FIELD TRIALS OF *TOLYPOCLADIUM CYLINDROSPORUM* AGAINST LARVAE OF *AEDES POLYNESIENSIS* BREEDING IN CRAB HOLES IN FIJI[1]

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ABSTRACT. The New Zealand isolate of the entomopathogenic fungus *Tolypocladium cylindrosporum* was field-tested against larvae of the crab hole breeding mosquito *Aedes polynesiensis*. A reduction of approximately 87% was achieved in the larval populations of all crab holes treated with *T. cylindrosporum* by day 21 of spore application. However, the mosquito populations recovered to the pretreatment level within two months indicating that any residual activity of this pathogen was insignificant.

INTRODUCTION

The genus *Tolypocladium* was established by Gams (1971) for a group of hyphomycetes isolated from soil samples. One of these species, *T. cylindrosporum,* has since been reported as a pathogen infecting larvae of the tree hole breeding mosquito *Aedes sierrensis* (Ludlow) in northern California (Soares et al. 1979). Another isolate of this fungus was discovered independently in New Zealand from larvae of the mosquito *Ae. australis* (Erichson) which breeds in brackish rock pools on the southern coast of the South Island (Weiser and Pillai 1981).

Laboratory studies have shown that this fungus is pathogenic to a number of mosquito species from different parts of the world including species that breed in brackish water (Pillai[4]). The New Zealand isolate has also exhibited broad tolerances to several environmental conditions, eg., to both temperature (4–35°C) and salinity (0–7% NaCl) (Gardner et al. 1982).

Aedes polynesiensis Marks was chosen as the target species for the biological control experiments. The mosquito is the principal vector of subperiodic filariasis over a vast region of the South Pacific where it is adapted to diverse breeding habitats. The supralittoral crab hole made by several species of crabs belonging to genus *Cardisoma,* is regarded as the most important source of the mosquito (Pillai and Engber 1982). All but the crab hole habitat lend themselves to source reduction procedures. The latter is virtually impossible to eliminate and thus often constitutes the main vector source, which makes the interruption of disease transmission rather more difficult (Burnett 1960).

In this paper we are reporting the results of our experiment to control *Ae. polynesiensis* in crab holes in Fiji by the New Zealand isolate of *T. cylindrosporum.*

MATERIALS AND METHODS

A culture of the New Zealand isolate of *Tolypocladium cylindrosporum* was grown at the Vector Control Unit, Suva, Fiji on DIFCO Sabouraud Dextrose (SAB) agar slopes at room temperature (27 ± 2°C). After 5 days, the conidia were harvested by rubbing the surface of the culture with a sterile rubber bulb. The conidia were suspended in sterile distilled water at a concentration of 2 x 10^8 conidia/ml and stored at 6°C until required. Spore viability was assessed prior to the trials by calculating the germination rate following incubation on SAB agar for 24 hr at room temperature. The inoculum was also tested against laboratory reared *Ae. polynesiensis* larvae to assess both the virulence of the pathogen and the susceptibility of the target population. A laboratory colony of *Ae. polynesiensis* was established so that these laboratory bioassays could be conducted in conjunction with the field trials.

For the laboratory experiments, the mosquito larvae were preadapted to standard size 150 ml plastic bioassay cups one day prior to the application of spores. Each cup contained 20 larvae in 99 ml of distilled water. One ml of the appropriate spore suspension was added to the cups containing the test larvae. The concentrations tested were 10^4, 10^5, 10^6 and 10^7 conidia/ml with 5 dishes for each concentration. One ml of distilled water was added to each of the control cups which were otherwise prepared in the same way as the test cups. The bioassay was monitored for 10 days and any dead larvae were removed and examined microscopically for mycoses of the coelomic

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cavity as evidence of fungal infection. Larvae were fed on liver powder on the day prior to the trial and again on days 3 and 7. The percentage mortality rates were corrected by Abbott's formula (Abbott 1925) and a probit analysis was performed to obtain an estimate of the LC50 value, using the SPSSx statistical package compiled by N. H. Nie and published by McGraw Hill, 1983.

The field trials were conducted in a mangrove swamp containing a large number of crab holes (formed by the *Cardisoma* spp.) at Nukui on the Rewa Delta, approximately 26 km from Suva. The holes were 10–15 cm in diameter often reaching a depth of about 2 m and containing up to 4 liters of water with numerous *Ae. polynesiensis* larvae. The twisting nature of the tunnels made sampling difficult and a 4.5 liter hand-operated sprayer, especially modified (Goettel et al. 1981), was used to collect the water samples. As the water level was affected by the tide, sampling was carried out close to high tide when the water volume was at a maximum.

A preliminary trial was commenced in December 1984 in 15 crab holes (10 treated and 5 untreated). The test site was situated approximately 50 m from the control site. Each crab hole was first sampled and as much water as possible was recovered. The volume of water recovered fluctuated within the range of 1–4 liters. The number of larvae collected was counted and the inoculum was then added before the sample was returned to the crab hole. A separate count was made for the younger larvae (1st–2nd instars), older larvae (3rd–4th instars) and the pupae. As it was not possible to determine the precise volume of water present in a particular crab hole, a standard spore preparation of 1×10^9 conidia, was applied. The mosquito populations of the untreated crab holes were also counted by the same sampling technique. A random sample of 10 larvae was collected from each crab hole and returned to the laboratory for identification and examination.

Following the application of the spores, the site was revisited to assess the effect of the fungus on the population of larvae in each crab hole. Any dead larvae were collected from the crab holes and returned to the laboratory for examination. Random larval samples were also collected and later examined for evidence of infection and the temperature and salinity conditions of the site were monitored. This trial was abandoned after a month following severe damage to the field site by two cyclones.

A larger field trial was begun at the same site in May 1985 with 26 treated crab holes and 10 controls. The trial was conducted in the same way as the preliminary trial with an inoculum of 1×10^9 conidia being applied on day 23 to each of the treated holes. The site was monitored until July, depending on weather conditions, at approximately 10 day intervals till day 85. Further monitoring was not possible due to prolonged drought conditions, which followed in the wake of the cyclones.

RESULTS

The viability of the inoculum was confirmed with the germination rate of the conidia preparation being assessed at 90–95%. The laboratory bioassays demonstrated the susceptibility of the *Ae. polynesiensis* larvae to infection with *T. cylindrosporum*. The LC_{50} value was calculated at 1.6×10^5 conidia/ml.

The results of the preliminary field trial showed a substantial reduction in the immature populations of each of the 10 crab holes following treatment. The mean immature population of these crab holes was reduced from 341.4 ± 68.1 at day 0 to 56.4 ± 46.9 after 7 days. However, after this initial decline the populations began to recover and 21 days after treatment the mean population was 271.5 ± 106.3. In comparison, the mean population of the untreated crab holes increased steadily during the trial from 252.4 ± 90.2 (day 0) to 262.5 ± 34.5 (day 7) to 332.7 ± 53.3 (day 21). Unfortunately further sampling was not possible due to climatic disruptions.

The results obtained for the second trial are shown in Fig. 1. From the original sample, 25 treated and 9 untreated crab holes were monitored through day 54, and by day 82, 20 treated and 5 untreated crab holes remained sufficiently intact to enable sampling. In this trial a marked decrease in the immature population occurred within 21 days of treatment, i.e., by day 44. The total population was reduced by approximately 87% with all immature stages affected. After this initial substantial reduction the numbers increased steadily and by day 80 had almost recovered to their pretreatment levels.

Larvae collected from the test site during both trials were covered with saprophytes making examination difficult, but live specimens that were brought to the laboratory and dissected showed clear evidence of *T. cylindrosporum* infection in the coelomic cavity and/or the outgrowth of aerial mycelia from the exocuticle.

Throughout both of the field trials the water temperature and saline content fluctuated within the ranges 18–22 °C and 0–0.9% NaCl respectively.

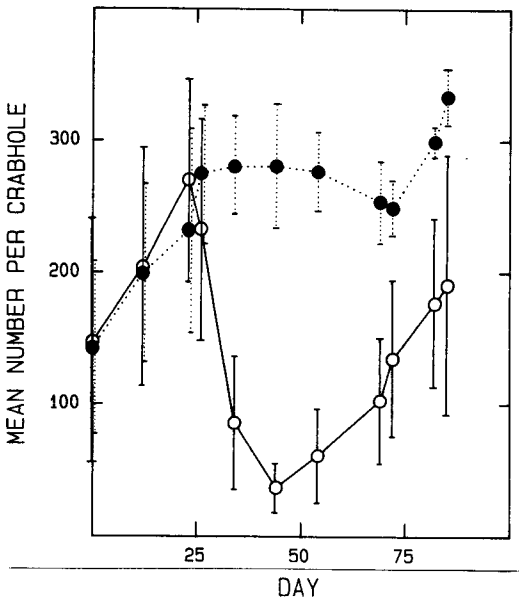

Fig. 1. Mean total population of *Aedes polynesiensis* larvae and pupae in crab holes following an application of *T. cylindrosporum* (1 x 10^9 conidia/crab hole) on Day 23.

○ Test crab holes ● Control crab holes

DISCUSSION

The application of conidia resulted in a significant initial decrease in the population of larvae and pupae in the crab holes. In the second trial, the total population of each crab hole was reduced by approximately 87% after 21 days. However, the emergence of young larvae, and the apparent lack of any significant residual activity of the fungus, allowed the population to steadily recover. After 62 days, the larval populations of most of the test crab holes had reached their pretreatment level. By this stage the populations of the untreated crab holes had increased by a further 40% and whether the test populations would also have increased by the same amount could not be assessed as drought conditions prevented any further sampling after day 85.

Tolypocladium cylindrosporum kills susceptible hosts by invasion through the gut wall following the ingestion of conidia. Therefore, death occurs more slowly than if the larvae were exposed to a chemical insecticide or a faster-acting pathogen such as *Bacillus thuringiensis* var. *israelensis*. Consequently, a precise field evaluation is difficult as infection may develop within the living larvae for several days before death occurs. Because of the continued emergence of young larvae, from newly laid eggs, the full effects of the fungus can be disguised. These problems may be avoided by monitoring the larval populations, with separate records for the young larvae (1st and 2nd instars), old larvae (3rd and 4th instars) and the pupae. By this grouping, it was possible to show that all stages of larval development were killed following the application of conidia and no significant increase in the number of young larvae occurred for about 30 days.

Tolypocladium cylindrosporum has the potential for recycling by forming a sporulating layer on the exterior surface of dead larvae. However, it was not possible to verify this in the trials as the larvae were hidden from view making selective sampling difficult. Since the level of control obtained declined with time it would indicate that little, or no, significant residual activity was occurring to prevent the population from recovering to its original level.

The short-term control achieved was significant but this must be weighed against the high dosage rate of fungal spores that was required and the absence of any long-term control.

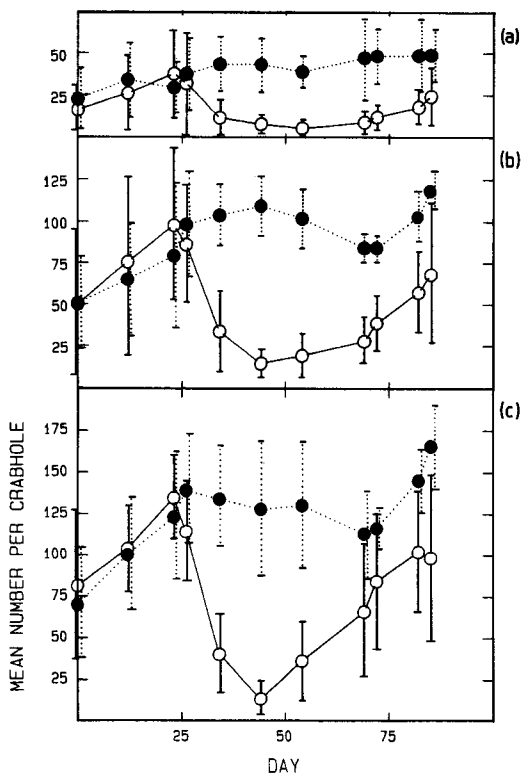

Fig. 2. (a) Mean population of *Aedes polynesiensis* 1–2nd instar larvae, (b) 3–4th instar larvae, and (c) pupae in crab holes following an application of *T. cylindrosporum* (1 × 10^9 conidia/crab hole) on Day 23.

○ Test crab holes ● Control crab holes

Furthermore, although the production cost for this fungus is low, its application in this habitat would currently be prohibitive because of the additional expense of reintroduction at regular intervals. However, as there are currently no alternative control measures available for crab hole breeding mosquitoes the use of biological agents appears to be the logical choice. Agents such as larvivorous fish or preparations of *Bacillus thuringiensis* var. *israelensis* would be highly impractical. The crab holes are very numerous and the breeding pool is usually located at the end of a twisting tunnel often 2–3 m below the surface. The fluctuating water level in the chamber is not conducive to the use of larvivorous fish. *Bacillus thuringiensis* var. *israelensis* preparations would be effective, but since it does not recycle, its frequent application in the environment will be time consuming and cost prohibitive. The only biocontrol agent that can be of practical value is one that is capable of persisting and recycling in the crab hole environment and *T. cylindrosporum* is known to possess these biological attributes.

Since the temperature and salinity ranges of the habitat are well within the tolerance limits of the fungus, *T. cylindrosporum* will continue to be a leading contender. If the larvicidal action of the fungus can be enhanced or if residual activity can be prolonged then its use in the crab hole control programs would be more acceptable. Therefore, the main priority now is to isolate and select strains of the fungus for improved performance. The possible establishment of such an improved strain within the field site and the continual recirculation of the fungal spores through rain and water action may increase the exposure rate of the target population to infectious material.

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