NOTE

Resistance Mechanism in the Bamberg Strain of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae)[1, 2]

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Malathion resistance in the Bamberg strain of *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) collected in September 1992 has been stable during 1.5 years of laboratory culture with no pesticide selection pressure (Baker, J. Econ. Entomol., In press). The 2,800-fold organophosphate resistance is the highest level of resistance thusfar documented for a hymenopterous parasitoid. In addition, based on the LD_{50} of the Bamberg strain of the rice weevil, *Sitophilus oryzae* (L.), with which it was collected (Baker and Weaver, Biol. Cont. 3: 233-242, 1993), the parasitoid is about 200-fold less sensitive to malathion than this host. To determine the general mechanism of malathion resistance in the parasitoid, we tested several synergists (inhibitors of four types of detoxification enzymes) (Raffa and Priester, J. Agric. Entomol. 2: 27-45, 1985) in combination with malathion against both the laboratory (Savannah) and Bamberg strains of *A. calandrae.*

Two types of laboratory bioassays were used. In the first bioassay, mortality of Bamberg *A. calandrae* was determined in treated glass vials (Baker and Weever, Biol. Contr. 3: 233-242, 1993). Adults were exposed to malathion (0.69 µg/vial), a dose equal to 2x the LD_{99} of the susceptible Savannah strain, plus 5 parts by weight of inhibitor (3.45 µg) for 24 h at 25° C. Results are based on 2-3 tests on different dates, 3 to 5 replicates per test, and 10-12 adults per replicate. There was no significant difference between data obtained on the different dates so mortality data were pooled and corrected for control mortality with Abbott's formula (Abbott, J. Econ. Entomol. 18: 265-267, 1925).

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[2] Mention of a commercial or proprietary product does not constitute a recommendation by the USDA.

Serial-time response bioassays (Baker et al., J. Econ. Entomol. In press) were used to evaluate possible effects of two carboxylesterase inhibitors on uptake of malathion from the vial surface by the Savannah strain of *A. calandrae*. The Savannah strain was used because much lower doses of malathion and shorter time periods were required for mortality. For these bioassays, glass vials were treated with malathion + inhibitor at weight ratios of 1:1 or 1:5. Mortality of adults was determined at 10 min intervals. Results are based on three separate tests with 3 replicates per treatment and 10-11 adults per replicate for each test. Results of the three tests were combined and analyzed with a matrix-based probit procedure for correlated data (Throne et al., J. Econ. Entomol. In press). Chi square analysis was used to determine best fit of six transformations of mortality data.

Detoxification mechanisms, inhibitors tested, and results of the bioassays with Bamberg *A. calandrae* are given in Table 1. Corrected mortality was negligible in vials that contained malathion combined with piperonyl butoxide or MGK 264, two mixed function oxidase inhibitors. *t*-4-Phenyl-3-buten-2-one and diethyl maleate, two inhibitors of gluthathione-S-transferase, were also ineffective. However, the two carboxylesterase inhibitors, triphenyl phosphate (TPP) and S,S,S-tributyl phosphorotrithioate (DEF), were effective in abolishing the resistance to malathion in this strain. With the tested doses, TPP increased 24 h mortality to about 50% and near complete mortality was obtained in vials that contained DEF + malathion. The LD_{50} for the Bamberg strain of *A. calandrae* is 387 µg/vial (Baker, J. Econ. Entomol. In press). TPP or DEF combined with malathion in the vials allowed a significantly lower malathion dose to be effective. In these tests the amount of malathion tested made a difference because complete mortality of Bamberg *A. calandrae* could be obtained when 100 µg malathion/vial was combined with TPP. Nevertheless, test doses closer to the LD_{99} of the susceptible laboratory strain provide a more realistic basis for characterization of the resistance mechanism in this species.

Presence of DEF increased the rate of mortality of the susceptible Savannah strain of *A. calandrae* exposed to malathion (Fig. 1). In contrast, TPP slowed malathion toxicity. A log-logit transformation gave the best overall fit to mortality data. Based on non-overlap of 95% confidence limits of LT_{50}s, DEF (1:1) significantly decreased the LT_{50} and TPP (1:5) significantly increased the LT_{50} for the Savannah strain. LT_{50}s with 95% CL were as follows: malathion, 71.1 min (65.3-77.3 min); malathion + DEF, 1:1, 54.6 min (50.2-59.3 min); and malathion + TPP, 1:5, 121.2 min (107-137 min).

Plapp et al. (J. Econ. Entomol. 56: 643-649, 1963) found that DEF and TPP were effective synergists for malathion against houseflies and certain mosquitos. Dyte et al. (J. Stored Prod. Res. 1: 223-234, 1966) and Dyte and Rowlands (J. Stored Prod. Res. 4: 157-173, 1968) documented the synergistic effect of TPP on malathion-resistant strains of *Dermestes maculatus* DeGeer and *Tribolium castaneum* (Herbst), respectively. They concluded that the resistance in these species was due to enhanced degradation of malathion by carboxylesterases which were inhibited by the synergists. Subsequently, TPP has been used to characterize malathion resistance in a number of stored product insects as being due to a malathion specific carboxylesterase (Haliscak and Beeman, J. Econ. Entomol 76: 717-722, 1983; Navarro et al., Phytoparasitica 14: 273-280, 1986;

Table 1. Effect of six synergists on toxicity of malathion to the Bamberg strain of *A. calandrae* at 25° C and 75% RH.

Detoxification mechanism	Synergist**	Mortality at 24 h* R/N	Pcorr
–	Controls (solvent)	17/118	–
–	Malathion (alone)	12/111	0.00
Type I Mixed function oxidase	Piperonyl butoxide	12/100	0.00
Type II Mixed function oxidase	MGK 264	11/99	0.00
Carboxylesterase	Triphenyl phosphate (TPP)	62/110	0.49
Carboxylesterase	S, S, S-tributyl phosphorotrithioate (DEF)	112/114	0.98
Glutathion-S-transferase	*t*-4-phenyl-3-buten-2-one	12/99	0.00
Glutathion-S-transferase	Diethyl maleate	10/99	0.00

* R = responders, N = total number tested. Pcorr = proportion killed corrected for control mortality with Abbott's formula. Negative corrected values were set to 0.00. No significant mortality occurred in vials treated with inhibitor alone. The malathion: inhibitor ratio tested was 1:5 wt/wt (0.69 µg malathion + 3.45 µg inhibitor per vial).

** Piperonyl butoxide and MGK 264 obtained from McLaughlin Gormley King Co., Minneapolis, MN; Tpp, *t*-phenylbutenone, and diethyl maleate obtained from Aldrich Chemical Co., Milwaukee, WI; DEF obtained from Miles Inc., Kansas City, MO.

Bansode and Bhatia, J. Ent. Res. 14: 189-191, 1990; Zettler and Cuperus, J. Econ. Entomol. 83: 1677-1681, 1990). Malathion resistance in a field strain of *Xylocoris flavipes* (Reuter), an anthocorid predator of a number of stored product insect pests, was also abolished with TPP (Baker and Arbogast, J. Econ. Entomol. In press). Results of current studies with TPP and DEF provide evidence that a specific carboxylesterase may be involved in the malathion resistance found in the Bamberg strain of *A. calandrae*. However, results of the synergism studies do not preclude the possibility of additional resistance related to insensitive or altered cholinesterase (Plapp, Ann. Rev. Entomol. 21: 179-197, 1976) in this species.

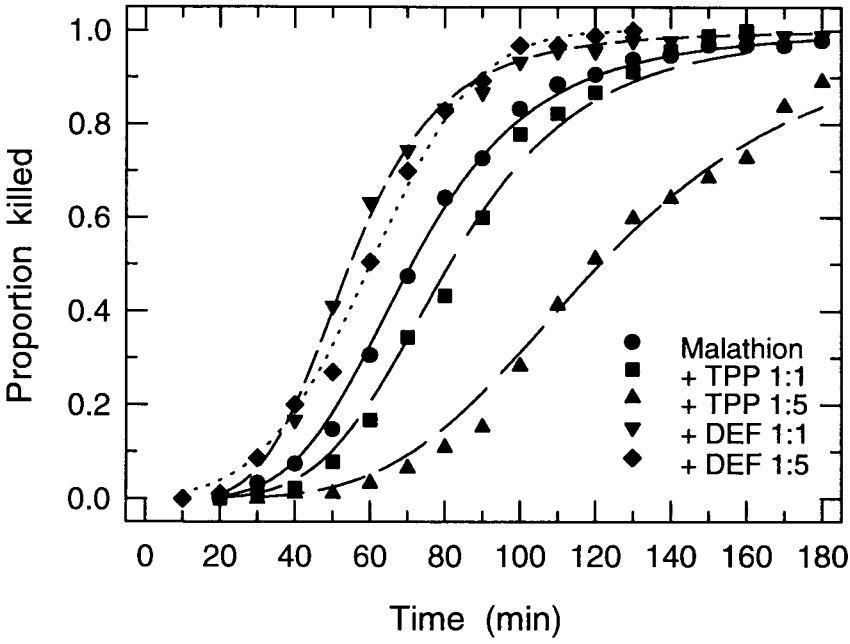

Fig. 1. Probability of dying curves (lines) obtained from back transformation of time-mortality data (symbols) describing effect of weight ratios of TPP and DEF on toxicity of malathion to the susceptible laboratory (Savannah) strain of *A. calandrae* at 22° C. All transformations (obtained from the probit procedure of Throne et al., J. Econ. Entomol., In press) described by the lines were log-logit except for the combination of malathion + DEF (1:5) (dotted line) which was a probit mortality transformation.

TPP is known to reduce the effectiveness of malathion and other organophosphates against some insect species (Plapp et al., J. Econ. Entomol. 56: 643-649, 1963; Dyte et al., J. Stored Prod. Res. 1: 223-234, 1966; Baker et al., J. Econ. Entomol., In press). It has been postulated that this antagonistic effect results from interference with the uptake of malathion through the insect cuticle. The serial-time bioassay was very effective in demonstrating the delayed sensitivity of the susceptible laboratory strain of *A. calandrae* to malathion combined with TPP, particularly when the malathion-TPP ratio was 1:5.

In contrast to the antagonistic effect of TPP, DEF enhanced the toxicity of malathion against the laboratory strain of *A. calandrae* in the timed-response bioassay. DEF caused some mortality with mosquito larvae (Plapp et al., J. Econ. Enomol. 56: 643-649, 1963) and adults of *Bracon hebetor* Say (Baker et al., J. Econ. Entomol., In press). *B. hebetor* was assayed with the same glass vial bioasssay as used for *A. calandrae*, but the DEF concentration tested with

B. hebetor was about 18-fold higher. DEF, at doses tested in the present study, caused no significant mortality with *A. calandrae* after 24 h. The more rapid effectiveness of malathion, in the presence of DEF, against the laboratory strain of *A. calandrae* may be due to an increased rate of uptake of malathion or may possibly result from an inhibition of low levels of malathion-degrading enzyme present in this susceptible strain.