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Possible selective advantage of *Anopheles* spp. (Diptera: Culicidae) with the oxidase- and acetylcholinesterase-based insecticide resistance genes after exposure to organophosphates or an insect growth regulator in Sri Lankan rice fields

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Abstract

Treatment of rice fields in Sri Lanka with monocrotophos at 10 mg/litre or pirimiphos-methyl at 0·1 mg/litre gave a selective advantage to larvae of *Anopheles subpictus* Grassi and *A. nigerrimus* Giles carrying the oxidase- and acetylcholinesterase-based resistance genes, respectively. However, this selective advantage was apparent for less than ten days after spraying. There was no mortality of any larval instar with monocrotophos 12 days after spraying, and no fourth-instar larval mortality with pirimiphos-methyl 17 days after spraying. The chemical degredation curves for these compounds indicated that this short duration of efficacy was due to the instability of the compounds in water under field conditions. In contrast, the new growth regulator S-31183 (2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine) sprayed at 0·1 mg/litre conferred no selective advantage to larvae with either resistance mechanism and had a total efficacy period of at least 71 days. This difference can be attributed to the greater toxicity of the growth regulator and its slower chemical degradation under field conditions when compared to the organophosphates.

Introduction

Insecticide resistance is becoming a major problem in many areas. In Sri Lanka, there is broad spectrum resistance in *Anopheles nigerrimus* Giles and *A. subpictus* Grassi throughout the island, while in some locations malathion-specific resistance is developing in *A. culicifacies* Giles. Many of the *Culex* species in Sri Lanka also have some organophosphate- and carbamate-resistance, although this has been well documented mechanistically only in *C. quinquefasciatus* Say (Hemingway *et al.*, 1986; Hemingway *et al.*, 1987; Herath *et al.*, 1987; Villani *et al.*, 1983). Resistance may be due to either selection by insecticide control measures aimed directly at the mosquito population, such as house spraying with malathion against *A. culicifacies*, or exposure of the mosquito population to

compounds used for agriculture, such as the spraying of rice fields against pests. The selective advantage of the resistance genes have rarely been measured under field conditions. The present study was undertaken to determine the level and duration of protection conferred by oxidase- and acetylcholinesterase-based resistance genes after larviciding of rice fields in Sri Lanka. The two organophosphates monocrotophos and pirimiphos-methyl were used, as these were commonly used by farmers for rice treatment in Sri Lanka.

New compounds to which there is no cross-resistance from existing mechanisms, will be valuable in future control programmes. Such compounds need evaluating in terms of their efficacy, stage specificity, environmental effect and cost, such that they can be successfully integrated into insect control programmes as effectively as possible. A new growth regulator, S-31183 (2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine), has been developed to which the acetylcholinesterase resistance gene should, in theory, give no cross-resistance (Hatakoshi *et al.*, 1986). The effectiveness of this growth regulator against organophosphate- and carbamate-resistant and susceptible mosquitoes, and the duration of active control was, therefore compared to that of the two organophosphates.

Materials and methods

Monocrotophos and pirimiphos-methyl were supplied as 60% emulsifiable concentrates (e.c.) and were sprayed on 40 × 20-m rice-field plots at dosages of 10 and 0·1 mg active ingredient/litre, respectively. The insect growth regulator was supplied as a 10% e.c. and was sprayed at 0·1 mg active ingredient/litre.

Before spraying, water samples were taken from each plot and assayed with first- and fourth-instar larvae of *A. nigerrimus* and *A. subpictus*. There was no mortality 24 h after the start of each test and only low level mortality (<10%) when the larvae were maintained in the water with light feeding until they either died or successfully developed into adults. Larvae were found in all the rice field plots immediately prior to the insecticide treatment.

Mosquitoes used were as follows:

(a) Susceptible and resistant colonies of *A. nigerrimus*, established by selection and single family rearing. As this species does not readily mate in cages, the colonies were actively maintained by hand-mating a large number of females every generation. The F_6 and F_7 generations, which bred true for susceptibility and resistance by WHO bioassay, were used in these experiments. The resistance mechnanism in this species is an altered acetylcholinesterase (AChE), which gives broad spectrum organophosphate and carbamate resistance (Hemingway *et al.*, 1986).

(*b*) The F_1 progeny of wild-caught females of *A. subpictus* were also used. Eggs were obtained by isolating single females in vials. The families were classified as resistant, mixed or susceptible by WHO bioassay susceptibility tests on half the family as one-day-old adults. Larval resistance in this species is controlled by two genes, an oxidase-based mechanism, which gives broad spectrum organophosphate resistance, and an esterase mechanism, which gives some carbamate resistance and possibly enhances the organophosphate resistance (Hemingway *et al.*, 1987).

Water samples were taken from all rice plots plus the untreated control plot at regular intervals after spraying. Water (1000 ml) was collected from top right and bottom left sectors of each plot and assayed separately to determine homogeneity of treatment. Larvae of each test species of each resistant type were used for two replicate bioassays. Bioassays from organophosphate-treated plots were run for 24 h, after which mortality was scored. First- or fourth-instar larvae were used in all tests. Where possible, 25 larvae were used in each replicate, but in a few cases lower numbers of one particular genotype were available for testing; the minimum number in any one replicate was ten larvae. Bioassays on water samples from the plots treated with growth regulator were run until all larvae had either died or developed into adults. All tests used early fourth-instar larvae of each species and genotype. Controls were always run for both type of test with water from the untreated rice field, and occasional controls were run with distilled water.

Degradation curves of the pesticides were determined by taking 2 × 4-ml water samples from the top right and bottom left corners of each test plot on the days that bioassays were carried out. These samples were immediately stored at −70°C to reduce or eliminate further degradation of the pesticide. They were extracted with 4 ml dichloromethane immediately before being transferred to London. In the laboratory, samples were evaporated to dryness under a stream of air and stored at −70°C. Prior to high performance liquid chromatography (HPLC) analysis, samples were resuspended in 2 ml of HPLC grade methanol and passed through a 0·22-μm filter. At least two replicates of 20 μl of each sample were analysed by HPLC, and standards of known concentration were run at regular intervals to ensure accurate calibration of the machine. All samples were analysed on an Ultrasphere ODS reverse phase column. Mobile phase conditions for monocrotophos and pirimiphos-methyl were 80:20 methanol:water for 5 min, then a 5-min gradient from 80 to 95% methanol, followed by 2–4 min at 95%, using a standard flow rate of 0·8 ml/min. The mobile phase for the growth regulator was 85:15 methanol:water for 10 min at a flow rate of 1 ml/min. Peak detection for all compounds was 280 nm, and peak areas were integrated using a Beckman Sp 4270 integrator. Calibration of peak areas was by laboratory standards of the individual pesticides.

Rainfall data in the Colombo area over the experimental period (December 1986–February 1987) was collated from figures obtained from the Sri Lankan meteorological office.

Results

The degradation curves for monocrotophos, pirimiphos-methyl and the growth regulator are given in Fig. 1. The decline in active ingredient on the first day after spraying was greatest with the growth regulator (Fig. 1*c*), but the subsequent rate of loss of monocrotophos (Fig. 1*a*) was then much faster than that of the other two compounds. There was a rapid decline in pirimiphos-methyl at 8–15 days after spraying (Fig. 1*b*), whereas the loss of growth regulator was more gradual from day 8 onwards. There was no measurable amount of monocrotophos or pirimiphos-methyl 15 and 30 days, respectively, after spraying, whereas it took 76 days for the level of the growth regulator to fall below our detection limits.

The 24-h bioassay mortalities of fourth-instar larvae of the two test species of *Anopheles* with monocrotophos and pirimiphos-methyl are given in Fig. 2. There was no control mortality after 24 h in first- or fourth-instar larvae exposed to water collected from the untreated rice field. Under field conditions, the duration of the selective effect of both pirimiphos-methyl and monocrotophos was very short. The organophosphate-resistant genotype of *A. nigerrimus* (RR) had some selective advantage over its susceptible counterpart for <5 and <10 days as fourth- and first-instar larvae respectively, after monocrotophos was sprayed. Fourth-instar resistant larvae of *A. subpictus* (RS) also had a selective advantage for less than ten days over their susceptible counterparts. (The effect on first-instar larvae was not measured.) After ten days, the mortality in all genotypes of both species was negligible. This correlates well with the degradation curve in Fig. 1 and laboratory log-dosage probit mortality data on *A. nigerrimus*, from which it could be predicted that monocrotophos, being a relatively poor mosquito larvicide, would fall to concentrations which would give no fourth-instar larval mortality 1·5 days after spraying and no mortality of any instar one week after spraying.

Similar results were obtained with pirimiphos-methyl, although the initial decline in larval mortality was not as rapid as that observed for monocrotophos over the first four days (Fig. 2). The selective advantage of the altered AChE gene in *A. nigerrimus*, in the initial four days after spraying was much less than that conferred to monocrotophos. This correlates well with the laboratory results, which indicate that the level of resistance conferred to pirimiphos-methyl by the altered AChE gene is low (3–10-fold). A full comparison of the selective advantage of resistant and susceptible genotypes of *A. subpictus* is not possible from these data as homozygous resistant families were not available on all test dates for the pirimiphos-methyl assays. However, there is evidence

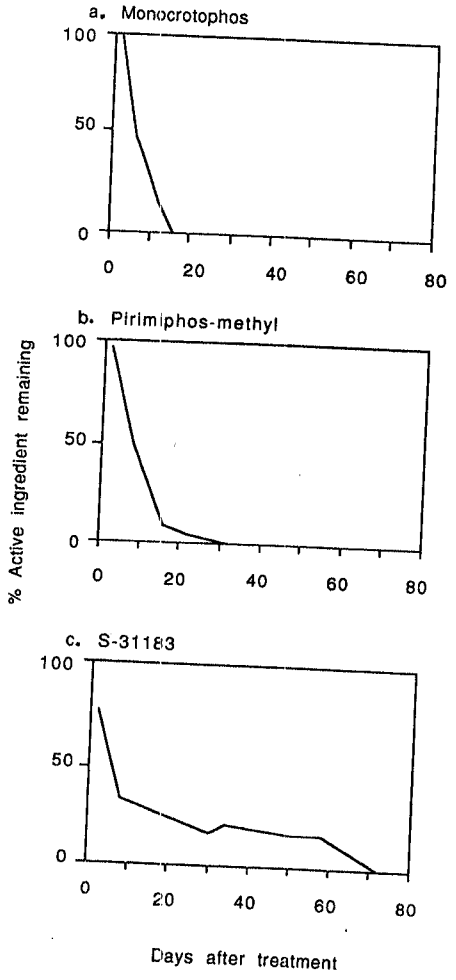

Fig. 1.—Chemical degradation curves for the active ingredients of (*a*) monocrotophos e.c., (*b*) pirimiphos-methyl e.c. and (*c*) Sumitomo growth regulator S-31183 e.c. after spraying in rice fields.

from the resistant heterozygotes that the oxidase resistance mechanism confers some selective advantage to these individuals.

The result of tests of water treated with the growth regulator against the two test species are shown in Fig. 3. In contrast to the organophosphates, this compound gave high mortalities for up to 2·5 months after treatment. Assays were stopped after 76 days as lack of rainfall towards the end of the test period led to the treated sites drying out. The data in Fig. 4 show that there was heavy rainfall from 42 to 46 days after treatment and very little rainfall subsequent to this.

There was no significant difference in the mortality of the organophosphate- and carbamate-resistant and susceptible strains of *A. nigerrimus*; both suffered at least 90% mortality up to 50 days after treatment. The decline in mortality after day 50 followed the

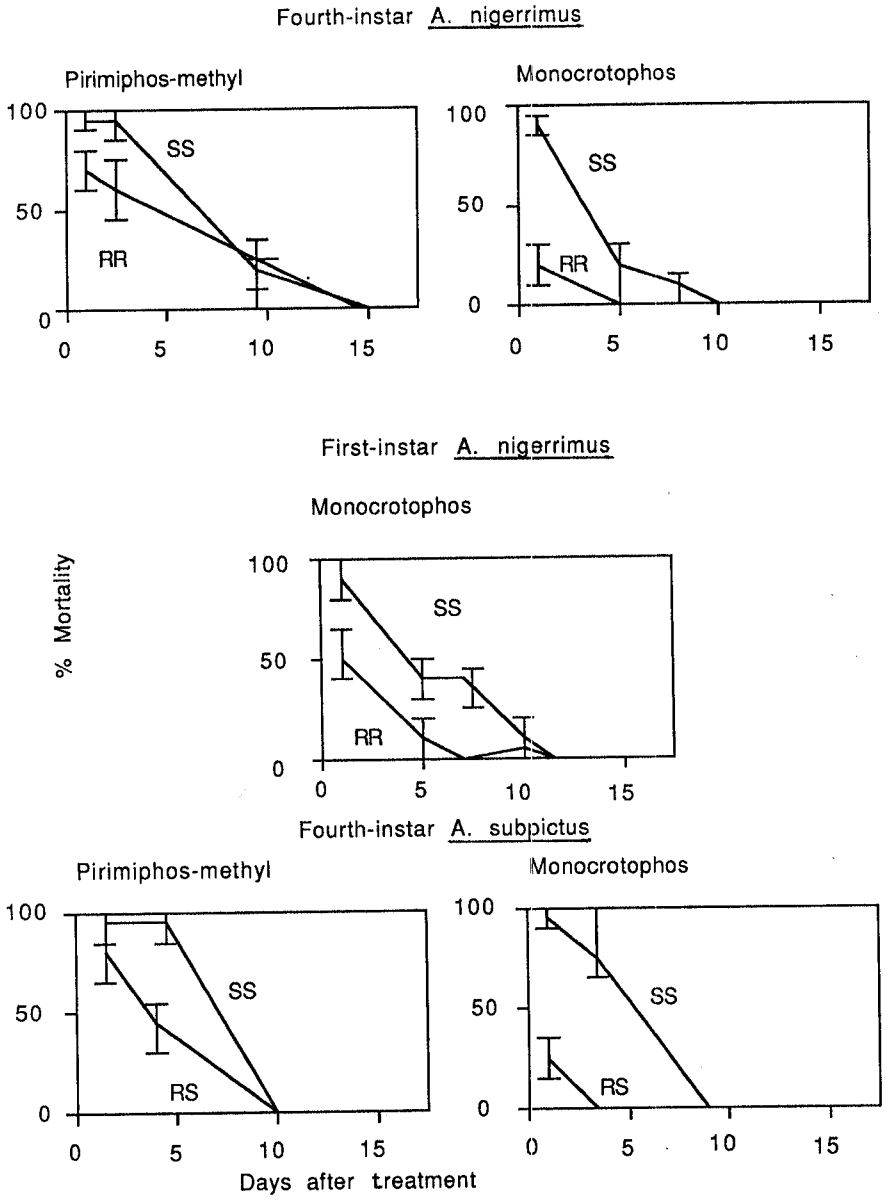

Fig. 2.—Percentage mortalities of larvae of *Anopheles nigerrimus* and *A. subpictus* after exposure for 24 h to water from the rice fields treated with pirimiphos-methyl or monocrotophos.

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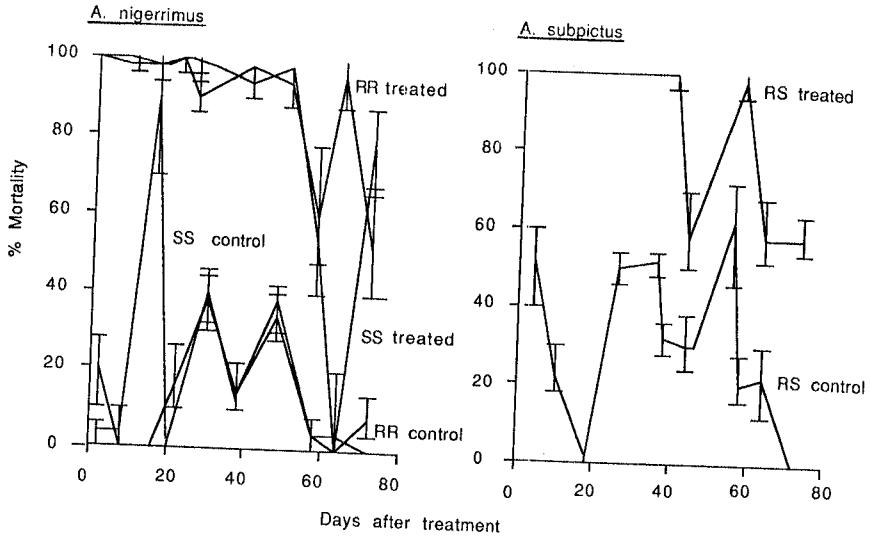

Fig. 3.—Percentage mortalities of *Anopheles nigerrimus* and *A. subpictus* after holding in water from rice fields treated with the growth regulator S-31183. Fourth-instar larvae were held until either they died or adults successfully emerged in both the control and test experiments.

heaviest period of rainfall during the test and corresponded to a decline in the amount of active ingredient in the test site (Fig.1*c*). A similar pattern of results was obtained for *A. subpictus*, where there was high mortality until day 43, which followed the heaviest night of rainfall in the experimental period (Fig. 4).

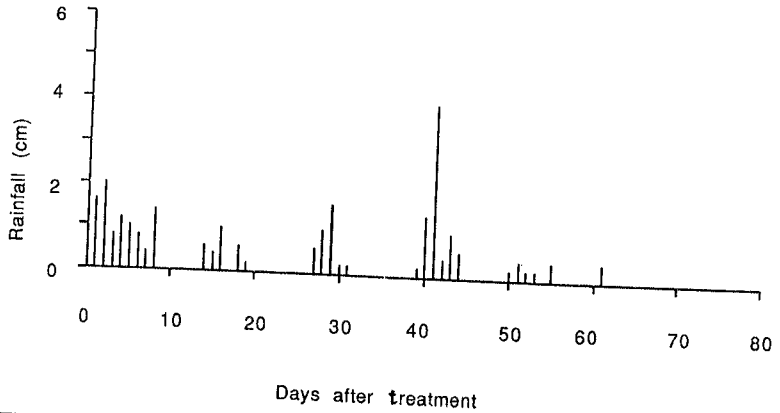

Fig. 4.—Daily rainfall data for the Colombo area during the experimental test period December 1986 to February 1987.

Growth regulators do not kill larvae directly at low concentrations. Larval exposure to these compounds causes the rate of development to be slowed and high mortality at the points of pupation and adult emergence. In these tests, the larvae remained at the fourth-instar stage for up to 14 days. In the controls, there was no mortality after 24 h, but some mortality resulted if control larvae were kept until adult emergence. These control

mortalities for all species are given in Fig. 3. They show a similar pattern for all species (with the exception of susceptible individuals of *A. nigerrimus* on day 15). The peaks of highest control mortality roughly coincided with the periods of heaviest rainfall, and may indicate the leaching of toxins from the surrounding rice fields into the control plot. However, the results clearly show that the mortalities from the growth regulator treatment were significantly higher than the baseline control mortalities.

Discussion

Monocrotophos and pirimiphos-methyl are the two organophosphates most commonly used on rice fields for control of pests in Sri Lanka. Both had a selective effect on mosquitoes breeding there, but the effect was very short-lived. Monocrotophos sprayed at or slightly above the manufacturer's recommended dosage for rice treatment, caused individuals with either an altered AChE or an oxidase-based resistance mechanism to be at a selective advantage for less than ten days after spraying. In the presence of pirimiphos-methyl, individuals heterozygous for the oxidase-based mechanism have some selective advantage, whereas individuals homozygous for AChE-based resistance have a smaller advantage. The short duration of larvicidal efficacy and the rapid degradation of these organophosphates in the rice fields, suggests that there will be no selective effect of these compounds due to run-off into nearby breeding sites, as has been suggested by some authors.

In contrast, the insect growth regulator was effective for much longer periods. High mortality (>75%) was observed 2·5 months after treatment when the amount of growth regulator in the treated water was below the level of detection of the HPLC system. This is in agreement with laboratory bioassay data, which indicated that the growth regulator was effective down to extremely low concentrations (1×10^{-4} mg/litre). At present, there is no manufacturer's recommended field dosage for this compound, but 0·1 mg/litre may be higher than necessary given the frequent spraying cycles of mosquito larviciding programmes. A more realistic dosage for operational use may be 0·01 mg/litre. Data from field tests in Tanzania, using a granular formulation of the same compound at 0·01 mg/litre, showed a 20-day control period at a time when there was heavy rainfall (Hemingway & Magauka, unpublished).

Bioassays with organophosphate- and carbamate-resistant and susceptible *Anopheles* strains showed that there was no selective advantage to either the altered AChE- or oxidase-based resistance mechanisms in the presence of the growth regulator. This is as expected for the former mechanism, as there is no evidence to suggest that the compound primarily acts by affecting the central or peripheral nervous system of the insect. However, the structure of the growth regulator suggests that it may be susceptible to oxidative metabolism within the insect. The oxidase-based resistance in *A. subpictus* from the present results, does not appear to increase the metabolism of the growth regulator to a level which gives measurable resistance to the compound by bioassay. Other oxidative resistance mechanisms may, however, be more effective, so the lack of cross-resistance in this case should not automatically be extrapolated to all cases.

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