PACIFIC REGIONAL AGRICULTURAL PROGRAMME

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EVALUATING INSECTICIDAL ACTIVITY OF MICROBIALS

IN A FOUR-STEP BIOASSAY SYSTEM

The value of an insect active agent can only be conclusively assessed in a field situation. However before committing the resources required for such an evaluation the potential of the component can be examined in a more opportune way.

An economical way in terms of material, time and effort to test a high number of candidate control agents is a tiered assay system. In this scheme all candidates are tested in an easy first step with high exposure to the agent; a selection goes into the next tier(s) where the agent is challenged more thoroughly; finally agents that show potential are tested in a real field trial.

The use of a tiered bioassay system is discussed, illustrated with examples from our tests on taro beetles, *Papuana* spp.: fungi (*Metarhizium anisopliae*) against adult beetles and bacteria (*Bacillus popilliae*) against larvae. The principle is also applicable to other microbials and chemicals.

First Tier: Direct Application

In the first tier the component is tested by direct application to the test insect. The insect is exposed to a high dose of the microbial with no chance of escape. The mode of action of the microbial determines the method of application.

As fungal spores infect insects by penetrating the integument and no ingestion is needed, the virulence against taro beetles was tested by topical application. Spores were produced on agar in petri dishes. A small amount of spores (10^6) in a small volume of liquid (3–5 µl) was pipetted onto the exoskeleton of adult *Papuana*. About 40 isolates of the fungus *Metarhizium anisopliae* were tested. Tests were checked weekly and cadavers removed to prevent possibilities of additional infection.

Alternatives for this topical application would have been to dust the insects with spores, to let them crawl over the spores (for a certain time) or to spray the insect with spores (in a spray tower). In the first two cases the dose administered is not known but is high (maximum exposure), in the latter case the dose the insects receive can be calculated from the exposed surface and the dose sprayed per surface unit.

The bacterium *Bacillus popilliae* has to be ingested by the insect to be infective. It can be tested by feeding larvae with a known number of spores in a small volume of water (we used 10^7 spores in 10 µl) (Photo 1). Tests were checked after 1 week and then every two days and cadavers removed to prevent extra infection.

Similarly insects can be fed with small amounts of feed containing a known number of units of the agent, insects that do not ingest the required dose in the set time can be excluded from the assay. If the insect is large enough, it can be force fed by bringing a small tube (e.g. blunt injection needle) into the oesophagus and injecting the required dose straight into the gut.

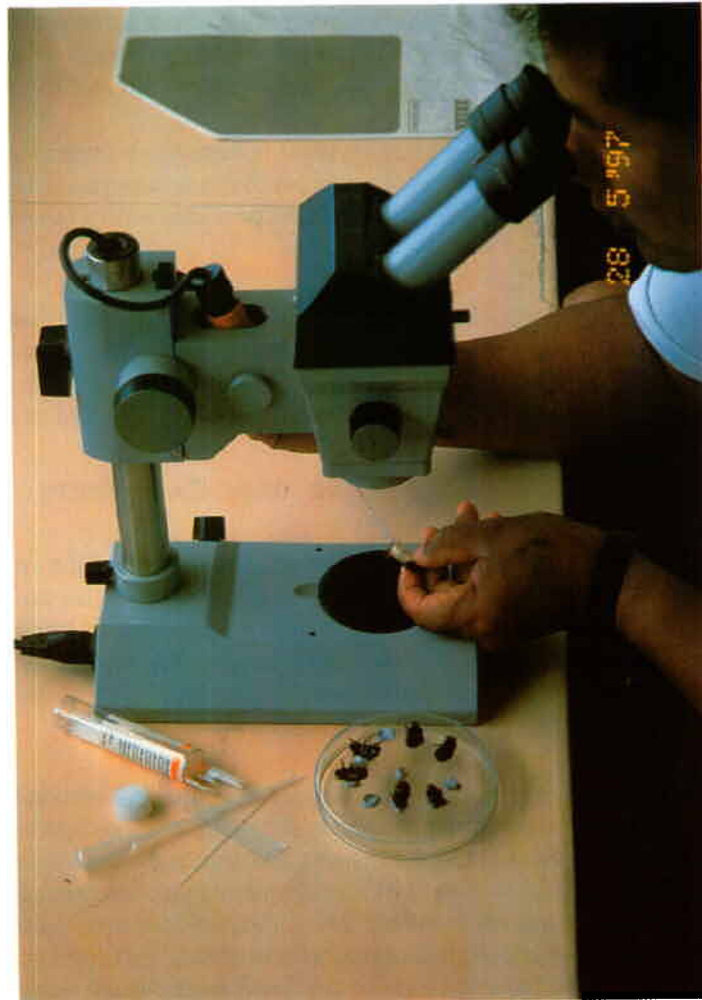

Photo 1. Feeding of larvae and adults with pathogens in a small amount of fluid using 10 mm capillaries. Ingestion is checked by observing the insects through a stereomicroscope.

Results obtained from these laboratory tests should of course be interpreted carefully and can generally not be extrapolated to the field situation since both the doses used and the infection process are quite unnatural. For example high doses of pathogens present in soil can be administered to insects that feed on leafs and may result in high infection rates; this indicates that the insects are susceptible; natural infection is however very improbable since the insects hardly ever contact the soil and even when they do they would not come into contact with such a high concentration of the pathogens.

Second Tier: Indirect Application

In the second tier the exposure is indirect, the agent is added to the environment of the insect (medium, food). The natural (feeding) behaviour of the insects partly determines the bioassay. The bioassay is, where possible, associated with the expected field situation.

The insects are exposed to a certain amount per kg or per m^2 rather than to a certain dose per insect. Although the individual insects will/may receive a smaller dose than in the first tier, a greater quantity of the tested agent will be required.

Ten of about 40 fungal strains were selected to be tested against adult taro beetles. Spores were produced on rice in small mass cultures. The spores were mixed with soil and adult beetles were added and provided with a taro corm. Tests were checked as above.

Two *Bacillus popilliae* strains were tested against taro beetle larvae. Spores were collected from a high number of infected (naturally or artificially) larvae and prepared as a dust formulation. They were mixed with cowdung/sawdust medium which is the larval habitat as well as feed. Tests were checked as above.

Third Tier: Cage-Tests

In field trials there is a great number of factors related to the plants, the soil and the weather which can cause variable results. Much of the variability can be associated with the insects: the target pest may not be present in sufficient numbers in the field test area during the test period or target pest density is sufficient but only part of the field test was affected. This causes (high) variability of the data and will necessitate a (high) number of repetitions to allow analysis.

By using caged plants to which insects are added at certain times after planting some factors can be controlled, such as the number of insects tested, the density and the developmental stage of the insects, the time and duration of the attack. The variability of the results will be lower so less plants per treatment are necessary. This makes it feasible to test among other things the persistence of the microbial (by challenging the microbial at certain times after treatment), different concentrations, application methods, number and times of applications, different densities of insects, etc.

The cages used depend on the insects (and host plants) and can vary from a real net and wire cage in the case of flying pests to simple marked plots if there is no danger of the pest escaping.

In our trials with fungal spores against adult beetles the cage consisted of a sheet metal cylinder (30 cm tall, 50 cm diameter), pushed into the soil to a depth of about 20 cm and surrounding the taro plant (cover photo). A pair of beetles was added, the leaves of the taro plant cut close to the ground and the top end of the cylinder closed with insect gauze held in place by an elastic band.

Ten plants for each treatment were tested at various times after planting. The required number of plants was determined and treated at planting time.

Pairs of adults beetles were added to ten plants for each treatment on planting day and evaluated 3 weeks later. The cages were then moved to another set of 10 plants for each treatment. Again adult beetles were added and the whole process of evaluat-

Photo 2. Cages used for third instar larvae of a taro beetle species in Papua New Guinea (Papuana woodlarkiana) consisted of simple wooden squares (50 x 50 x 15 cm) with sugarcane in the centre, on the roots of which the larvae feed.

Photo 3. Cages used for third instar larvae of a taro beetle species in Solomon Islands (Papuana uninodis) consisted of sheet metal cylinders pushed into the soil to a depth of about 20 cm and filled with small logs under which the larvae feed.

ing and setting up cages was repeated every 3 weeks until the end of the growing season. We assumed that if there were changes in effectiveness of the treatments, the biggest differences would be found in the early weeks/months. Therefore tests were done every 3 weeks, at week 0, 3, 6, 9 and 12, and then every 6 weeks (week 18, 24, 30). To prevent soil/location related factors from influencing the test, plants were completely randomly assigned to a treatment and time of evaluation. Using this method, a taro growing season of 6–9 months can be covered with 100–150 plants for each treatment (with another 100–150 plants in the control treatment).

Similarly, the bacteria were tested on larvae of the taro beetle. The 'cages' depended on the species of taro beetle (Photo 2). Simple wooden squares of 50 x 50 x 15 cm deep around a plot with sugarcane or the above-mentioned metal cages (without the insect gauze) fitted with small legs were used (Photo 3). The required number of plots was prepared and treated. The treatments and times the plots were to be tested were allocated at random. Ten larvae were added to 4 plots of each treatment 3 weeks after preparing/treating the plots and evaluation was done 4 weeks later. The procedure was repeated with 4 different plots for each treatment 5, 10, 20, 30, 40, 50 weeks after treatment, with evaluation always 4 weeks later. To test the persistence of the bacteria over these 7 intervals with 40 larvae (10 larvae/plot and 4 plots) requires 28 plots per treatment.

Fourth Tier: Field Trials

The final field trial is of course dependent on the insect/plant/farming system. The data from the cage trial can indicate which microbial, at which concentration(s) should be field tested and how, when, and how often the agent should be applied. The number of repetitions will depend on the expected variability. A statistical formula to decide the needed repetitions is given in *PRAP leaflet* no. 1 (How to lay out, maintain and record a randomised block trial).

For example, the fungus could be tested in a field trial consisting of four Latin squares, with each Latin square consisting of 9 plots (3 reps each for the control and 2 concentrations of fungal spores), with 36 plants in each plot. Including guard rows this required 2000 plants.

The bacteria are difficult to test in a regular field trial. They are tested by introducing the spores to (artificial) breeding sites and examining the larval population in the area at intervals for symptoms of the disease.

Conclusion

To evaluate the insecticidal activity of microbials (chemicals), a tiered bioassay system can be used. This method makes it feasible to test many candidates in an economical way by testing all in an easy first step and narrowing down the number of agents in the more expensive, time consuming and laborious step(s).

Before testing the selected component(s) in a regular field trial a cage trial can be used. Test insects are added to the cages (real cages or just mini-plots) which are evaluated after a certain interval.

Since in these cage trials the number, density and developmental stage of the insects and the time and duration of the attack are controlled, the data are less variable than in regular field trials.

Because of the relative ease of this setup it then becomes feasible to address other questions (persistence of treatment, application method, concentration and timing of treatment).

When the selected microbial(s) is finally tested in a field trial the cage test can indicate which concentration, application method, etc., are advisable.

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