-Cox (Box and Cox 1964) transformation to adjust for unequal variances among some treatments. TableCurve 2D and TableCurve 3D (Jandel 1993a, b) were used to fit equations to the data. We tested equations for lack of fit by using the method of Draper and Smith (1981).

Oviposition of *S. oryzae* in Wheat Treated with Malathion. To determine if *S. oryzae* would oviposit into malathion-treated wheat, wheat was treated with 0, 2.5, 5, or 10 ppm malathion and divided into 20 subsamples (25 g) per concentration. For both Bamberg and Savannah strains of *S. oryzae*, 10 adult weevils (unsexed) were placed in each of 10 subsamples per concentration and held at 28°C and 50–60% RH. Weevils (dead and alive) were removed from each vial after 14 d. The number of weevils emerged in each vial was determined after 55 d. Data were analyzed as described above.

Malathion Dissipation. Samples of wheat were sprayed with malathion to achieve application levels of ≈ 5 and 12 ppm; the samples were held at 28°C and 50–60% RH. Subsamples from each treatment were analyzed for malathion at 0, 1, 2, 3, and 4 d after application. Results are based on 3 separate samples per concentration with duplicate determinations for each sample at each time interval. Data were analyzed with the GLM procedure and equations fit to the data in TableCurve2D as described above.

Results

Malathion Application. A summary of the desired amount and mean \pm SD observed amounts of malathion applied with the spray treatments for all applications was: 2 ppm (2.6 ± 0.7 , $n = 8$); 5 ppm (5.2 ± 1.4 , $n = 34$); and 10 ppm (11.4 ± 2.2 , $n = 40$).

Mortality of *A. calandreae* on Wheat Treated with Malathion. We observed total mortality of the Savannah strain of *A. calandreae* at 72 h on wheat treated with 5 or 10 ppm. Corrected proportional mortality was 1.00 for both concentrations. In contrast, corrected mortality of the Bamberg strain was 0.09 at 5 ppm and < 0.0 at 10 ppm after 72 h on treated wheat.

Based on overlapping confidence intervals at the LT_{50} and z values < 1.96 for slope and intercept comparisons, the effect of malathion concentration on mortality of Bamberg *A. calandreae* in the serial

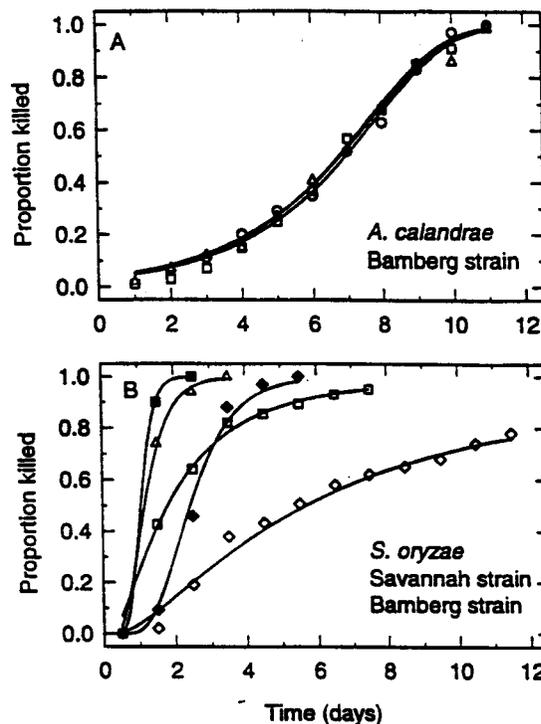


Fig. 1. (A) Mortality of the Bamberg strain of *A. calandreae* on wheat treated with malathion: 0 ppm, open circles; 5 ppm, open squares; and 10 ppm, open triangles. (B) Mortality of the Bamberg (open symbols) and Savannah (solid symbols) strains of *S. oryzae* on malathion-treated wheat: 10 ppm, triangles; 5 ppm, squares; 2 ppm, diamonds. Symbols are observed data and lines are back transformations to proportion killed, based on an original Gompertz transformation of mortality data in (A) and log time-probit mortality transformation of mortality data in (B).

time-response petri dish bioassay on treated wheat (Fig. 1 A) was not significant. Back-transformed mortality curves were nearly coincident at concentrations of 0, 5, and 10 ppm. A Gompertz transformation of mortality gave the best overall fit to the data. LT_{50} values were 6.8 d for 0 ppm (95% CL = 5.8–7.6; slope = 0.43 ± 0.04 ; $\chi^2 = 16.28$; $P = 0.026$), 6.9 d for 5 ppm (95% CL = 6.4–7.4; slope = 0.45 ± 0.04 ; $\chi^2 = 8.47$; $P = 0.194$), and 6.7 d for 10 ppm (95% CL = 6.2–7.2; slope = 0.45 ± 0.04 ; $\chi^2 = 8.01$; $P = 0.201$). Thus longevity of the Bamberg strain of *A. calandreae* was apparently not affected by malathion dose within the range that we tested. In contrast, complete mortality of the Savannah strain of the parasitoid occurred in ≈ 2 h on wheat treated with 10 ppm malathion in the same petri dish bioassay.

Mortality of *S. oryzae* on Wheat Treated with Malathion. Log-probit transformations gave the best overall fit for mortality data obtained with all strain-concentration combinations for *S. oryzae* on wheat treated with malathion. Back transformations of analyses of these serial time data are pre-

sented in Fig. 1 B. Based on t tests of slope and intercepts, the Bamberg strain of *S. oryzae* was significantly more tolerant of 2 ppm and 5 ppm malathion than was the Savannah strain (t values > 1.96). Complete mortality of the Savannah strain occurred at 10 ppm in <1 d, so these data were not analyzed with the matrix procedure. However, of overall interest is the fact that the Bamberg strain of *S. oryzae* was much more sensitive to malathion than was the Bamberg strain of *A. calandrac* (compare Fig. 1 A with B).

Emergence of *S. oryzae* from Wheat Treated with Malathion. There was no effect of concentration ($F = 0.31$; $df = 4, 15$; $P = 0.87$) on emergence of Bamberg weevils from wheat treated with malathion when the larvae were 16- to 23-d-old. Mean \pm SD number of weevils emerging per replicate ($n = 4$) was 198.8 ± 7.8 at 0 ppm, 197.3 ± 7.2 at 1 ppm, 197.2 ± 14.5 at 5 ppm, and 202.3 ± 19.8 at 10 ppm. Similarly, concentration had no significant effect ($F = 1.50$; $df = 2, 24$; $P = 0.24$) on emergence of weevils from wheat treated with malathion when the larvae were 0- to 7-d-old. Mean \pm SD number of weevils emerging per replicate was 60.9 ± 8.4 at 0 ppm, 66.4 ± 7.3 at 5 ppm, and 67.6 ± 10.2 at 10 ppm.

Concentration had no significant effect ($F = 0.96$; $df = 3, 16$; $P = 0.43$) on emergence of Savannah *S. oryzae* from wheat treated with malathion when the larvae were 20 d old. Mean \pm SD number of weevils emerging per replicate ($n = 5$) were 91.2 ± 8.9 at 0 ppm, 90.2 ± 3.9 at 2 ppm, 90.8 ± 5.7 at 5 ppm, and 96.0 ± 4.4 at 10 ppm.

Interaction of Bamberg *A. calandrac* and Bamberg *S. oryzae* on Wheat Treated with Malathion. Results of the 2 tests describing the effectiveness of the parasitoid on Bamberg weevils were nearly identical. In test 1, weevils emerging increased as the amount of malathion applied was increased ($F = 3.20$; $df = 2, 24$; $P = 0.05$). Mean \pm SD number of weevils ($n = 10$) emerging was 66.6 ± 13.6 at 0 ppm, 72.3 ± 15.6 at 5 ppm, and 76.5 ± 20.1 at 10 ppm. The presence of the Bamberg parasitoid caused a significant reduction in number of emerging weevils ($F = 76.5$; $df = 1, 24$; $P < 0.01$). Mean number of weevils ($n = 15$) emerging in the presence of Bamberg *A. calandrac* was reduced from 85.4 ± 9.9 to 58.2 ± 8.8 weevils per replicate in vials with 5 added parasitoids. We found no significant interaction effect between concentration of malathion and presence of parasitoid on number of emerging weevils ($F = 1.54$; $df = 2, 24$; $P = 0.23$).

In test 2, concentration of malathion had no significant effect on emergence of weevils from the treated wheat ($F = 0.69$; $df = 2, 24$; $P = 0.51$). Mean number \pm SD of emerging weevils was 66.2 ± 13.9 at 0 ppm, 69.2 ± 19.4 at 5 ppm, and 70.8 ± 13.1 at 10 ppm. The presence of the Bamberg parasitoid caused a significant reduction in number of emerging weevils ($F = 56.84$; $df = 1, 24$; $P < 0.01$). Mean number of weevils ($n = 15$) emerging

Table 1. ANOVA of 3 tests determining effects of release rates of the Bamberg strain of *A. calandrac* parasitizing rice weevils in wheat treated with different levels of malathion

Independent variable ^a	Weevils emerged		Wasp progeny		Frass produced	
	F	P	F	P	F	P
Test 1 ^b						
Pesticide level	1.88	0.17	0.61	0.55	387.3	<0.01
Parasitoid level	731.6	<0.01	236.8	<0.01	738.7	<0.01
Interaction	2.64	0.05	0.26	0.90	31.31	<0.01
Test 2 ^b						
Pesticide level	2.80	0.10	0.21	0.65	228.4	<0.01
Parasitoid level	863.0	<0.01	236.9	<0.01	736.7	<0.01
Interaction	2.87	0.05	1.19	0.33	37.66	<0.01
10 vs 5 + 5	7.36	0.01	1.17	0.29	3.50	0.07
Test 3 ^c						
Pesticide level	7.05	0.01	7.96	0.01	41.05	<0.01
Parasitoid level	330.6	<0.01	175.3	<0.01	188.6	<0.01
Interaction	2.23	0.10	1.60	0.21	10.87	<0.01
10 vs 5 + 5	0.24	0.63	2.57	0.12	0.21	0.65

^a Ten versus 5 + 5, comparison of a single release of 10 *A. calandrac* with 2 releases of 5 *A. calandrac* 5 d apart.

^b Degrees of freedom: pesticide level = 2, 36; parasitoid level = 2, 36; interaction = 4, 36.

^c Degrees of freedom: pesticide level = 1, 32; parasitoid level = 3, 32; interaction = 3, 32; 10 versus 5 + 5 = 1, 32.

in the presence of Bamberg *A. calandrac* was reduced from 81.3 ± 7.1 to 56.4 ± 10.2 weevils per replicate in vials with 5 added parasitoids. As in test 1, there was no significant interaction between concentration of malathion and presence of parasitoids ($F = 0.44$; $df = 2, 24$; $P = 0.65$).

In both tests, numbers of weevils in vials with parasitoids added was reduced by 30%. Mean \pm SD number of emerging parasitoids ($n = 15$) was 14.5 ± 3.2 in test 1 and 16.8 ± 5.0 in test 2. We found no significant effect of malathion concentration or interaction between concentration and presence of parasitoids on number of emerging parasitoids per vial in either test.

Interaction of Bamberg *A. calandrac* and Savannah *S. oryzae* on Wheat Treated with Malathion. Overall results indicated that malathion applications had no significant effect on effectiveness or fecundity of the Bamberg strain of *A. calandrac* parasitizing *S. oryzae* in wheat (Table 1). Reductions in numbers of emerging weevils exceeded 90% in these tests. Equations and equation parameters describing the data in tests 1, 2, and 3 are presented in Table 2.

Because concentration of malathion had no significant effect on numbers of emerging weevils (Table 1) (Fig. 2 A and D) or *A. calandrac* progeny produced (Fig. 2 B and E) in tests 1 and 2, respectively, data are plotted as a function of number of parasitoids released. In test 3, with malathion treatments of 0 and 10 ppm, we found a slight but

Table 2. Parameters of equations obtained from three separate tests describing effects of release rate of the Bamberg strain of the parasitoid *A. calandracae* combined with malathion application levels on emergence of the Savannah strain of the host rice weevil, number of parasitoids produced, and frass production (damage) on wheat at 28°C and 50–60% RH

Comparison	Eqn ^b	Equation parameters ± SEM			Goodness-of-fit ^a		
		a	b	c	R ²	F _L	P > F
Test 1							
Weevils emerged vs parasitoid level	1	14.60 ± 1.923	108.4 ± 3.330	—	0.96	0.35	0.89
<i>A. calandracae</i> produced vs parasitoid level	2	-2.986 ± 0.2833	50.35 ± 2.453	—	0.93	<0.01	1.00
Frass produced vs malathion level vs parasitoid level	3	4.778 ± 0.1013	-0.6328 ± 0.0252	2.084 ± 0.1022	0.98	1.90	0.11
Test 2							
Weevils emerged vs parasitoid level	1	25.67 ± 2.308	151.5 ± 3.997	—	0.98	1.74	0.20
<i>A. calandracae</i> produced vs parasitoid level	2	-4.692 ± 0.2616	82.63 ± 2.267	—	0.98	<0.01	1.00
Frass produced vs parasitoid level at 0 ppm malathion	1	99.99 ± 10.03	620.7 ± 17.37	—	0.99	0.96	0.35
Frass produced vs parasitoid level at 10 ppm malathion	1	73.80 ± 5.658	214.9 ± 9.800	—	0.97	1.15	0.30
Test 3							
Weevils emerged vs parasitoid level at 0 ppm malathion	1	13.01 ± 3.314	141.4 ± 5.739	—	0.98	0.23	0.64
Weevils emerged vs parasitoid level at 10 ppm malathion	1	22.37 ± 3.678	126.0 ± 6.371	—	0.97	0.10	0.76
<i>A. calandracae</i> produced vs parasitoid level at 0 ppm malathion	2	-3.933 ± 0.7778	71.90 ± 6.736	—	0.93	<0.01	1.00
<i>A. calandracae</i> produced vs parasitoid level at 10 ppm malathion	2	-4.040 ± 0.6327	80.75 ± 5.479	—	0.96	<0.01	1.00
Frass produced vs parasitoid level at 0 ppm malathion	1	61.88 ± 13.56	466.9 ± 23.50	—	0.97	0.14	0.71
Frass produced vs parasitoid level at 10 ppm malathion	1	53.16 ± 8.124	156.7 ± 14.07	—	0.91	0.05	0.83

^a All R² values were 99–100% of the maximum R² possible. F_L is the F value for lack of fit (Draper and Smith 1981).

^b Equations were (1) $y = a + be^{-x}$, (2) $y = ax^{1.5} + bx^{0.5}$, and (3) $\ln_2 = a + bx^{0.5} + ce^{-y}$.

significant increase in both the number of weevils (Fig. 2G) and number of parasitoids (Fig. 2H) emerging from wheat treated with 10 ppm malathion.

Generally, dependent variables were not significantly affected by multiple release treatments compared with a single release of the parasitoid in these bioassays. However, compared with a single release of 10 *A. calandracae* per vial in test 2, multiple releases of 5 + 5 parasitoids resulted in a small but significant reduction in weevils emerging from treated wheat (Table 1).

Frass production was significantly ($P < 0.05$) affected by both pesticide level and parasitoid in all tests. Frass production in test 2 (Fig. 2F) and test 3 (Fig. 2I) was significantly reduced in malathion-treated wheat in the absence of parasitoids because most weevils that emerged in these vials subsequently died.

Oviposition of *S. oryzae* in Wheat Treated with Malathion. Malathion concentration significantly reduced ovipositional response of *S. oryzae* in treated wheat ($F = 30.52$; $df = 3, 72$; $P = 0.00$) (Fig. 3). Some oviposition of the Savannah strain occurred in wheat treated with 10 ppm. However, oviposition response between the Bamberg and Savannah strains was not significantly different ($F = 0.49$; $df = 1, 72$; $P = 0.49$) nor was interaction between malathion dose and strain significant ($F = 1.85$; $df = 3, 72$; $P = 0.15$). In the presence of malathion, the oviposition response for both strains was best described by a negative exponential equation ($y = a(x/b)$), where y = progeny produced and x = malathion concentration. Because there was no strain effect, data for both strains were combined (parameters ± SEM were $a = 97.64 ± 7.935$; $b = 1.818 ± 0.391$).

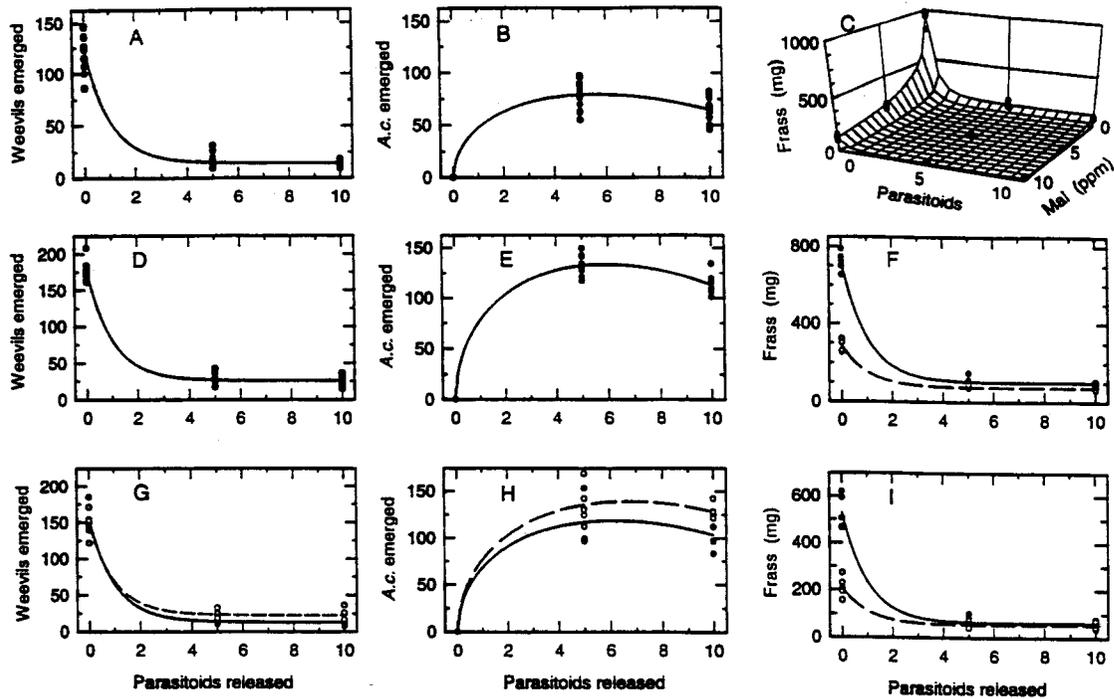


Fig. 2. Interaction of Bamberg *A. calandrarum* with the host Savannah *S. oryzae* on wheat treated with malathion. (A, D, and G) Reduction in number of weevils emerged per replicate as a function of number of parasitoids released. (B, E, and H) *A. calandrarum* progeny produced per replicate as a function of number of parasitoids released. (C, F, and I) Frass produced per replicate as a function of malathion level and number of parasitoids released. In A, B, D, and E there was no significant malathion concentration effect so combined data are plotted. (F, G, H, and I) Solid symbols, 0 ppm; open symbols, 10 ppm malathion. Equations and equation parameters used to prepare these graphs are given in Table 2.

Dissipation of Malathion. Mean initial levels of malathion applied to the wheat were 6.8 and 13.6 ppm. At 28°C and 50–60% RH, a 40% loss of malathion occurred at the lower concentration and a 36% loss of malathion occurred at the higher con-

centration after 4 d (Fig. 4). These data were described by a decay curve ($y = a/[(1 + 2a^2bx)^{0.5}]$), where y = malathion (ppm) and x = time (d) at 28°C. Parameters were: $a = 6.779 \pm 0.139$, $b = 0.0053 \pm 0.0004$ for the low concentration; and $a = 13.64 \pm 1.271$, $b = 0.0009 \pm 0.0004$ for the high concentration.

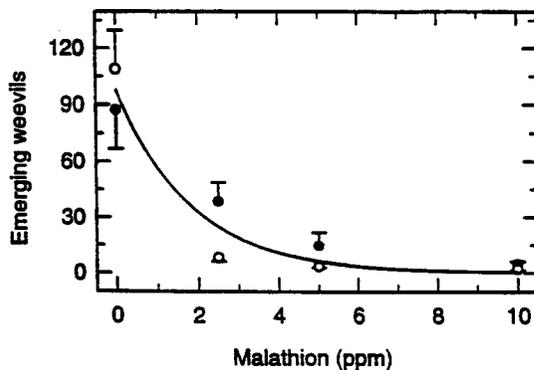


Fig. 3. Oviposition response of Bamberg (solid circles) and Savannah (open circles) strains of *S. oryzae* in wheat treated with malathion. Symbols are observed mean \pm SEM numbers of weevils emerging per replicate. There was no significant difference between strains so the line represents equation $y = 97.64(x/1.818)$ fit to combined data.

Discussion

Interaction of Insecticide with Parasitoid. Results of our experiments provide no evidence that the longevity, fecundity, or effectiveness of the resistant Bamberg strain of *A. calandrarum* parasitizing *S. oryzae* in wheat was reduced by malathion. Reduction in populations of hosts were >90% in the presence of malathion residues. Our study provides the first documentation of efficacious control of an insect pest by an insecticide-resistant parasitoid in the presence of insecticide levels that actually exceeded the label application rate. Although the bioassays were of a no-choice type, results also indicated that levels of malathion tested had no apparent detrimental effect on any behavioral mechanism(s) involved in host location by the parasitoid in the small masses of wheat.

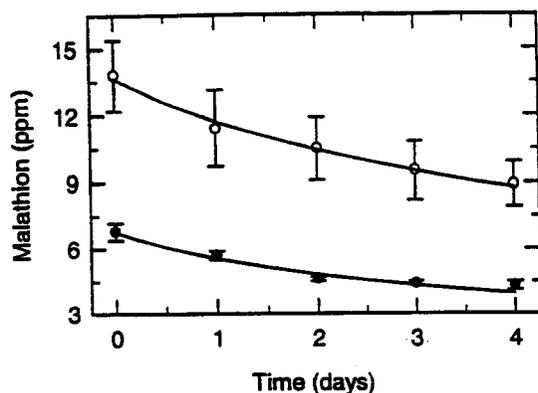


Fig. 4. Degradation of malathion on wheat with a moisture content of 13.7% at 28°C and 50–60% RH. Open circles, mean initial deposit, 13.6 ppm; solid circles, mean initial deposit, 6.8 ppm. Symbols are observed data (mean \pm SEM). Lines represent a decay equation ($y = a/(1 + 2a^2bx)^{0.5}$) fit to both initial malathion levels (parameter values in text).

In our studies, Bamberg *A. calandreae* were placed on wheat several hours after treatment. Even though 40% of the malathion dissipated in 4 d with our experimental conditions, the parasitoids were initially exposed to very high levels of pesticide. These initial high doses had no observable effect on this resistant strain. In contrast, parasitoids with laboratory selected resistances required some residue aging or degradation before significant survival occurred. For example, 52% of a laboratory selected strain of *Trioxys pallidus* Haliday (Hymenoptera: Aphididae), a parasitoid of the walnut aphid *Chromaphis juglandicola* (Kaltenbach), died after a 24-h exposure to 1-d-old residues of azinphosmethyl, whereas mortality was reduced to 15% after residues had aged for 4 d (Hoy and Cave 1989). Approximately 18 d of weathering of carbaryl residues on citrus were required before 50% of a genetically improved strain of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), a parasitoid of California red scale, *Aonidiella aurantii* (Maskell), would survive (Rosenheim and Hoy 1988). This strain of *A. melinus* was \approx 20 times resistant compared with a standard susceptible strain. However, this degree of resistance still did not permit survival on initial application levels of carbaryl. Although results of laboratory bioassays are not always correlated with observations in the field (Hoy 1990, Elzen et al. 1987), results from glass vial bioassays that were used to document a 2,800-fold resistance to malathion for the Bamberg strain of *Anisopteromalus calandreae* (Baker 1995) were a good indication of the subsequent tolerance of this parasitoid for field application rates of malathion on wheat.

Although malathion did not affect efficacy, Bamberg *A. calandreae* was more successful in parasitizing Savannah *S. oryzae* than Bamberg *S. oryzae*. Bamberg weevils are significantly smaller than Sa-

vannah weevils (mean adult weight of 1.36 mg for Bamberg compared with 2.44 mg for Savannah) (Baker and Weaver 1993). Because host size can influence success of pteromalids attacking internal grain insects (Wen et al. 1995, Smith et al. 1995), the small size of the Bamberg weevils may be primarily responsible for the reduced success of *Anisopteromalus calandreae* on Bamberg weevils in these tests. However, the developmental rate of Bamberg *S. oryzae* is slower than that of Savannah *S. oryzae* (unpublished data). As a result, better control of Bamberg weevils may be possible with appropriate adjustments for time of parasitoid release.

Interaction of Insecticide With Insect Host. Bamberg *S. oryzae* was \approx 1.6 times resistant to malathion compared with the Savannah strain in glass vial bioassays (Baker and Weaver 1993). This low level of resistance was reflected in the increased longevity of the Bamberg strain on wheat treated with malathion in the study described here. However, malathion had no significant effect on subsequent emergence of immatures of either the Bamberg or Savannah *S. oryzae* strain from treated wheat. These results support the findings of Strong and Sbur (1964). They tested 11 insecticides at 10 and 20 ppm for control of immature *S. oryzae* in wheat and found that malathion caused little mortality among the immature stages. Dichlorvos was effective in controlling immatures possibly because of the fumigative action of this compound (Desmarchelier et al. 1977).

Malathion significantly reduced oviposition of *S. oryzae* in treated wheat. Despite the toxicity of tested concentrations to adults, some oviposition and progeny production nevertheless occurred in both the Bamberg and Savannah weevil strains in wheat treated with 5 or 10 ppm malathion.

Few specific studies have described the effects of application levels of grain protectants on oviposition by *Sitophilus* weevils. Most information on oviposition has been obtained secondarily. For example, Arthur et al. (1992) treated wheat with a calculated concentration of 6 ppm chlorpyrifosmethyl (actual deposition, 4.4 ppm) and found essentially no progeny production by *S. oryzae* on wheat that had been stored for 2 mo at 25°C. However, oviposition occurred after longer storage periods and further degradation of the insecticide. Similar results were obtained with chlorpyrifosmethyl residues on corn infested with maize weevil, *S. zeamais* Motschulsky (Arthur et al. 1991). Storage periods of 4–6 mo at 30°C were required before F_1 progeny were produced. In both these studies, degradation of insecticide to low levels had to occur before any progeny were produced. Wheat treated with low levels (0.6 or 1 ppm) of fenitrothion (another organophosphate) that were slightly toxic to adults did not significantly affect fecundity of *S. oryzae* (Thaung and Collins 1986). Our results differed from those of the previous studies because both strains of *S. oryzae* oviposited

on wheat that had relatively high levels of malathion.

Interaction of Malathion with Host Food. Results of our studies on effect of malathion applications on immature weevils provide evidence that malathion does not penetrate significantly into the endosperm of the wheat kernels, at least in the time span of our studies. Kadoum and LaHue (1977) found that the flour (endosperm) fraction from milled wheat contained the least amount of malathion residue. Our bioassays confirm results of these distribution studies.

Loss of malathion residue depends on temperature and moisture content of the grain during storage (Kadoum and LaHue 1976, 1979; Abdel-Kader et al. 1980). Residue loss can exceed 36% in 24 h at 27°C in grain with 16% moisture content (Kadoum and LaHue 1979). In our study, we detected a 35–40% loss in residue on wheat held at 28°C and 50–60% RH. These results are similar to those obtained by Kadoum and LaHue (1976). It is apparent that the dissipation rate of malathion applied to grain can be significant during bin loading and storage in the warm, humid conditions present in the southeastern United States.

Resistance Development in *Anisopteromalus calandrae*. A scenario for development of malathion resistance in *Anisopteromalus calandrae* in the southeast (detailed in Baker and Weaver 1993) included (1) single applications of malathion on grain during bin loading and subsequent degradation of residue throughout the storage period; (2) immature weevil hosts that are not exposed to malathion applications and that are generally protected from effects of the insecticide; and (3) parasitoid life history parameters (for example, rapid development times, a low propensity to disperse, and relative host specificity) that were thought to favor resistance development (Croft and Morse 1979).

Our findings confirm the rather rapid dissipation of malathion on wheat, as well as the availability of host weevil larvae in the presence of field rates of malathion. The latter results corroborate the importance of hidden host stages in development of parasitoid resistance (Rosenheim and Hoy 1986), and also provide support for the food limitation hypothesis as one basis for evolution of insecticide resistance in natural enemies (Tabashnik 1986). This hypothesis suggests that insecticide resistance development must occur in the host before significant resistance can develop in the parasitoid or predator. However, in the *Anisopteromalus-Sitophilus* system, the internal location of immature weevils in grain kernels, rather than any significant development of insecticide resistance by the host insect, is primarily responsible for providing a readily available source of food for the parasitoid, even when the parasitoid is under selection pressure. Differences in life history and ecological factors are known to influence insecticide resistance

development in predator/prey systems (van de Baan and Croft 1990).

Resistant Beneficial Insects in Stored Grain Insect Pest Management. Although malathion has been used as a grain protectant in the United States since the late 1950s, the Environmental Protection Agency has given notification in 1995 of receipt of requests from several registrants to delete the registration for malathion use on stored commodities. Nevertheless, our results demonstrate that a combination of malathion as an adulticide for *Sitophilus* weevils and simultaneous use of the resistant strain of *A. calandrae* as a larvicide for these species is feasible and could be an effective pest management system for stored grain. Along with appropriate physical and cultural insect control practices in bulk storage of grain, this combined parasitoid-insecticide system could allow for a significant reduction in insecticide use. Reduced application levels of insecticide could still kill those weevils initially present in the grain during bin loading but less residual control of adults throughout storage would be necessary.

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