

PYRETHROID-FORMAMIDINE INTERACTIONS AND BEHAVIORAL EFFECTS IN PYRETHROID SUSCEPTIBLE AND RESISTANT TOBACCO BUDWORMS<sup>1</sup>Thomas C. Sparks<sup>2</sup>, B. Roger Leonard and Jerry B. Graves

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## ABSTRACT

The co-application of chlordimeform (CDF) to surfaces treated with C<sup>14</sup>-permethrin or C<sup>14</sup>-*lambda*-cyhalothrin often increased the amount of pyrethroid picked up by third instar larvae of the tobacco budworm. Compared to the pyrethroid susceptible (PYR-S) tobacco budworm larvae, larvae of the pyrethroid resistant (PYR-R) strain consistently picked up less radiolabeled insecticide. However, when vials were coated with H<sup>3</sup>-Juvenile hormone III (JH III), PYR-R larvae consistently picked up less radiolabeled material in the presence of non-labeled permethrin than they did in the absence of non-labeled permethrin, suggesting that modified behavior may play a role in pyrethroid resistance. Using the uptake of H<sup>3</sup>-JH III from a coated vial as a relative measure of larval movement, both CDF and BTS-27271 (NOR-AM 49844; *N'*-(2,4-dimethylphenyl)-*N*-methylformamidine) were effective in increasing the movement of PYR-S larvae, but amitraz was not. When the effect of these three formamidines on C<sup>14</sup>-permethrin uptake was evaluated, only CDF was found to result in a significant increase in the amount of permethrin picked up by the PYR-S larvae of the tobacco budworm. Therefore, compared to amitraz and BTS-27271, CDF was more consistent in enhancing larval movement, and hence increased uptake of radiolabeled JH III and permethrin, by larvae of the tobacco budworm.

## INTRODUCTION

Pyrethroid resistance in insects can be the result of one or several mechanisms including reduced penetration, knock-down resistance (*kdr*) and increased metabolism (Sawicki 1985). In addition to these relatively common mechanisms, insects can also respond to insecticide selection pressure through a variety of behavioral mechanisms (Georghiou 1972, Lockwood et al. 1984, Pluthero & Singh 1984, Sparks et al. 1989). As with other classes of insecticides, behavioral resistance has been associated with pyrethroid resistance in a variety of insect and mite species (Sparks et al. 1989).

The tobacco budworm, *Heliothis virescens* (F.), has a history of developing resistance to insecticides (Sparks 1981), including DDT, organophosphorus insecticides

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and, most recently, the pyrethroids (Plapp et al. 1987, Luttrell et al. 1987, Campanhola & Plapp 1988, Graves et al. 1988, Leonard et al. 1988). Pyrethroid resistance in the tobacco budworm has been shown to be associated with several biochemical and physiological resistance mechanisms including altered penetration, *kdr*, enhanced monooxygenase and enhanced esterase activity (Nicholson & Miller 1985, Dowd et al. 1987, Sparks et al. 1988, Little et al. 1989). However, information concerning the role of behavioral resistance in pyrethroid resistant larvae of the tobacco budworm is lacking. A recent review of behavioral resistance suggests that this mechanism of resistance may be more widespread than generally perceived, and that resistance mechanisms such as *kdr* may have more than one form of expression, including possible behavioral responses (Sparks et al. 1989).

The formamidines, such as chlordimeform (CDF), appear to act as octopamine agonists (Hollingworth & Lund 1982), resulting in a variety of behavioral effects in lepidopterans and mites (Hollingworth & Lund 1982, Knowles 1982). CDF has been shown to increase the movement of tobacco budworm larvae on plants and to reduce feeding (Treacy et al. 1987). CDF also synergizes the toxicity of the pyrethroids in laboratory and field studies (Plapp 1987, Campanhola & Plapp 1987, 1988). However, the effects of the CDF induced movement on the amount of insecticide picked up by an insect remains unclear as does the response of pyrethroid resistant and susceptible larvae to the presence of pyrethroids on a treated surface. The present study was undertaken to determine if formamidines could alter the amount of insecticide picked up by pyrethroid resistant and susceptible larvae of the tobacco budworm.

## MATERIALS AND METHODS

**Insects.** The pyrethroid susceptible strain (PYR-S) of the tobacco budworm was established in 1977 and has been in continuous culture without insecticide exposure. The pyrethroid resistant strain (PYR-R; ICI-87) was kindly provided by ICI Americas. All larvae were reared on an artificial diet (Leonard et al. 1988) at  $27 \pm 3^\circ\text{C}$  with a 14L:10D photoperiod and ca 40% relative humidity.

**Bioassays.** Topical bioassays were performed on third instar (20-30 mg) larvae as described previously (Leonard et al. 1988). For all bioassays, the dose-response data were analyzed by probit analysis (MicroProbit 3.0, T. C. Sparks & A. Sparks) after Finney (1971). For the insecticide bioassays, lack of overlap of the 95% fiducial limits was used as the criterion for statistical significance.

**Insecticides.** Analytical grade (99%) samples of permethrin (40:60 *cis:trans*) and *lambda*-cyhalothrin (PP-321, Karate), a 1:1 mixture of the  $\alpha$ -S,1R3R and  $\alpha$ -R,1S3S isomers of cyhalothrin, along with radiolabeled (99% radiochemical purity) permethrin ( $\text{C}^{14}$ , 27.6 mCi/mM) and *lambda*-cyhalothrin ( $\text{C}^{14}$ , 58.1 mCi/mM) were provided by ICI Americas (Goldsboro, NC). Technical grade (>95%) amitraz and NOR-AM 49844 (BTS-27271; *N'*-(2,4-dimethyl-phenyl)-*N*-methylformamidine) were provided by NOR-AM. Chlordimeform was from Chem Sources (West Chester, PA). Radiolabeled juvenile hormone III (JH III;  $\text{H}^3$ -C10, 11 Ci/mmol) was from NEN.

**Formamidine-Pyrethroid Interactions.** Twenty ml glass scintillation vials were washed and then coated with the radiolabeled pyrethroid (0.03  $\mu\text{g}$  permethrin,  $\approx 4,800$  dpm, or 0.003  $\mu\text{g}$  *lambda*-cyhalothrin,  $\approx 895$  dpm) alone or in combination, with and without CDF (at 10x the pyrethroid dose), in 0.5 ml acetone which was allowed to evaporate. Larvae (3/vial) were then introduced into the vial and held

at  $27 \pm 3^\circ \text{C}$ . At selected posttreatment intervals (3, 6 & 18 hr) the larvae and any fecal material were carefully removed and immediately homogenized in 0.5 ml of ice-cold ( $4^\circ \text{C}$ ) methanol from which duplicate 100  $\mu\text{l}$  aliquots were taken and quantified by liquid scintillation counting (LSC). This approach provides an estimate of the total amount of insecticide external *and* internal in the test insects. The amount of radiolabeled material remaining in the treated vials was also analyzed by LSC. A comparison of the radioactivity present in the larval homogenate (external + internal) with the amount remaining provided a measure of the amount of pyrethroid taken up by the larvae. All assays were replicated three times at each time point. Treatment data at each time point were analyzed by analysis of variance and contrast (Wilkinson 1987).

Similar experiments were also performed using permethrin (0.03  $\mu\text{g}$ ) or JH III ( $\approx 40,000$  dpm) alone and in combination with CDF, amitraz or BTS-27271 (at 10  $\mu\text{g}/\text{vial}$ ). Larvae (2-3/vial) were then introduced into the vial and held at  $27 \pm 3^\circ \text{C}$ . At 3 hr posttreatment the larvae and any fecal material were carefully removed and assayed as described above. All assays were replicated at least 3 times and analyzed as described above.

## RESULTS

**Insecticide Bioassays.** The co-application of CDF (10:1) with permethrin did not produce any significant increase in the toxicity of permethrin towards the susceptible PYR-S strain of the tobacco budworm (Table 1). Compared to the PYR-S strain, the PYR-R pyrethroid resistant strain was 2905 and 150 times less susceptible to cypermethrin and *lambda*-cyhalothrin, respectively (Table 1).

TABLE 1. Topical Toxicity of Selected Insecticides to Pyrethroid Susceptible and Resistant Tobacco Budworm Larvae

Compound(s)	LD-50 ( $\mu\text{g}/\text{g}^a$ )	95% Fiducial Limits	Slope	RR <sup>b</sup>
SUSCEPTIBLE (PYR-S)				
Chlordimeform (CDF)	>400.0	-	-	-
Permethrin	1.20	1.04-1.96	2.39	-
Permethrin + CDF (1:10)	1.16	0.72-1.68	1.84	-
Cypermethrin	0.28	0.24-0.36	2.32	-
<i>lambda</i> -Cyhalothrin	0.12	0.08-0.12	3.45	-
RESISTANT (PYR-R)				
Cypermethrin	813.6	632.0-1016	2.00	2905
<i>lambda</i> -Cyhalothrin	17.92	13.28-24.24	2.51	150

<sup>a</sup>Third instar larvae, 20-30 mg.

<sup>b</sup>Resistance ratios (RR) is LD-50 of resistant strain (PYR-R) / LD-50 of susceptible strain (PYR-S).

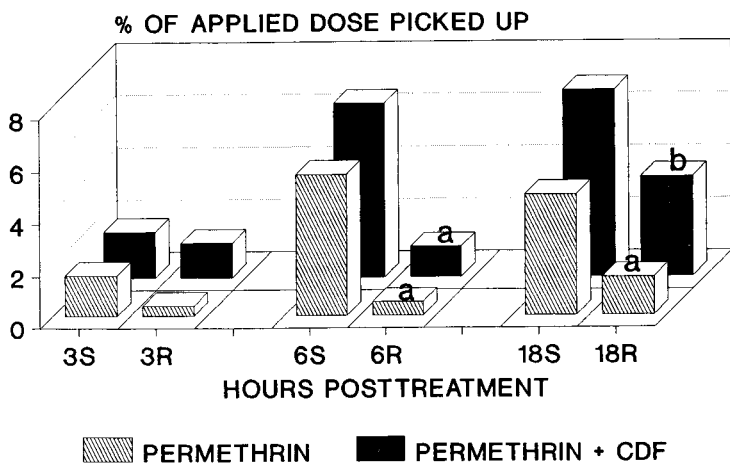


FIG. 1. The effect of CDF on the amount of  $C^{14}$ -permethrin picked up by pyrethroid susceptible (S) and resistant (R) tobacco budworm larvae at 3, 6 and 18 hr posttreatment. a = values for S vs R at that indicated time point and treatment are significantly different ( $P < 0.05$ ). b = values for permethrin + CDF vs permethrin alone are significantly different ( $P < 0.05$ ) for the indicated strain and time point.

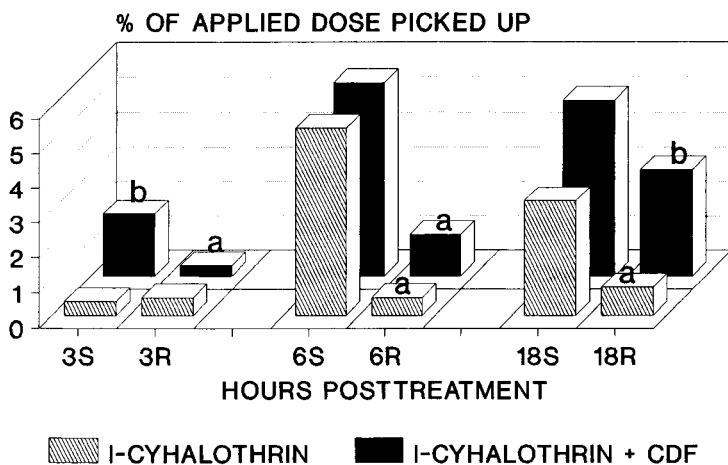


FIG. 2. The effect of chlordimeform (CDF) on the amount of  $C^{14}$ - $\lambda$ -cyhalothrin picked up by pyrethroid susceptible (S) and resistant (R) tobacco budworm larvae at 3, 6 and 18 hr posttreatment. a = values for S vs R at that indicated time point and treatment are significantly different ( $P < 0.05$ ). b = values for  $\lambda$ -cyhalothrin + CDF vs  $\lambda$ -cyhalothrin alone are significantly different ( $P < 0.05$ ) for the indicated strain and time point.

**Formamidine-Pyrethroid Interaction.** Although the addition of CDF to the vial resulted in a generally higher mean quantity of  $C^{14}$ -permethrin picked up by the larvae, only for the resistant strain at 18 hr posttreatment was the difference between the permethrin + CDF and the permethrin alone significantly different (Fig. 1). A similar trend was observed for *lambda*-cyhalothrin with significant differences observed for the susceptible larvae at 3 hr posttreatment and the resistant larvae at 18 hr posttreatment (Fig. 2). Thus, in some cases the co-application of CDF with the pyrethroid increased the amount of pyrethroid picked up by tobacco budworm larvae.

The quantity of permethrin or *lambda*-cyhalothrin picked up by resistant larvae of the tobacco budworm was consistently and significantly less (3.1 to 8.2-fold) than the quantity picked up by susceptible larvae 6 and 18 hr posttreatment (Figs 1 & 2), and for the CDF plus *lambda*-cyhalothrin 3 hr posttreatment (Fig. 2). These observations suggest that resistant larvae may move less than susceptible larvae in the presence of pyrethroids.

JH III is a relatively non-toxic, lipophilic molecule. Because of these properties, the amount of JH III picked up by larvae potentially provides a relative measure of larval movement in vial coated with radiolabeled JH III. When PYR-S larvae were placed in vials containing either radiolabeled JH III alone or radiolabeled JH III plus permethrin, there was no significant difference in the amount of JH III picked up by the larvae 3 hr posttreatment (Fig. 3). In the same experiment with larvae from the PYR-R strain, larvae in the vials containing JH III plus permethrin picked up significantly less JH III than larvae in vials containing only JH III (Fig. 3) suggesting that permethrin may cause the PYR-R larvae to move less.

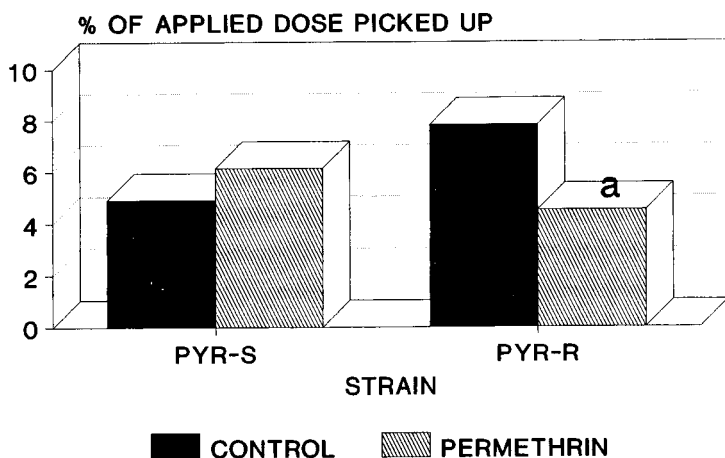


FIG.3. The effect of permethrin ( $0.01 \mu\text{g}/\text{vial}$ ) on the amount of  $H^3$ -JH III picked up by pyrethroid susceptible (PYR-S) and resistant (PYR-R) tobacco budworm larvae 3 hr posttreatment. a = value is significantly different ( $P < 0.05$ ) from adjacent column.

CDF, amitraz and BTS-27271 were evaluated for effects on the uptake of H<sup>3</sup>-JH III and C<sup>14</sup>-permethrin from a treated vial by PYR-S third instar larvae of the tobacco budworm. CDF and BTS-27271 caused a significant increase in the quantity of JH III picked up, while amitraz did not (Table 2). When the effects of these three formamidines on uptake of radiolabeled permethrin from treated vials was investigated, only CDF was found to result in a significant increase in quantity of permethrin picked up (Table 2). Thus, for BTS-27271 there may be an interaction (at the dose tested) with permethrin that alters its action.

TABLE 2. Effect of Selected Formamidines (10 µg/vial) on Amount of Radiolabeled Juvenile Hormone III or Permethrin Picked up by Susceptible Larvae of the Tobacco Budworm.

Compound	JH III		Permethrin	
	Mean picked up (% of total)	Ratio <sup>1</sup>	Mean picked up (% of total)	Ratio <sup>1</sup>
Control	1.99	1.0	0.36	1.0
+ Chlordimeform	6.93	3.5*	4.48	12.4*
+ Amitraz	2.52	1.3	0.82	2.3
+ BTS-27271	6.46	3.2*	0.77	2.1

<sup>1</sup>Ratio: formamide treated / control. For ratios followed by an \*; means are significantly (P<0.05) greater than the control.

## DISCUSSION

Pyrethroid-Formamide Interactions. CDF has been shown to synergize pyrethroid activity in the laboratory using coated vial (contact) bioassays (Campanhola & Plapp 1987, 1988). However, in our study the topical application of CDF-pyrethroid combinations produced no observable synergism (Table 1). Recent studies suggest that the movement of tobacco budworm larvae is increased on CDF treated plants (Treacy et al. 1987). Results of the present study indicate that CDF causes a general and in several instances a statistically significant increase in the amount of pyrethroid (internal and external) present on and/or in larvae of the tobacco budworm (Figs. 1 & 2, Table 3). Thus, one potential effect that formamidines, such as CDF, have when co-applied with other insecticides to a surface is to cause an increase in larval movement thereby increasing the amount of insecticide the larva contacts. However, not all formamidines are equally effective. In the absence of any pyrethroid, CDF and BTS-27271 both produced significant increases in the amount of H<sup>3</sup>-JH III picked up by tobacco budworm larvae while amitraz did not. In contrast, only CDF produced a significant increase in the quantity of radiolabeled insecticide picked up when the formamidines were co-applied with permethrin. Both BTS-27271 and amitraz produced only slight non-significant changes. Thus, of the three formamidines evaluated in the present study,

CDF was the most effective in enhancing the uptake of material from a treated surface for larvae of the tobacco budworm.

Although CDF was the most effective of the three formamidines examined in the present study, these results may, in part, be influenced by the type of bioassay, dosages and insect species being used. For example, amitraz is more effective than either CDF or BTS-27271 in causing walkoff behavior in twospotted spider mites (Franklin & Knowles 1984), while BTS-27271 is more effective than either CDF or amitraz in causing detachment of cattle ticks (Knowles 1982).

**Behavioral Resistance.** An interesting result from our study is the observation that in the presence of a 72 hr LD<sub>50</sub> dose for susceptible larvae, resistant larvae consistently picked up less pyrethroid than the susceptible larvae. Such a response on the part of resistant larvae may constitute a specific behavioral response to the presence of the pyrethroid. This type of behavioral response could be beneficial to the insect by reducing the amount of insecticide that must be dealt and consequently, the effectiveness of any other resistance mechanisms present. An alternative explanation for the reduced movement by the PYR-R strain compared to the PYR-S strain is that the resistant larvae are inherently less active. However, this explanation seems unlikely since the resistant larvae pick up as much radiolabeled material in the absence of permethrin as do the larvae of the susceptible strain. Thus, the available data suggest that the observed behavioral response is a genuine response on the part of the PYR-R strain to the presence of the pyrethroid. Therefore, in addition to enhanced metabolism, reduced penetration and *kdr* (Sparks et al. 1988, Little et al. 1989) a behavioral component may also exist that contributes the resistance of the PYR-R strain.

Because behavior is the sum of a series of physiological and biochemical events (Sparks et al. 1989), a reduced level of movement by resistant larvae in the presence of permethrin could be a function of a reduced sensitivity in the nervous system to the pyrethroids. *Kdr* involves an alteration of the insect nervous system (Miller and Adams 1982) and, like many parts of the insect nervous system, the sensory nervous system is also affected by pyrethroid insecticides (Miller & Adams 1982, Bloomquist 1988). *Kdr* also appears to be one of the resistance mechanisms present in larvae of the PYR-R larvae (Sparks et al. 1988). However, the modified behavior displayed by larvae of the PYR-R strain to pyrethroids may, or may not, be related to the presence of *kdr* or some other resistance mechanism. Regardless of the basis for this apparent behavior, such a behavioral modification clearly can improve the chances of survival for a larva in a pyrethroid containing environment and, therefore, constitutes a form of behavioral resistance. As we have discussed previously (Lockwood et al. 1984, Sparks et al. 1989), it is important to recognize the significance of such modified behaviors and to consider them when investigating the basis for resistance in the tobacco budworm or any other insect.

The present study has shown that formamidines such as CDF can enhance the amount of pyrethroid insecticide picked up from a surface by larvae of the tobacco budworm. These results suggest that one mechanism by which CDF, and potentially other formamidines, increase insecticide toxicity is through increased movement and, hence, contact with the insecticide (but also see Liu and Plapp 1990). In addition to these observations, our results also suggest that pyrethroid resistant larvae exhibit an altered behavior (i.e. reduced movement) in the presence of pyrethroids which could potentially be associated with one of the other resistance mechanisms including

*kdr*. This behavioral resistance is but one of several mechanisms potentially contributing to pyrethroid resistance in the tobacco budworm.

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