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Insecticidal effects of organotin(IV) compounds on *Plutella xylostella* (Linnaeus) larvae I: Topical application toxicity and antifeedant effect

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Structure–activity relationship studies were conducted with early fourth-instar larvae of a highly resistant strain of the diamondback moth, *Plutella xylostella* (Linnaeus) on (1) toxicity by topical appliction of 43 organotin compounds, and (2) the antifeedant effect of a selected number (17) of these compounds on treated *Brassica chinensis* (Chinese cabbage) leaves.

The toxicity data revealed that the triorganotins (R_3SnX) were, without exception, more toxic than the commercial sample of malathion (84% active ingredient) used in the tests. Among the diorganotins, phenylcyclopentyltin oxide proved to be as active as malathion. Within the triorganotin series, the tricyclohexyltins were generally more toxic than the triphenyltins, the most active tricyclohexyltin compound being (c-$C_6H_{11})_3$Sn(2-pyridinethiolato *N*-oxide) (LC_{50} 0.03 μg μl^{-1}), which was almost 500-fold more active than malathion. The most active compound in the triphenyltin class was *O,S*-bis(triphenyltin)mercaptoacetate (LC_{50} 0.30 μg μl^{-1}). Variations in the anionic X group resulted only in marginal changes in activity in the (c-$C_6H_{11})_3$Sn series, but significant changes in activity were obtained with the Ph_3Sn compounds, especially the ring-substituted phenoxyacetates, (4-$ZC_6H_4)OCH_2(O)COSnPh_3$. In the mixed triorganotin compounds an increase in activity was observed when one of the phenyl groups in Ph_3SnOH was replaced by the *p*-chlorophenyl group.

In the antifeedant tests, the tricyclohexyltins were found to be generally more effective than the triphenyltins. In most cases, antifeedant activity paralleled the toxicity by topical application trends in the (c-$C_6H_{11})_3$Sn series, but in the Ph_3Sn series an inverse trend was observed. The diorganotin compound (c-C_5H_9)PhSnO exerted a relatively pronounced antifeedant activity which was comparable with that of a number of triphenyltin derivatives.

It was established from histological studies of the mid-gut cross-sections of the treated larvae that, in most cases, the organotins affected the columnar cells physiologically; an exception was noted for $Ph_3SnOC(O)C_6H_4COOH$-4 which, like malathion, caused severe morphological damage to the cell membrane.

Keywords: Organotins, *Plutella xylostella*, toxicity by topical application, antifeedant effects, histological studies

INTRODUCTION

Plutella xylostella (Linnaeus), more commonly known as the diamondback moth (DBM), is a major universal pest of cruciferous vegetables. The moths have an average life span of about one to three weeks, of which an average of six days are spent in the four larval stages; the third- and fourth-instar larvae are extremely destructive and can skeletonize a cruciferous plant within hours.[1] The resistance spectrum of DBM larvae covers all major groups of chemical insecticides,[2–6] i.e. chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids and benzoylureas, as well as microbial insecticides,[7] such as *Bacillus thuringiensis* Berliner and Agrimek®. In view of the seriousness of the resistance problem, especially in the tropics where DBM produces 21 generations per year,[8,9] insecticides with novel modes of action are constantly being sought. This has prompted investigations in our laboratory on

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the potency of organotin compounds as control agents for this pest.

As a structural class, the triorganotin compounds, R_3SnX, possess useful biological properties which have enabled their commericalization, particularly as fungicides (R = Bu, Ph) and miticides ($R = c\text{-}C_6H_{11}$).[10] Several of these have also been demonstrated to exert strong insecticidal activity against pests including the orders Diptera,[11,12] Homoptera,[13] Coleoptera[14–16] and Lepidoptera.[17–19] Among Lepidoptera, organotin derivatives were found to be effective against the larvae of *Heliothis* sp., *Trichoplusia ni* (Hübner), *Spodoptera littoralis* (Boisduval), *Cnaphalocrocis medinalis* (Guenée) and *Chilo polychrysus* (Meyrick).[17–19]

In addition to toxicity effects, a number of triorganotin compounds are known to manifest 'antifeedant' effects, a property of potential value in crop protection.[20,21] The term 'antifeedant' refers to the ability of a compound to inhibit the feeding of an insect on the plant without either repelling or killing it.[22]

Evidence for antifeedant activity has previously been reported among the commercially known triphenyltin[20,21,23] and tricyclohexyltin[24,25] biocides as well as some hexaorganoditins[26,27] in tests against several lepidopterous larvae such as *Prodenia litura* (Fabricius), *Agrotis ypsilon* (Rottemburg), *Boarmia selenaria* (Schiffermüller), *Spodoptera littoralis* (Boisduval) and *Epilachna varivestis* (Mulsant).

We report in this paper our results on the structure–activity relationship studies conducted on the toxicity and antifeedant effects of a range of organotins against the early fourth-instar larvae of a highly resistant local strain of DBM.

MATERIALS AND METHODS

Insects

Fourth-instar larvae of a highly tolerant DBM strain (R-strain) obtained locally from Kea Farm, Cameron Highlands, were used throughout this study. The larvae were reared in 30 cm × 30 cm × 30 cm muslin-mesh cages at 28 ± 2 °C, 90 ± 6% relative humidity and a photoperiod of 12:12 (L:D) without exposure to any insecticide. The adults were fed with drops of Holloway medium on cellophane[2] and the larvae on fresh *Brassica chinensis* (Chinese cabbage) leaves.

Insecticides

A total of 43 organotin compounds and four commercially procured insecticides were used in the toxicological studies. The organotin compounds were synthesized according to established methods[28] and were of analytical-grade purity. The standard insecticides (methomyl, dichlorvos, malathion and fenitrothion) used were of technical-grade quality.

Topical bioassay

Early fourth-instar larvae in batches of ten, of average weight 28 ± 3 mg, were lightly anaesthesized with carbon dioxide and treated topically on the dorsal surface with 1.0 µl of test solution using a Drummond microcap applicator. Treatments were carried out at six concentrations for each test compound, while the controls were treated with solvent (acetone) only. For a complete test, three batches of ten larvae each were treated with each of the six concentrations of a given test compound. The treated larvae were then transferred onto fresh *Brassica* leaves and kept at 28 ± 1 °C in plastic finger bowls provided with perforated covers to provide for ventilation.

Mortality was assessed at 24 h and 48 h after the topical applications. Larvae that failed to respond to gentle mechanical stimulation were considered dead. The bioassay data were analysed by the probit method of Finney[29] to obtain the lethal concentration index values, LC_{50}.

Evaluation of antifeedant effects

Standard-size, tender *Brassica* leaves, of average weight 2.0 ± 0.3 g per leaf, were chosen for the test. Aqueous suspensions of the test compounds were prepared incorporating a surfactant (50 µl Teepol per 10 ml test suspension) to ensure complete wetting of the leaf surfaces. Both sides of the leaf were carefully coated with a fixed volume of test suspension (50 µl) using a fine-tipped 100 µl Eppendorf micropipette, and the leaf was air-dried.

Ten active early fourth-instar larvae, prestarved for 6 h, and of total average weight 30 ± 3 mg, were released onto each treated leaf. The leaf petiole was placed in a 50 ml conical flask filled with tapwater and the mouth of the flask was covered with aluminium foil in order to minimize evaporation losses and ensure turgidity of the leaf. The larvae were weighed at the end of 24 h. All treatments were replicated at least five times for each of the six concentrations of a given

test compound. The experimental larvae exposed to adequate concentrations of the compounds gained less weight than the control larvae. The difference between the mean weight gain in the control larvae and the mean weight loss of starved larvae (kept for 24 h without food) was defined as 100% starvation. Percentage starvation in the larvae was calculated by the method of Ascher and Nissim:[21]

$$\text{Starvation} = \frac{C_w - E_w}{C_w - S_w} \times 100\%$$

where C_w = mean weight gain of control larvae within 24 h (*positive control*),
E_w = mean weight gain of test larvae at test concentration within 24 h,
S_w = mean weight gain of starved control larvae within 24 h (*negative control*),
$C_w - S_w$ = 100% starvation.

The data obtained for percentage starvation were subjected to log dose–probit analysis. The term SC_{95} is defined as the concentration that results in 95% starvation.

Histological studies

Larvae that had been exposed for 24 h to treated leaves were used for the histological studies. The larvae were initially fixed overnight in warm Bouin's fluid. This caused the larval body to relax to elongated postures. They were then further fixed overnight in 70% ethanol. This was followed by sequential washings of the larvae in 85% ethanol (30 min), twice in 95% ethanol (30 min each), twice in toluene (45 min each) and finally in 1:1 (v/v) toluene–paraffin mixture (45%). The larvae were then embedded in paraffin wax and serial sections, 7 μm thick, were prepared and stained with Harris alum haematoxylin and eosin and mounted in Canada balsam. Transverse sections of larvae were made and photomicrographs of the mid-gut taken.

RESULTS AND DISCUSSION

Acute toxicity evaluations

The acute toxicity results, expressed conventionally in terms of LC_{50} values, for the full range of compounds tested are given in Table 1. Toxicity data for five commercial insecticides are also included in the Table. The LC_{50} values were determined by the method of Finney,[29] and a minimum of six concentrations of the toxicant were chosen for this purpose such that at least two of these concentrations were above and two below the LC_{50} value. The unit of the LC_{50} value is $\mu g\ \mu l^{-1}$ per larva, but it has been additionally expressed in the Table in $mmol\ l^{-1}$ (i.e. $mmol\ dm^{-3}$) to enable direct comparisons to be made between compounds within a given structural class, such as the triorganotin series (R_3SnX), where systematic variations in the R and X groups were investigated. The molar LC_{50} values thus serve to compensate for differences in molecular weights between the compounds compared.

It is seen from the data for the symmetrical triorganotins in Table 1(a) that the toxicity is especially pronounced when the R group is cyclohexyl, the most toxic compound being tricyclohexyltin 2-pyridinethiolato-*N*-oxide, with an LC_{50} value of 0.03 $\mu g\ \mu l^{-1}$. Indeed, the tricyclohexyltin compounds are seen to be 10- to 10^2-fold more active than methomyl, dichlorvos, malathion or fenitrothion, but not deltamethrin (LC_{50} 0.01 $\mu g\ \mu l^{-1}$). This is also the case for a number of triphenyltin compounds with LC_{50} values ranging from 0.36 to 14.63 $\mu g\ \mu l^{-1}$ [Table 1(b)], although as a class the triphenyltins are relatively less toxic than the tricyclohexyltins.

Whereas the variation in LC_{50} values with different X groups was less pronounced in the more toxic cyclohexyltins, significant differences were observed in the triphenyltin series. This is clearly demonstrated in the triphenyltin carboxylates where, relative to the parent triphenyltin acetate, the compounds containing substituents in the ester unit showed either increased or decreased toxicity, as assessed from the magnitudes of their molar LC_{50} values. Triphenyltin benzoylpropionate (molar LC_{50} 1.84 $mmol\ l^{-1}$) showed a higher activity than triphenyltin acetate (molar LC_{50} 6.06 $mmol\ l^{-1}$) or triphenyltin levulinate (molar LC_{50} 7.00 $mmol\ l^{-1}$); conversion of the γ-keto group of the levulinate to the semicarbazide or thiosemicarbazide had little impact on activity. In the phenoxyacetates the presence of a *p*-carboxyl substituent led to a 7-fold enhancement in activity relative to the case with *p*-nitro or *p*-chloro as ring substituents. Triphenyltin indole-3-acetate (molar LC_{50} 27.9 $mmol\ l^{-1}$) was less toxic than either triphenyltin acetate or triphenyltin hydroxide; by way of contrast, tricyclohexyltin

Table 1 Toxicity (48 h) by topical application on fourth-instar larvae of DBM (R-strain)[a]

Compound	LC_{50} ($\mu g\ \mu l^{-1}$)	Molar LC_{50} ($mmol\ l^{-1}$)
(a) Tricyclohexyltins, $(c\text{-}C_6H_{11})_3SnX$		
X = —$SC_5H_4N\rightarrow O$	0.03 ± 0.01	0.07
—$OCO(CH_2)_2C(O)NHPh$	0.05 ± 0.01	0.09
—O_3SCH_3	0.05 ± 0.02	0.12
—$OC(O)(CH_2)_2C(O)Ph$	0.07 ± 0.01	0.14
—$OC(O)CH_2(3\text{-}C_8H_7N)$[b]	0.08 ± 0.02	0.14
—$O_3SC_6H_4CH_3\text{-}4$	0.10 ± 0.02	0.18
—$ON(Ph)C(O)Ph$	0.16 ± 0.02	0.28
—$OB[OSn(C_6H_{11})_3)]_2$	0.39 ± 0.17	0.43
—$OC(O)CH_2(8\text{-}C_9H_6NO)$[c]	0.11 ± 0.02	0.57
—NCH:NCH:N	0.28 ± 0.02	0.64
—OH	0.43 ± 0.05	1.13
(b) Triphenyltins, Ph_3SnX		
X = —$OC(O)CH_2SSnPh_3$	0.36 ± 0.22	0.45
—$OC(O)CH_2OC_6H_4COOH\text{-}4$	0.37 ± 0.20	0.70
—$OC(O)CH_2SC(O)NH(4\text{-}ClC_6H_4)$	0.53 ± 0.19	0.90
—$OC(O)C_6H_4SSnPh_3$	0.82 ± 0.15	0.96
—$SC_6H_4NH_2\text{-}o$	0.73 ± 0.18	1.58
—$OC(O)(CH_2)_2C(O)Ph$	0.97 ± 0.16	1.84
—$OC(O)CH_2SC:NC_6H_4S$	1.18 ± 0.22	2.05
—$OC(O)(CH_2)_2C[:NNHC(S)NH_2]CH_3$	1.12 ± 0.53	2.08
—$OC(O)(CH_2)_2C[:NNHC(O)NH_2]CH_3$	1.16 ± 0.34	2.22
—$OC(O)C_5H_4N\rightarrow O$	0.75 ± 0.17	2.24
—$OC(O)CH_2OC_6H_4Cl\text{-}4$	1.21 ± 0.14	2.26
—$SC:N—N:C(NH_2)S$	1.11 ± 0.25	2.31
—$OC(O)CH_2OC_6H_3Cl_2\text{-}2,4$	2.48 ± 0.40	4.35
—$OC(O)CH_2OC_6H_4NO_2\text{-}4$	2.39 ± 0.43	4.38
—$OC(O)C(O)CH_3$	1.97 ± 0.28	4.50
—$OC(O)CH_2NC(O)C_6H_4C(O)$	2.67 ± 0.41	4.82
—OH	1.91 ± 0.21	5.22
—OAc	2.47 ± 0.31	6.06
—$SSnPh_3$	4.43 ± 1.16	6.06
—$OC(O)(CH_2)_2C(O)CH_3$	3.23 ± 0.32	7.00
—NCH:NCH:N	9.78 ± 0.53	23.40
—$OC(O)CH_2(3\text{-}C_8H_7N)$	14.63 ± 0.64	27.94
(c) Mixed aryltins		
$(4\text{-}ClC_6H_4)Ph_2SnOH$	0.29 ± 0.20	0.73
$(4\text{-}ClC_6H_4)_2PhSnOH$	0.94 ± 0.12	2.16
$(3,4\text{-}Cl_2C_6H_3)Ph_2SnOH$	1.02 ± 0.20	2.34
$(4\text{-}ClC_6H_4)Ph_2SnOC(O)CH_2SC(S)N(CH_3)_2$	1.21 ± 0.14	2.36
$(4\text{-}ClC_6H_4)Ph_2SnBr.C_9H_7NO$	2.79 ± 0.55	4.58
$(4\text{-}CH_3C_6H_4)_3SnCl$	5.22 ± 0.38	12.22
(d) Diorganotins		
$[(3,5\text{-}Cl_2C_6H_3)PhSnO]_n$	385.4[d]	—
$[(4\text{-}ClC_6H_4)PhSnO]_n$	288.0[d]	—
$[PhOctSnO]_n$	33.0[d]	—
$[(c\text{-}C_5H_9)PhSnO]_n$	11.4[d]	—

[a] Comparison LC_{50} values for commercial samples of methomyl, dichlorvos, malathion, fenitrothion and deltamethrin are 2.32, 0.58, 14.08, 8.28 and 0.01 $\mu g\ \mu l^{-1}$, respectively. [b] 3-Indolylacetyl. [c] 8-Quinolinoylacetyl. [d] Extrapolated values from probit analysis.

indole-3-acetate was more toxic than tricyclohexyltin hydroxide.

Triphenyltin hydroxide is comparable in activity with the acetate, but triphenyltin 1,2,4-triazole is a significantly weaker toxicant. In general, it is noted that the triphenyltin mercaptides are somewhat stronger toxicants than either triphenyltin hydroxide or acetate.

It is noteworthy that replacement of a phenyl group in Ph_3SnOH by the *p*-chlorophenyl group [Table 1(c)] led to a 7-fold increase in toxicity; the related derivatives $(4\text{-}ClC_6H_4)_2PhSnOH$ and $(3,4\text{-}Cl_2C_6H_3)Ph_2SnOH$ were only twice as active.

Inasmuch as the toxicity tests included four diorganotin oxides [Table 1(d)], it was of interest to compare their activities with those of the triorganotins. Their LC_{50} values (extrapolated from probit analysis) are also listed in Table 1. The data indicate that $[c\text{-}C_5H_9PhSnO]_n$ (LC_{50} 11.4 $\mu g\,\mu l^{-1}$) is as active as triphenyltin 1,2,4-triazole, but this is not the case with $[PhOctSnO]_n$ (LC_{50} 33.0 $\mu g\,\mu l^{-1}$, $[(4\text{-}ClC_6H_4)PhSnO]_n$ (LC_{50} 288.0 $\mu g\,\mu l^{-1}$) or $[(3,4\text{-}Cl_2C_6H_3)PhSnO]_n$ (LC_{50} 385.4 $\mu g\,\mu l^{-1}$). The wide variation in the LC_{50} values is especially instructive in that it admits the possibility that equally efficacious diorganotin toxicants can be synthesized by a judicious choice of substituents on tin.

Table 2 Antifeedant action of sublethal amounts of selected organotin compounds tested against early fourth-instar DBM larvae (R-strain) by the larval starvation method

Compound	SC_{95} (% a.i.)
(a) Tricyclohexyltins, $(c\text{-}C_6H_{11})_3SnX$	
X = —OH	0.06
—$SC_5H_4N\rightarrow O$	0.06
—$OC(O)(CH_2)_2C(O)NHPh$	0.07
—O_3SCH_3	0.09
—NCH:NCH:N	0.13
—$OC(O)(CH_2)_2C(O)Ph$	0.38
(b) Triphenyltins, Ph_3SnX	
X = —NCH:NCH:N	0.14
—OAc	0.67
—$OC(O)(CH_2)_2C(O)Ph$	0.71
—$OC(O)CH_2OC_6H_4COOH\text{-}4$	1.00
—$OC(O)CH_2C[:NNHCSNH_2](CH_3)$	34.00
(c) Mixed aryltins	
$(4\text{-}ClC_6H_4)Ph_2SnOH$	0.58
$(3,4\text{-}Cl_2C_6H_3)Ph_2SnOH$	0.82
$(4\text{-}ClC_6H_4)Ph_2SnOC(O)CH_2SC(S)N(CH_3)_2$	0.83
$(4\text{-}ClC_6H_4)_2PhSnOH$	3.67
(d) Diorganotins	
$[(c\text{-}C_5H_9)PhSnO]_n$	1.94
$[PhOctSnO]_n$	10.00
Malathion (commercial sample 84% a.i.)	9.40

Antifeedant activity

A total of 17 organotin compounds along with a commercial sample of malathion, were investigated for their antifeedant properties and the data obtained are presented in Table 2. Using the 95% starvation concentration (SC_{95}) as an index of the antifeedant effect, the best results were obtained with the tricyclohexyltins ($(c\text{-}C_6H_{11})_3SnX$ [Table 2(a)], where the trend of SC_{95} values with variations in X groups was largely similar to that of their LC_{50} values. Thus:

$$SC_5H_4N\rightarrow O < OCO(CH_2)_2CONHPh$$
$$< O_3SMe < NCH:NCH:N$$

On the other hand, tricyclohexyltin hydroxide, which was a relatively weaker toxicant than tricyclohexyltin 2-pyridinethiolato-*N*-oxide cf. Table 1(a), appeared to be as effective as the latter in its antifeedant action, and also more effective than tricyclohexyltin 3-benzoylpropionate.

In the Ph_3SnX series [Table 2(b)], the trend in antifeedant activity was generally the reverse of that observed in the toxicity tests. Thus, triphenyltin(1,2,4-triazole) was respectively 5-, 7- and 243-fold more effective than triphenyltin acetate, *p*-carboxy phenoxyacetate and levulinate thiosemicarbazate in its antifeedant activity whereas, on the basis of the toxicity data [Table 1(b)], triphenyltin *p*-carboxyphenoxyacetate was, respectively, 3-, 9- and 33-fold more toxic than the corresponding levulinatothiosemicarbazate, acetate and 1,2,4-triazolyl derivatives.

The mixed triorganotin compound, $(4\text{-}ClC_6H_4)Ph_2SnOH$ [Table 2(c)], exhibited a stronger antifeedant effect than $(4\text{-}ClC_6H_4)_2PhSnOH$. On the other hand, the compound $(3,4\text{-}Cl_2C_6H_3)Ph_2SnOH$, with two chlorine atoms in the one ring, showed an activity comparable with that of $(4\text{-}ClC_6H_4)Ph_2SnOH$.

Of some significance is the observation of a

strong antifeedant effect exerted by the diorganotin compound, phenylcyclopentyltin oxide [Table 2(d)]. The SC_{95} value of 1.9% a.i. for this compound is comparable with that of some of the triphenyltin biocides. By way of contrast, the SC_{95} value for the other diorganotin compound studied, phenyloctyltin oxide, was 10.0% a.i. The result for phenylcyclopentyltin oxide appears to be the first recorded observation of a strong antifeedant effect manifested by a diorganotin compound. As for the case of the triorganotins, the SC_{95} values for the two diorganotins also correspond to sublethal concentrations (below LC_{50}), with no larval mortality observed during the experiments. The treated leaves showed numerous test-bites by the larvae, in contrast with the control leaves. This rules out the element of repellency by the organotins.

Chapman[30] classifies antifeedants into two groups: those perceived by specialized receptor cells in the insect, which lead to avoidance behaviour; and those which suppress the activity of neurons, and thus may be expected to have a general physiological effect on all insects. Organotin compounds would appear to belong to the latter class, at least from visual observations, including histological examination (*vide infra*), made in this study.

Histological studies and mode of action

A histological study was conducted to determine how the organotin compounds in the antifeedant tests affected the morphology and physiology of the epithelial cells in the larval mid-gut. It was observed that, except in the tests with malathion and triphenyltin *p*-carboxylphenoxyacetate which induced severe structural damage to the larval mid-gut region, the other organotins used as antifeedants only physiologically affected the columnar cells of the mid-gut. The columnar cells are concerned with the production and secretion of enzymes along with the absorption of digestive products.[31]

Figures 1–4 are photomicrographs of cross-sections of the larval mid-gut following the antifeedant tests. Relative to the control (Fig. 1), the test with commercial malathion (84% a.i.), showed near-complete rupture of the cell membranes of the columnar cells (Fig. 2).

A similar damage to the columnar cells resulted upon ingestion by the larvae of triphenyltin *p*-carboxylphenoxyacetate. Although an aqueous dispersion of the compound showed a pH of *ca* 6, it is conceivable that the metabolic generation of either the *p*-carboxyphenoxyl or the *p*-carboxyphenoxyacetyl moiety in the gut might lead to

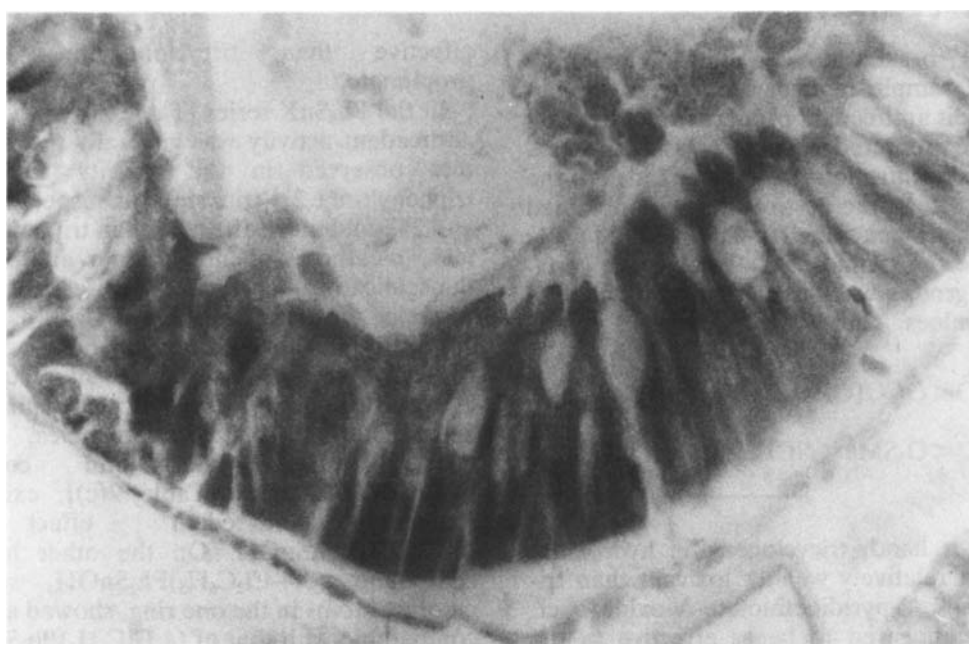

Figure 1 Mid-gut cross-section of control larva; magnification, ×400.

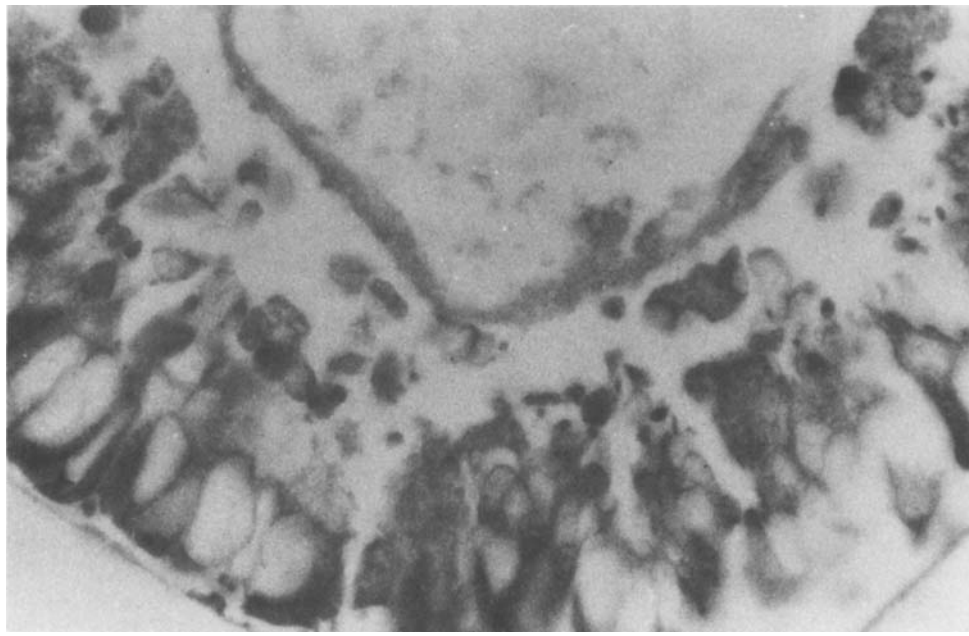

Figure 2 Mid-gut cross-section of larva exposed to malathion-treated leaf, showing damage and rupture of columnar cells; magnification, ×400.

higher acidities than the stannyl ester. According to Chapman,[31] the pH encountered in the mid-gut of lepidopterous larvae is normally in the range 8–10. In the case of other phenyltin compounds, ingestion resulted in inhibition of the secretory activities of the digestive cells (columnar cells). Cross-sections of larvae treated with *O,S*-bis(triphenyltin)mercaptoacetate (Fig. 3), triphe-

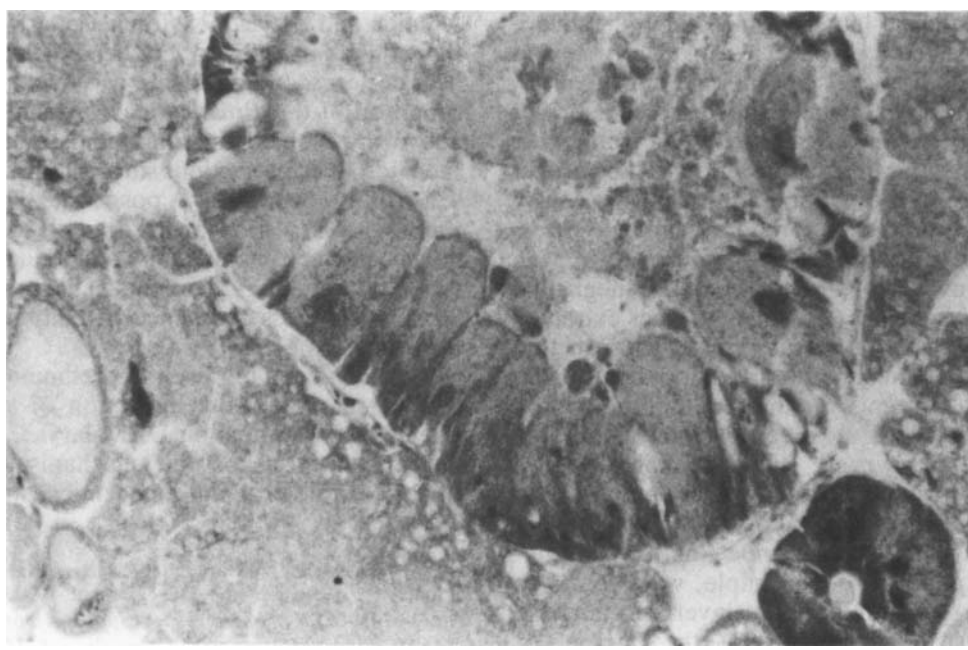

Figure 3 Mid-gut cross-section of larva exposed to *O,S*-bis(triphenyltin)mercaptoacetate-treated leaf, showing enlarged columnar cells; magnification, ×400.

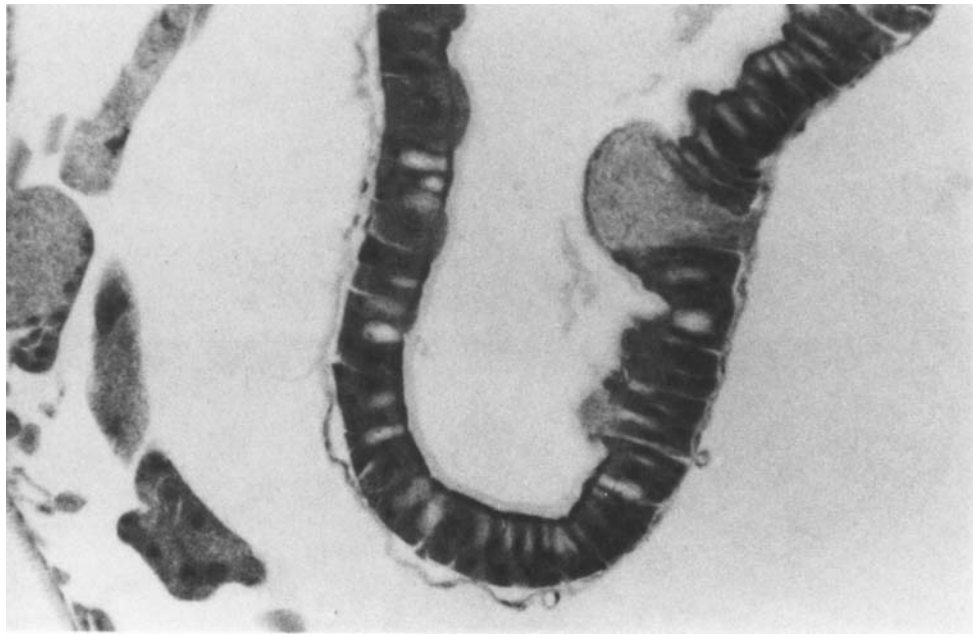

Figure 4 Midgut cross-section of larva exposed to (phenylcyclopentyl)tin oxide-treated leaf, showing columnar cells with severe hypertrophy; magnification, ×200.

nyltin 3-benzoylpropionate (not illustrated) and phenylcyclopentyltin oxide (Fig. 4) showed the columnar cells to be severely affected, causing hypertrophy and lack of discharge of secretory materials into the lumen of the mid-gut. Indeed, large undigested food particles are clearly seen in Fig. 3; this, however, is not the case in the control experiment (Fig. 1), where only digested debris of food is seen. It is tempting to infer from these observations that the manifestation of the antifeedant property of organotin compounds is consequent upon their ingestion by the larvae.[32] Some representative triorganotin compounds have been shown in a previous study[33] to have negligible effect *in vitro* on the digestive enzymes extracted from the DBM larvae. This strongly suggests that the effects of the organotin compounds on the DBM larval enzymes are indirect, i.e. they act on the physiological system responsible for enzyme production. However, inhibition of digestive enzymes by triphenyltins has been demonstrated in studies with other insects. Triphenyltin acetate,[32] triphenyltin chloride[34] and other phenyltin compounds,[35] for example, were found to be good *in vitro* inhibitors of invertase, amylase and protease derived from the larvae of *Spodoptera littoralis* (Boisduval), *Tribolium castaneum* (Herbst) and *Tribolium confusum* (Jacquelin duVal), respectively.

Examination of larval mid-gut cross-sections treated with the tricyclohexyltin compounds revealed that tricyclohexyltin 2-pyridinethiolato-*N*-oxide and tricyclohexyltin succinanilate caused the columnar cells to be densely packed and dehydrated. Although there was evidence of consumption of the *Brassica* leaves treated with the above tricyclohexyltin compounds by the larvae, the mid-gut cross-sections surprisingly showed absence of food particles. A possible explanation for this is that ingestion of the treated leaves could have been accompanied by immediate regurgitation of the mid-gut contents. This would lead to larval weight loss and hence virtually high starvation values.

These findings represent a preliminary attempt to elucidate the mode of action of antifeedant effects of organotins on DBM larvae. Clearly, a proper appraisal of the mechanism(s) awaits further histological and biochemical studies.

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