FIELD EVALUATION OF AN INSECT GROWTH REGULATOR, PYRIPROXYFEN, AGAINST *ANOPHELES PUNCTULATUS* ON NORTH GUADALCANAL, SOLOMON ISLANDS

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ABSTRACT. Five pools containing immature stages of *Anopheles punctulatus* were treated with pyriproxyfen at 4 different dosages. Inhibition of adult emergence was observed in pupae collected from the test pools and/or those obtained by rearing of the 4th instar larvae. Adult emergence was inhibited completely for 2 months at a dosage of 0.1 ppm, for one month at 0.05 ppm and 0.01 ppm, and for 20 days at 0.02 ppm. Death of test insects were observed at the pupal stage and at adult emergence. The mortality rate at adult emergence increased with the duration of larval rearing and with the elapse of time after application.

INTRODUCTION

Anopheles punctulatus Dönitz is one of the main malaria vectors on the Solomon Islands and New Guinea. In the Solomons, this species was formerly endophagous and endophilic (Taylor 1975) and was susceptible to DDT before the malaria eradication programs (1969–75). The mosquito became scarce at the beginning of the eradication program, but in late 1972 it rapidly returned to a high density in the mountainous areas (Taylor and Maffi 1978). Although DDT spraying has continued since then, malaria is still hyperendemic. Larviciding is one of the recommended malaria control methods other than DDT residual spraying.

Anopheles punctulatus larvae are found mainly in unshaded temporary ground water accumulations in the mountainous regions (Lee et al. 1987). The size, number and water volume of these pools are highly influenced by weather conditions, usually unstable, and the number of larvae and pupae change temporally and spatially.

Although larviciding with synthetic chemicals is a popular and practical measure, it may have an adverse effect on nontarget organisms and usually requires much manpower, time and chemicals. Insect growth regulators (IGRs) are being developed to satisfy all of the factors that make larviciding more desirable.

Pyriproxyfen has been evaluated for efficacy against the larvae of the housefly, *Musca domestica* Linn. (Kawada et al. 1987), tea scale (Cooper and Oetting 1985) and mosquitoes (Estrada and Mulla 1986, Hatakoshi et al. 1987, Kawada et al. 1988). High activity of adult emergence inhibition was reported for *An. farauti* Laveran in a field experiment in the Solomon Islands (Suzuki et al. 1989). In this study, the efficacy of pyriproxyfen against *An. punctulatus* larvae in natural habitats in the Solomon Islands was observed.

MATERIALS AND METHODS

This study was carried out at Torovanihau Village, 10 km inland along the Tenaru River on the northern coast of Guadalcanal, Solomon Islands. In the village, *An. punctulatus* was the most abundant of the man-biting *Anopheles*. There were many larval habitats for this species along a nearby logging road. Most of the habitats were temporary water pools in ground depressions in the road. Their sizes were variable, from small to several meters in length, depending upon the rainfall. One large control pool that contained water for months and 5 test pools (I–V) were selected for the study. Pools I, III, IV and V were water accumulations in ground depressions in the road. Pool II and the control pool were water accumulations in wheel ruts. All pools were unshaded and unpolluted. All the pools except II were surrounded by grass except at the track margin, where people walked and mud was exposed without vegetation. Pool II was formed on bare ground and sparse grass grew along the margin. The day before larvicide application, they were measured for water volume. At the beginning of the study, people used to walk and small vehicles would drive along

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this road. Therefore the ground was bare on the track, and sometimes new wheel ruts were made. One month after the initiation of the study, parts of the road were eroded by floods. After that, no vehicles nor people came through. Vegetation returned, and the margins of the pools were covered with dense grass.

On November 23, 1988, pyriproxyfen granules (0.5%) were applied to the pools, by hand, at the following dosages: Pool I, 0.1 ppm (372 g); Pool II, 0.1 ppm (10 g); Pool III, 0.05 ppm (14 g); Pool IV, 0.02 ppm (8 g) and in Pool V, 0.01 ppm (22 g). Observations of pools and larval sampling continued for about 200 days after application. During the study period there were often heavy rains. Rainfall data were available at Henderson Airport, 7 km north of the study area. The cumulative amount of rainfall reached 2,100 mm during the study period. Sixteen days had more than 30 mm of rainfall. Heavy rains caused flooding of the main river and small tributaries.

To determine the activity and longevity of the compound, pupae collected from the test pools and those obtained by rearing of field-collected larvae were observed for emergence inhibition. The pools were examined for pupae and 4th instar larvae once a week in the first month, then at 2-wk intervals thereafter. At times no larvae or pupae were found. When pupae and 4th instar larvae were found, they were collected with the water of the respective test pool and brought back to the laboratory in Honiara. Thirty larvae were transferred to plastic containers (12 cm diam. × 12 cm high) with 700 ml of the pool water and reared with dry yeast at 29 ± 2°C air temperature. After pupation, each pupa was transferred to a small plastic container (3 cm diam. × 5 cm high) and adult emergence was recorded. Observations were continued until all the pupae emerged or died. Adults remaining attached to their pupal skins were recorded as dead. Mortality rate was calculated daily for pupae. To calculate adult emergence inhibition rates, results from the pupae collected from the test pools and those pupae obtained by one day 4th instar larval rearing were used.

RESULTS

Daily change of adult emergence inhibition rates in relation to duration of larval rearing after sampling: The inhibition rate was closely related to the length of time of larval rearing. It was complete or high in pupae that had a lengthy larval rearing period and in field collected pupae from test pools (Fig. 1). Complete inhibition lasted longer at high dosages than at low ones. The duration of complete inhibition became shorter and the rate dropped earlier with elapsed

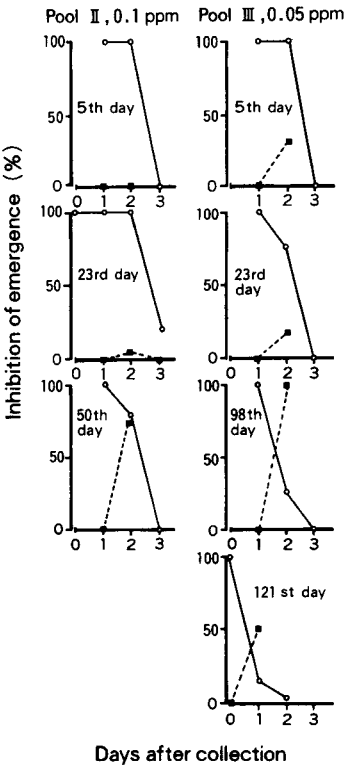

Fig. 1. Daily change of adult emergence inhibition rate at 0.1 and 0.05 ppm, during 121 days after application of pyriproxyfen. Circles indicate the change of inhibition rate for pupae. Dotted lines and squares indicate rate of mortality at adult emergence against total inhibition.

time after application, especially at the lower dosages. Complete inhibition was produced in pupae obtained by larval rearing for 2 days after sampling, both at 5 and 23 days post-application of 0.1 ppm. The rate decreased sharply in pupae resulting from larvae reared for 3 days after sampling. Complete inhibition was observed in pupae obtained by one day larval rearing, 50 days post-application. The rate dropped to 84% for pupae produced from 2 day larval rearing. At a dosage of 0.05 ppm, complete inhibition resulted for pupae produced from 1 and 2 day rearings after sampling 5 days post-application, and for 1 day 23 and 98 days post-application.

Mortality occurred either in the pupal stage or at adult emergence. Mortality occurred more frequently in the pupal stage at high dosages. High pupal mortality was observed in pupae obtained from short time larval rearings, irrespective of dosages. The mortality rate at adult emergence increased with the time of larval rearing and/or with the time elapsed after larvicidal application.

Duration of activity of pyriproxyfen: Pool II (0.1 ppm, Fig. 2A): Observations were continued for about 180 days after application of the compound. Adult emergence was completely inhibited for the first 105 days, despite several floodings of the pool from heavy rains. Afterwards, the pool became filled with sand and dried up. At the 140th day post-treatment, pigs dug a wallow at the site of the pool, and rain water accumulated. Larvae of *An. punctulatus* appeared soon afterwards. Inhibition was still complete initially in the new pool, but the rate rapidly decreased 2 wk later.

Pool III (0.05 ppm, Fig. 2B): Unlike Pool II, this pool retained water throughout the study period, though the volume varied. Complete inhibition resulted for at least one month. The rate fluctuated between 100 and 60% in the period from 50 to 145 days post-application. During this period, pigs sometimes wallowed in the water and stirred up the mud. At 192 days post-application, the inhibition rate was 30%.

Pool IV (0.02 ppm, Fig. 2C): Similar to Pool III, this pool retained water and was never swept by floods. Complete inhibition lasted for 20 days post-application. On the 50th day post-application, 70% emergence inhibition was observed.

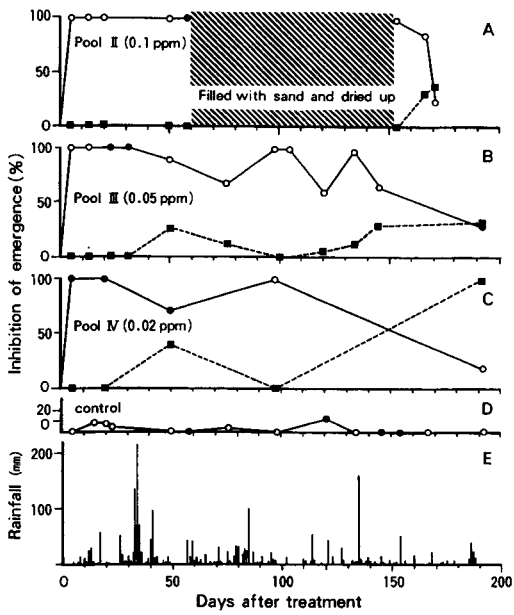

Fig. 2. Change of adult emergence inhibition rate in test pools (A–C), control pool (D) and daily rainfall at Henderson Airport (E). Solid circles = 5–9 pupae observed and open circles = 10 or more pupae. Dotted lines and squares indicate rate of mortality at adult emergence against total inhibition.

Pool I (0.1 ppm, Fig. 3A): Complete inhibition was observed for 15 days post-application. After sampling on the 15th day, the pool disappeared due to erosion from the flooded Tenaru River.

Pool V (0.01 ppm, Fig. 3B): Complete inhibition was observed for at least 30 days post-application. After 30 days the water surface became covered with water hyacinths, and no further larvae of *An. punctulatus* were found.

Control pool (Fig. 2D): Mortality in the control pool was always less than 8% except for the 121st day, when it was 12.5%. There was a tendency for the mortality rate at adult emergence against total inhibition to be higher when the inhibition rate was low ($r = 0.559$, significant $P < 0.05$).

Color change of larvae after pyriproxyfen treatment: The body color of the larvae whitened soon after the treatment of the pools. The white color remained for a considerable period of time, but became weak and eventually returned to the natural color as the inhibition rate decreased.

DISCUSSION

Due to the small size of the larval habitats, larval and pupal populations were small and their age composition was variable. This made it impossible to collect pupae and 4th instar larvae of the same age at every sampling. The stage of the immatures used for evaluation affected the inhibition rate, though they were reared in water taken from the test pools. A similar phenomenon was reported by Kawada et al. (1987), in which the inhibition rate was lower in pupae obtained by larval rearing in water taken from the test pond compared to pupae taken directly from the test pond. The reason for the lower rate is uncertain. Heterogeneity of the compound concentration in the breeding pools was one of the possible reasons for this phenomenon. The granular type of pyriproxyfen was used, which sank to the bottom and was slowly released. The concentration of active ingredient may have been higher in the bottom than in the upper portion of the pool. Larvae usually occur at the margin of the pool, where the water is very shallow, and may submerge on to the mud. Water samples were taken from the surface at the deeper portion, and may have contained less compound than the water at the margins.

In this study, the young 4th instar larvae were less affected by the IGR than the older larvae. Therefore, we used field collected pupae from the test pools and pupae obtained by one day rearing of larvae.

The body color of *An. punctulatus* whitened after the application of pyriproxyfen. Suzuki et

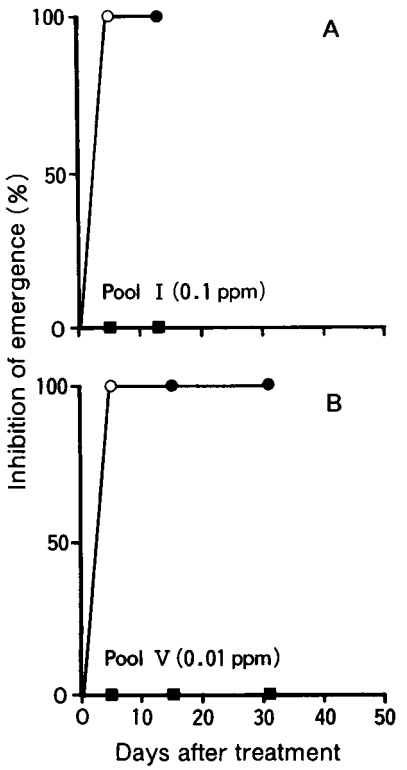

Fig. 3. Change of adult inhibition rate in test pools I and V. Solid circles = 5–9 pupae observed and open circles = 10 or more pupae. Squares indicate rate of mortality at adult emergence against total inhibition.

al. (1989) reported a similar color change in *An. farauti*. If white color is closely related to the inhibition rate, it can be used as an indicator of the activity of the compound and whether an additional application may be necessary.

Pyriproxyfen was effective in inhibiting adult emergence of *An. punctulatus*. Granules of this compound inhibited emergence completely for 2 months at a dosage of 0.1 ppm, even after several floodings by heavy rains; for 1 month at the lower dosages of 0.01 and 0.05 ppm; and for 20 days at 0.02 ppm. It was observed that 0.1 ppm pyriproxyfen had continued activity after dry conditions for 50 days. This long-lasting efficacy at low dosages indicates that this compound could make larviciding feasible in terms of costs and operations. A large scale field trial should be undertaken to determine if larviciding is effective in reducing the incidence and prevalence of malaria in an area.

ACKNOWLEDGMENTS

We express our deep gratitude to the staff of the entomology section of the Malaria Training and Research Center for their support. We also thank S. Meek of the World Health Organization, M. Sasa of Toyama International University and E. J. Gerberg of Insect Control & Research, Inc., for useful suggestions and assistance. Sumitomo Chemical Co. Ltd. provided us with the pyriproxyfen used in the present study. The Ministry of Health and Medical Services, Solomon Islands and the Japan International Cooperation Agency supported this study.

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