

# Toxicity and In Vitro Metabolism of *t*-Permethrin in Eastern Subterranean Termite (Isoptera: Rhinotermitidae)

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**ABSTRACT** Toxicity and metabolism of *t*-permethrin were evaluated in two colonies (UF and ARS) of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), collected in Gainesville, FL. The UF colony ( $LC_{50} = 1.86 \mu\text{g}$  per vial) was approximately twofold more tolerant of *t*-permethrin than the ARS colony ( $LC_{50} = 0.89 \mu\text{g}$  per vial) at the  $LC_{50}$ . The synergists piperonyl butoxide and S,S,S-tributylphosphorotrithioate increased *t*-permethrin toxicity four- and threefold (at the  $LC_{50}$ ) in the UF and ARS colonies, respectively. Despite these differences in *t*-permethrin susceptibility, microsomal oxidase activities toward surrogate substrates (aldrin epoxidase, and methoxyresorufin *O*-demethylase), cytochrome P450 content, and microsomal esterase activity toward  $\alpha$ -naphthyl acetate did not differ significantly between the colonies. Moreover, no significant differences in qualitative and quantitative metabolism of [<sup>14</sup>C]*t*-permethrin were observed between the UF and ARS colonies for three enzyme sources (microsomal oxidase, microsomal esterase, and cytosolic esterase). Based on in vitro metabolism assays, the major detoxification route of *t*-permethrin in the UF and ARS termite colonies appears to be hydrolysis catalyzed by microsomal esterases.

**KEY WORDS** insecticide detoxification, *t*-permethrin, subterranean termite

THE EASTERN SUBTERRANEAN termite, *Reticulitermes flavipes* (Kollar), is the predominant subterranean termite pest species east of the Mississippi River and from Montana to Mexico (Weesner 1965). The most common method of preventive and remedial control for active *R. flavipes* infestations in the United States is to drench the soil with insecticide, creating a barrier that effectively separates the termites in the soil from the structure (Mallis 1998). Organochlorine insecticides were used extensively for this purpose until their production was halted in 1987 and the Environmental Protection Agency suspended their use in 1988. Currently, pyrethroid, organophosphorus, and nitroguanidine insecticides are used as barriers for subterranean termites.

Despite near complete reliance on insecticides for subterranean termite control, little is known about the ability of termites to detoxify these chemicals. Several species are known to possess competent oxidative (Haritos et al. 1994, Valles et al. 1998), conjugative (Haritos et al. 1996), and hydrolytic (Davis et al. 1995) detoxification systems. However, direct measurement of insecticide metabolism as it compares with insecticide toxicity is lacking. Moreover, little information is available concerning comparative insecticide susceptibility among different colonies of the same subterranean termite species.

We examined the toxicity of *t*-permethrin and the effects of the synergists piperonyl butoxide (PBO) and S,S,S-tributylphosphorotrithioate (DEF) against two colonies of *R. flavipes* from Gainesville, FL. We also conducted in vitro *t*-permethrin metabolism studies on the same colonies to gain a better understanding of the detoxification ability of this economically important species.

## Materials and Methods

**Chemicals.** Technical-grade aldrin, dieldrin, *t*-permethrin, and DEF were purchased from ChemService (West Chester, PA). [<sup>14</sup>C]*t*-Permethrin with radio-carbon in the cyclopropyl ring position (1.96 GBq/mmol [53 mCi/mmol]) was generously provided by S. J. Yu (University of Florida). [<sup>14</sup>C]*t*-Permethrin was purified with two-dimensional thin-layer chromatography (TLC) plates (silica gel 60; Merck, Darmstadt, Germany) with benzene:ethyl acetate (6:1) and carbon tetrachloride:diethyl ether (3:1). Purity was verified by TLC with authentic standards. Glucose 6-phosphate, glucose 6-phosphate dehydrogenase, NADP, NADPH, EDTA, dithiothreitol (DTT), and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma (St. Louis, MO). PBO and 1-phenyl-2-thiourea (PTU) were purchased from Aldrich (Milwaukee, WI). All other chemicals were procured from commercial suppliers.

**Termites.** Termites were collected from bucket stations as described by (Su and Scheffrahn 1986) from

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Table 2. Detoxification enzyme activities in the UF and ARS colonies of *R. flavipes*

Detoxification enzyme	Specific activity (nmol/min/mg protein [ $\pm$ SEM])	
	UF colony	ARS colony
	Microsomal oxidases	
Cytochrome P450 <sup>a</sup>	0.28 $\pm$ 0.06	0.16 $\pm$ 0.02
Aldrin epoxidase	0.58 $\pm$ 0.12	0.67 $\pm$ 0.10
MRR O-demethylase <sup>b</sup>	0.014 $\pm$ 0.0005	0.013 $\pm$ 0.0003
Microsomal esterases		
$\alpha$ -Naphthyl esterase	882.5 $\pm$ 15.9	829.5 $\pm$ 28.4

<sup>a</sup> Nanomoles per milligram of protein.

<sup>b</sup> Methoxyresorufin O-demethylase.

Microsomal oxidase activities toward surrogate substrates (aldrin epoxidase, and methoxyresorufin O-demethylase) and cytochrome P450 content were not significantly different between the colonies (Table 2). Microsomal esterase activity toward  $\alpha$ -naphthyl acetate was also similar between the colonies.

*t*-Permethrin was metabolized in vitro by microsomal oxidases and esterases; and cytosolic esterases prepared from worker termites (Figs. 1-3). Metabolism by microsomes in the presence of an NADPH source and DEF (microsomal oxidases) was qualitatively identical for the UF and ARS colonies (Table 3); both produced at least four metabolites with *R<sub>f</sub>* values of 0.75, 0.60, 0.47, and 0.23. Quantitative metabolism by microsomal oxidases, as measured by total metabolite production, was not significantly different ( $t = 0.67$ ,  $df = 4$ ) for UF and ARS colonies (Fig. 1). Similar results were observed when cytosol was used as the enzyme source. No significant differences in the rate of *t*-permethrin metabolism (Fig. 2) or in the metabolites produced (Table 3) were observed. Finally, quantitative (Fig. 3) and qualitative (Table 3) microsomal esterase metabolism were similar between strains. Both colonies produced two major metabolites (*R<sub>f</sub>* = 0.15 and 0.13) and metabolized *t*-permethrin at a rate of 33.0  $\pm$  3.7 and 36.3  $\pm$  6.2 pmol/h/0.5 mg protein, respectively.

When metabolism data for both strains were combined by enzyme source, significantly greater metabolism ( $F = 27$ ;  $df = 15, 2$ ;  $P < 0.05$ ) occurred with

microsomes in the absence of NADPH (i.e., microsomal esterases). Microsomal esterases metabolized *t*-permethrin 2.7- and 3.8-fold faster than microsomal oxidases and cytosolic esterases, respectively.

## Discussion

Despite near complete reliance on insecticide-based control measures for subterranean termites, little information is available regarding insecticide metabolism in Isoptera. The UF and ARS *R. flavipes* colonies exhibited a competent detoxification enzyme system as reported for *R. virginicus* (Banks) in Valles et al. (1998). Cytochrome P450 content and catalyzed reactions (aldrin epoxidase and methoxyresorufin O-demethylase) were not statistically different between the UF and ARS colonies and were similar to the values reported for *R. virginicus* (Valles et al. 1998), *Mastotermes darwiniensis* Froggatt and *Coptotermes acinaciformis* (Froggatt) (Haritos et al. 1994).

Although significant differences in *t*-permethrin toxicity were observed among the UF and ARS colonies, in vitro analysis of *t*-permethrin metabolism by microsomal oxidases, esterases, and cytosolic esterases were qualitatively and quantitatively similar for both strains. Metabolism observed by microsomes in the presence of NADPH and DEF (i.e., microsomal monooxygenases; Fig. 1) was eliminated by removal of the NADPH source, indicating that cytochrome P450 monooxygenases were the enzymes responsible. Also, esterases were implicated as the enzymes responsible for *t*-permethrin metabolism in the cytosolic and microsomal (without NADPH) fractions because metabolism was eliminated by the addition of DEF (100  $\mu$ M). Furthermore, despite differences in *t*-permethrin susceptibility between the strains, the level of synergism by PBO and DEF was the same for each respective strain. However, the synergism data do not coincide with the in vitro *t*-permethrin metabolism results. Slightly greater synergism occurred in the toxicity bioassays by addition of PBO (synergist ratio = 4.2) compared with DEF (synergist ratio = 3), which implicates oxidative metabolism as the principal route of detoxification for *t*-permethrin. Conversely, based

Table 3. Percentage distribution of [<sup>14</sup>C] *t*-permethrin metabolites from various enzyme preparations of the UF and ARS colonies of *R. flavipes*

<i>R<sub>f</sub></i>	Distribution of radioactivity (% $\pm$ SEM)					
	Soluble fraction		Microsomes (-NADPH)			
	UF	ARS	Microsomes (-NADPH)		Microsomes (+NADPH, +DEF)	
			UF	ARS	UF	ARS
0.83	89.4 $\pm$ 1.4	88.2 $\pm$ 2.7	66.5 $\pm$ 4.4	65.0 $\pm$ 4.6	91.2 $\pm$ 3.3	94.2 $\pm$ 2.2
0.75	0	0	0	0	0.3 $\pm$ 0.3	0.3 $\pm$ 0.3
0.60	0	0	0	0	0.4 $\pm$ 0.3	1.0 $\pm$ 0.6
0.47	0	0	0	0	1.3 $\pm$ 0.9	1.6 $\pm$ 1.0
0.23	0	0	0	0	2.4 $\pm$ 2.0	1.6 $\pm$ 1.0
Origin <sup>a</sup>	8.9 $\pm$ 1.4	10.5 $\pm$ 3.0	33.0 $\pm$ 4.3	30.9 $\pm$ 7.0	0.4 $\pm$ 0.4	0.3 $\pm$ 0.3
Aqueous <sup>b</sup>	0.4 $\pm$ 0.3	0.3 $\pm$ 0.2	0.4 $\pm$ 0.1	1.6 $\pm$ 0.6	1.3 $\pm$ 0.4	2.0 $\pm$ 0.9
Remaining <sup>c</sup>	1.4 $\pm$ 0.7	0.9 $\pm$ 0.5	0.2 $\pm$ 0.2	2.5 $\pm$ 2.2	2.1 $\pm$ 0.8	1.3 $\pm$ 0.4
					0.2 $\pm$ 0.1	0.1 $\pm$ 0.1

<sup>a</sup> Includes TLC scraping of plate area of *R<sub>f</sub>* values 0 to 0.2.

<sup>b</sup> Radioactivity remaining in the aqueous fraction.

<sup>c</sup> Radioactivity remaining on the TLC plate after visible spots indicated on the autoradiogram were removed.

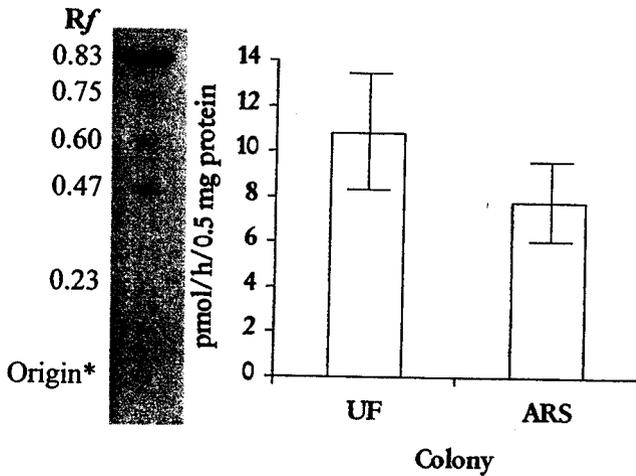


Fig. 1. Autoradiogram of a TLC separation of [<sup>14</sup>C]*t*-permethrin ( $R_f = 0.83$ ) and its metabolites by NADPH-fortified, DEF-inhibited microsomes (microsomal oxidases). Metabolism was quantified by combining all metabolites produced. Metabolites were not detected when NADPH was excluded from the reaction mixture. Autoradiogram from UF colony shown. \*Includes all metabolites in the region of  $R_f$  0–0.2.

on in vitro assays, microsomal esterases are evidently the major metabolic route for *t*-permethrin in these *R. flavipes* colonies.

An unusually high susceptibility to PBO by *R. flavipes* may explain this apparent discrepancy. By definition, synergists are not considered toxic alone (Matsumura 1985); however, we typically observed 100% mortality when a group of 50 termites (combined weight of  $\approx 140$  mg) was exposed to 40  $\mu$ g of PBO. German cockroaches (50 mg per cockroach), by contrast, are usually treated individually with 100  $\mu$ g of PBO in topical insecticide bioassays and show no apparent toxicity (Valles and Yu 1996). As a result, we suspect that PBO may have alone been toxic to *R. flavipes*.

The major route of *t*-permethrin detoxification in the ARS and UF colonies of *R. flavipes* is apparently hydrolytic as indicated by significantly greater metabolism of *t*-permethrin by microsomal esterases, a process inhibited by the addition of DEF. Microsomal esterases have been shown to be the major metabolic route of the trans isomers of permethrin and cypermethrin in mouse, rat, housefly [*Musca domestica* (L.)], and cabbage looper [*Trichoplusia ni* (Hübner)] (Shono et al. 1979). However, mouse microsomal oxidases metabolized *t*-permethrin at nearly the same rate as microsomal esterases, whereas very little oxidation of permethrin was observed in the rat, housefly, and cabbage looper (Shono et al. 1979) and in the *R. flavipes* colonies used in the current study. The ability

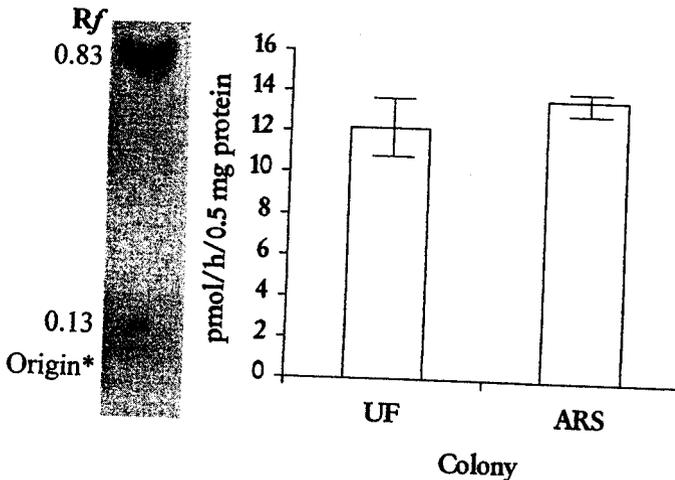


Fig. 2. Autoradiogram of a TLC separation of [<sup>14</sup>C]*t*-permethrin ( $R_f = 0.83$ ) and its metabolites by cytosol (cytosolic esterases). Metabolism was quantified by combining all metabolites produced. Metabolites were not detected when DEF (100  $\mu$ M) was added to the reaction mixture. Autoradiogram from UF colony shown. \*Includes all metabolites in the region of  $R_f$  0–0.2.

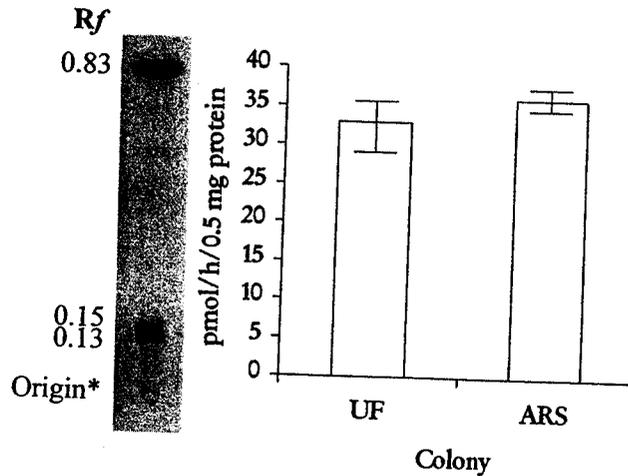


Fig. 3. Autoradiogram of a TLC separation of [ $^{14}\text{C}$ ]t-permethrin ( $R_f = 0.83$ ) and its metabolites by microsomes (microsomal esterases). Metabolism was quantified by combining all metabolites produced. Metabolites were not detected when DEF (100  $\mu\text{M}$ ) was added to the reaction mixture. Autoradiogram from UF colony shown. \*Includes all metabolites in the region of  $R_f 0-0.2$ .

of this economically important insect to readily detoxify t-permethrin (and probably other insecticides based on additional detoxification enzyme data provided) questions the long-term effectiveness of using insecticides as a barrier to protect structures from termites. Obviously, thin spots in the soil treatment or insecticide degradation by biotic and abiotic factors would eventually render the barrier ineffective.

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