

INSECTICIDE RESISTANCE AND RESISTANCE MANAGEMENT

Inheritance of Malathion Resistance in the Parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae)J. E. BAKER, J. PEREZ-MENDOZA,¹ AND R. W. BEEMAN

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J. Econ. Entomol. 90(2): 304-308 (1997)

ABSTRACT The genetic basis of malathion resistance in the Bamberg (R) strain of *Anisopteromalus calandrae* (Howard) was determined with reference to a susceptible (S) laboratory strain of the parasitoid. Evidence from bioassays of male and female progeny from the parent R and S strains, F1 hybrids, backcrosses, and inter se (self) crosses indicates that the malathion resistance in this haplo-diploid wasp is inherited as an incompletely dominant trait controlled by a single gene. This inheritance pattern will slow the diluting effect of interbreeding of progeny from released resistant wasps with susceptible wasps present in any resident population within a particular grain storage facility. It may also allow either an inoculative or inundative release strategy, depending on the size of the resident parasitoid population, within an integrated chemical-biological management program for grain weevils in stored cereals.

KEY WORDS *Anisopteromalus*, parasitoid, insecticide, malathion, resistance

MODERATE TO HIGH LEVELS of malathion resistance are present in field strains of 2 hymenopterous parasitoids (Baker and Weaver 1993, Baker et al. 1995) and 1 anthocorid predator (Baker and Arbogast 1995) collected from corn and peanut storages in the southeastern United States. Especially notable is the malathion resistance found in a strain of *Anisopteromalus calandrae* (Howard) collected in September 1992 from a farm storage near Bamberg, SC. The parasitoid is \approx 200-fold more tolerant of malathion than its host, the rice weevil, *Sitophilus oryzae* (L.), collected at the same facility (Baker 1995). Application levels of malathion up to 10 ppm applied to wheat had no significant effect on the longevity, fecundity, or effectiveness of the Bamberg strain of *A. calandrae* parasitizing weevil larvae present in treated kernels (Baker and Throne 1995). Results of this latter study indicate that the resistant Bamberg strain of *A. calandrae* has the potential to be incorporated into an integrated biological-chemical control program for internally feeding insects in stored grain.

Natural populations of stored grain insect parasitoids are found in many grain storages. However, an augmentative release strategy will probably be necessary to use these parasitoids effectively to control pest insect species in these ecosystems (Parella et al. 1992, Brower et al. 1995). Whether resistant *A. calandrae* are inundatively or inoculatively released into a grain storage, it would be im-

portant to know the genetic basis of resistance, so that the effects of interbreeding of the released resistant strain with the local strain can be incorporated into the pest management strategy (Caprio et al. 1991, Brown et al. 1992). The genetic basis of the malathion resistance in *A. calandrae* has not been examined previously. However, the resistance in this haplo-diploid wasp has been stable for at least 23 generations of laboratory rearing without any selection pressure (Baker 1995). The resistance mechanism has not been clarified, but there is evidence that a triphenyl phosphate-suppressible carboxylesterase may be involved (Baker 1994), similar to the malathion resistance found in other insect species (e.g., Beeman 1983).

The Bamberg strain carries by far the highest level of naturally occurring pesticide resistance ever recorded for a hymenopterous parasitoid. For this reason, and because of its importance as a major parasitoid of a severely damaging group of stored grain insect pests, we conducted studies on the inheritance of resistance in this species. Results of bioassay of male and female progeny from the original parent strains, hybrid progeny from reciprocal crosses of the resistant and susceptible strains, backcross progeny, and progeny from inter se crosses of hybrids are reported below.

Materials and Methods

Rearing of Insect Hosts and Parasitoids. Cultures of the host rice weevil, *S. oryzae*, were maintained on soft red winter wheat (primarily 'Florida 302') at 27°C and 60% RH. Cultures of *A. calandrae* were maintained by aspirating adult parasit-

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oids into 21-d-old weevil cultures held at the same temperature and humidity. The Savannah laboratory (susceptible, S) strain of *A. calandrae* has been maintained in culture for >20 yr. The Bamberg (resistant, R) strain of *A. calandrae* was collected in a farm storage in South Carolina in 1992 and has been reared continuously on a laboratory strain of the rice weevil without any selection pressure.

Isolation Procedures. Virgin males and females from the S and R parent strains of *A. calandrae*, as well as from appropriate crosses, were obtained by isolating kernels from batches of wheat containing parasitized weevil larvae. Generally, 5–6 wheat kernels were placed in glass test tubes (13 by 100 mm) with plastic caps and the tubes were observed daily for emergence of adult parasitoids.

Mating Success Between Parasitoid Strains. Reciprocal single pair crosses between Savannah (S) and Bamberg (R) strains of *A. calandrae* were made to determine mating success between strains and the number and sex ratio of progeny. The following crosses were made: S × S, S × R, R × R, R × S, unmated S females, and unmated R females (females are listed first in each cross). The virgin adults were paired in glass tubes (13 by 100 mm). After 24 h, during which time a source of honey was available to the isolated pair, each pair (or each unmated female) was placed in a clear plastic snap-cap prescription vial (3.2 by 8 cm, 16 drams) with screen lid that contained 25 g of wheat infested with 21-d-old rice weevil larvae. The number and sex of emerging parasitoids at 25°C and 75% RH were determined and compared between crosses.

Inheritance of Resistance. Virgin parasitoids from the S and R strains of *A. calandrae* were obtained by isolation of infested wheat kernels as described above. Dose–response bioassays with malathion were conducted on male and female progeny from S and R parent strains and on F1 male and female progeny from the following single-pair crosses: S × R, R × S, (S × R) × R, (R × S) × S, (S × R) self, and (R × S) self.

Expected ratios of genotypes and phenotypes for the above crosses are based on an assumption of a 1 gene–2 allele system and the presence of diploid females and haploid males in both parasitoid strains. Males are assumed to be produced from unfertilized, haploid eggs, an assumption consistent with our results (see below).

Dose–Response Bioassays. Dose–response bioassay of adult parasitoid progeny (2–7 d old) from the single pair crosses were conducted at 25°C and 75% RH in 20 ml glass vials (28 by 60 mm) with screen lids as described by Baker and Weaver (1993). Technical malathion (95% [AI], American Cyanamid, Princeton, NJ) was used. Concentrations in the test vials were as follows: 0, 0.2, 1, 10, 50, 100, 250, 500, 750, and 1,000 µg malathion [AI]/vial. Generally 10–14 adults were placed in each vial and 5 replicate vials were used for each concentration. After a 24-h exposure to the mala-

Table 1. Number and sex ratio (mean ± SE) of progeny from single pair crosses of the Savannah (S, susceptible) and Bamberg (R, resistant) strains of *A. calandrae* parasitizing rice weevil larvae in wheat at 25°C and 75% RH

Cross ^a	n	No. progeny ^b	Sex ratio ^c
S × S	9	75.2 ± 17.1a	2.8 ± 0.4a
S × R	4	66.5 ± 21.2a	2.3 ± 0.4a
R × R	7	97.1 ± 10.8a	2.2 ± 0.4a
R × S	8	83.7 ± 10.7a	2.6 ± 0.3a
S (unmated)	9	50.8 ± 9.9a	All males
R (unmated)	8	63.7 ± 9.3a	All males

^a By convention, females are listed first in each cross.

^b Means followed by the same letter are not significantly different (ANOVA, $F = 1.62$; $P > F = 0.178$).

^c Ratio of ♀/♂ progeny. Means followed by the same letter are not significantly different (ANOVA weighted by number of progeny, $F = 0.81$; $P > F = 0.501$).

thion, the parasitoids were removed from the vials and placed in plastic petri dishes (15 by 100 mm). Mortality was assessed after an additional 24 h.

Data Analyses. Number and sex ratio of progeny from single pairs of each parent strain and reciprocal hybrid crosses between strains were analyzed by analysis of variance (GLM) in SAS (SAS Institute 1987). The analysis of sex ratio was weighted by number of progeny produced by each pair.

Dose–mortality data were analyzed by probit regression in SAS (SAS Institute 1988). Degree of dominance was calculated according to Stone (1968). Chi-square analysis (Snedecor and Cochran 1971) was conducted on expected and observed ratios of backcross and selfcross progeny that were sensitive to discriminating concentrations of malathion.

Results and Discussion

Mating Between S and R Strains. There was no significant difference in total progeny or sex ratio of progeny between the parent strains, between reciprocal crosses, or between reciprocal crosses and the parent strains when adult females from these crosses were allowed to oviposit over their entire lifetime (Table 1). Unmated females of both the S and R strains produced only males. Mating was almost immediate when virgin males and females in the various crosses were combined in the glass tubes. Based on the numbers of progeny from each cross in these tests and the observed behavior of parasitoids, there is no evidence of mating incompatibility between the R and S strains of *A. calandrae*.

Inheritance of Resistance—Parent Strains. Malathion concentrations ranging from 1 to 100 µg per vial can effectively discriminate between progeny from the parent S and R strains of *A. calandrae* (Fig. 1A). All S wasps are killed by doses >0.9 µg per vial whereas doses >100 µg per vial are necessary before significant mortality occurs in the R strain. Probit parameters of progeny of the S

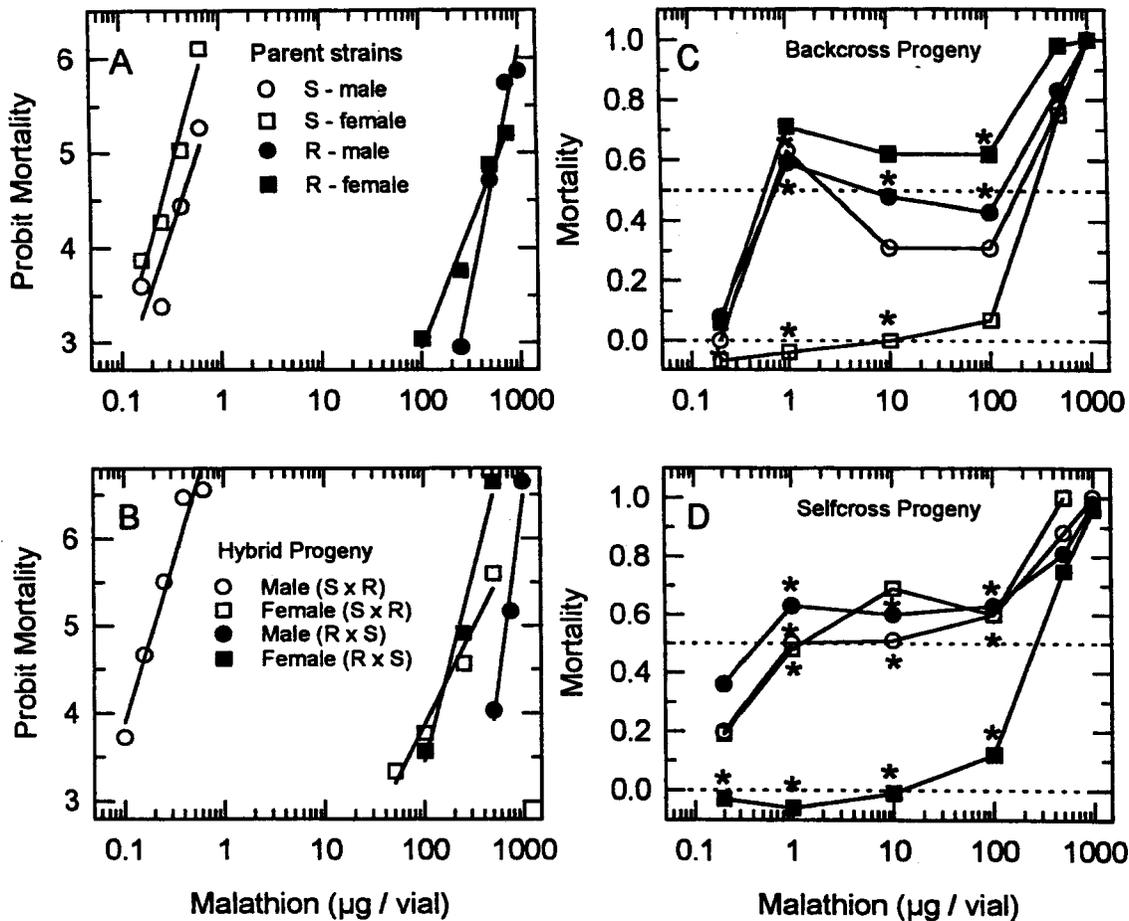


Fig. 1. Effect of malathion concentration on mortality of male and female progeny of *A. calandrae* from the following. (A) Parent R and S strains. (B) Hybrid crosses of parent R and S strains (open circles, males from $S\varnothing \times R\delta$; open squares, females from $S\varnothing \times R\delta$; solid circles, males from $R\varnothing \times S\delta$; solid squares, females from $R\varnothing \times S\delta$). (C) Backcrosses of virgin hybrid females backcrossed to virgin males of the opposite parent strain (open circles, males from $\varnothing[S/R] \times \delta R$; open squares, females from $\varnothing[S/R] \times \delta R$; solid circles, males from $\varnothing[R/S] \times \delta S$; solid squares, females from $\varnothing[R/S] \times \delta S$). (D) Inter se or selfcrosses of F1 hybrid progeny (open circles, males from $\varnothing[S/R] \times \delta S$; open squares, females from $\varnothing[S/R] \times \delta S$; solid circles, males from $\varnothing[R/S] \times \delta R$; solid squares, females from $\varnothing[R/S] \times \delta R$). Data points marked with stars in C and D are not significantly different (0.05 level) from expected mortality based on chi-square analysis.

strain in these tests were males ($n = 631$; $\chi^2 = 7.52$; slope \pm SE, 4.7 ± 1.6 ; $LD_{50} = 0.56 \mu\text{g}$ per vial [95% CL = undefined]); females ($n = 428$; $\chi^2 = 2.62$; slope \pm SE, 3.6 ± 0.6 ; $LD_{50} = 0.38 \mu\text{g}$ per vial [95% CL = 0.32–0.44]). Probit parameters of progeny from the R strain in these tests were males ($n = 258$; $\chi^2 = 6.82$; slope \pm SE, 4.2 ± 0.9 ; $LD_{50} = 529 \mu\text{g}$ per vial [95% CL = undefined]); females ($n = 318$; $\chi^2 = 1.26$; slope \pm SE, 2.9 ± 0.6 ; $LD_{50} = 611 \mu\text{g}$ per vial [95% CL = 515–765]). Based on these and previous data (Baker and Weaver 1993), there is no evidence of heterogeneity with respect to malathion-susceptibility or resistance in the 2 strains.

Inheritance of Resistance—Hybrids. Hybrid males from the S/R cross were fully susceptible to malathion (Fig. 1B). Probit parameters of these

males were ($n = 323$; $\chi^2 = 4.12$; slope \pm SE, 3.9 ± 0.4 ; $LD_{50} = 0.19 \mu\text{g}$ per vial [95% CL = 0.16–0.21]). Hybrid females from the S/R and R/S crosses, and males from the R/S cross, were resistant. Probit parameters of these progeny were females from S/R ($n = 372$; $\chi^2 = 2.14$; slope \pm SE, 2.6 ± 0.6 ; $LD_{50} = 325 \mu\text{g}$ per vial [95% CL = 257–400]); males from R/S ($n = 179$; $\chi^2 = 3.32$; slope \pm SE, 10.5 ± 3.8 ; $LD_{50} = 683 \mu\text{g}$ per vial [95% CL = 444–759]); females from R/S ($n = 276$; $\chi^2 = 4.07$; slope \pm SE, 23.4 ; $LD_{50} = 253 \mu\text{g}$ per vial [95% CL = undefined]).

Phenotypic responses of the male and female progeny from the hybrid crosses were as expected for an arrhenotokous system (Table 2). Male progeny from the S \times R cross were fully susceptible because males come from unfertilized eggs and

Table 2. Expected ratios of genotypes and the phenotypic response of male and female progeny to a discriminating concentration of malathion from hybrids, backcrosses, and selfcrosses originating from resistant (R) and susceptible (S) parent strains of the haplo-diploid wasp *A. calandreae*

Cross	Genotype of parents		Genotypes of progeny		Mortality at discriminating concn, %	
	♀	♂	♀	♂	♀	♂
	Hybrid	S/S	R	All S/R	All S	0
Hybrid	R/R	S	All R/S	All R	0	0
Backcross	S/R	R	½ S/R	½ S	0	50
Backcross	R/S	S	½ R/S	½ R	50	50
Selfcross	S/R	S	½ S/S	½ S	50	50
Selfcross	R/S	R	½ R/R	½ R	0	50

Assuming inheritance of resistance is based on a 1 gene-2 allele system with diploid females and haploid males (males produced from unfertilized eggs). Malathion concentrations of 1-100 µg per vial will discriminate between SS and RS genotypes.

males from this cross would contain only the maternal genotype. Similarly, hybrid males from the R × S cross were fully resistant because they contained only the genotype of the resistant mother. Resistance was incompletely dominant with a degree of dominance calculated to be 0.74 for hybrid females from the R × S cross. LD₅₀s of the resistant hybrid females were only slightly lower than those of the parent R strain females.

Because sex is controlled by ploidy in this species and in most other hymenopterous parasitoids, linkage can not be defined as being autosomal or through sex chromosomes.

Inheritance of Resistance-Backcrosses and Selfcrosses. Female progeny from the backcross (S/R × R) were unaffected by concentrations of malathion up to ≈100 µg per vial (Fig. 1C). These female offspring are expected to carry at least one R allele (Table 2) and should therefore all be resistant. Male progeny of the (S/R × R) backcross and male and female progeny of the (R/S × S) backcross had a broad plateau of mortality at approximately 50% kill, indicating a 1:1 segregation of resistant and susceptible phenotypes. This observation is consistent with the conclusion that malathion resistance in these wasps is controlled by a single, major gene. Although the possible contribution of additional minor genes or modifying genes cannot be totally discounted, the minor deviations from the expected plateau at 50% mortality over a range of discriminating concentrations of malathion (1-100 µg per vial) were in a direction opposite from that expected for polygenic inheritance (i.e., toward negative rather than positive slope values [Fig. 1C]).

Female progeny from the selfcross (R/S × R) were unaffected by concentrations of malathion up to ≈100 µg per vial (Fig. 1D). Mortality of these females in the dose range of 1-100 µg per vial was not significantly different from the expected 0% mortality by chi-square analysis (see Table 1). Al-

most none of the mortality values for females from this cross, or for males and females from the (S/R × S) selfcross, were significantly different from the expected 50% mortality in the dose range of 1-100 µg per vial based on the single, dominant gene model.

Bioassays with both backcross and selfcross progeny indicated broad plateaus of mortality across a 100-fold concentration range of malathion. These types of plateaus within dose-mortality curves provide convincing evidence for a monogenic inheritance of resistance (Tsukamoto 1983) and also fit the expected phenotypic response for the haplo-diploid *A. calandreae*.

Genetic Basis for Malathion Resistance in *A. calandreae*. Naturally occurring insecticide resistance among hymenopterous parasitoids is uncommon (Croft 1990), probably because of the difficulty in host location when both parasitoids and hosts are under selection pressure. Resistance in parasitoids may develop if the host insects themselves become resistant (Tabashnik 1986) or if unique ecological factors exist, such as those believed to be responsible for resistance development in *A. calandreae* (e.g., protection of host larvae hidden within grain kernels from exposure to insecticide applications [Baker and Weaver 1993, Baker and Throne 1995]). Laboratory selection procedures can be used to increase resistance frequencies in field strains. However, laboratory-selected resistance to azinphosmethyl in the parasitoid *Trioxys pallidus* Haliday (Hymenoptera: Aphidiidae) was inherited as a polygenic trait (Brown et al. 1992), a usual consequence of laboratory selection procedures (Roush and McKenzie 1987) that may complicate assessment of efficacy of released resistance factors, because resistance will tend to dissipate and fragment as polygenic resistant insects cross with wild (susceptible) counterparts after release.

Although resistance in parasitoids is rare, our results provide evidence that the naturally occurring malathion resistance found in the Bamberg strain of *A. calandreae* is inherited as an incompletely dominant trait controlled by a single gene. As such, not only is the mode of inheritance of malathion resistance in *A. calandreae* similar to that of malathion resistance in other insect species (Sawicki 1973, Beeman 1983), but it will allow a more effective use of this parasitoid in pest management strategies. Depending on the size of resident populations of this species in grain storages, inoculative or inundative release strategies can be used to increase or maintain a high frequency of the resistant phenotype within the parasitoid population.

There were no mating incompatibilities and no significant differences in fecundity observed between the susceptible and resistant strains of *A. calandreae* during these studies. Nevertheless, more detailed studies on fitness of the resistant phenotypes are necessary to evaluate fully the potential of this strain within an integrated chemical-biolog-

ical control pest management strategy for grain weevils.

Acknowledgments

We thank Robert P. Beuerline for maintaining the parasitoid and weevil cultures used in this study. We also thank John Brower, Barry Dover, Allan Dowdy, and Frederick Plapp, Jr., for critically reviewing the manuscript.

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Received for publication 8 April 1996; accepted 26 November 1996.