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An Investigation into Mycoinsecticides as Potential Control Agents of Sexava in Oil Palms in New Britain

Roy Bateman, IPARC, Imperial College, Silwood Park, UK and Charles Dewhurst, PNG Oil Palm Research Association , West New Britain, PNG

ABSTRACT

Three entomopathogenic fungus isolates in the genera *Metarhizium, Beauveria* and *Paecilomyces* were obtained, mass produced and tested against the important oil palm pest *Segestes decoratus*. Previous work had been marred by high control mortality in bioassays, but in the experiments described here, control mortality (using blank oil formulation) was reduced to an acceptable level. Two experiments were carried out: (a) a bioassay in which 5µL oil-based formulation was topically applied directly to the insects and (b) an arena/cage test in which the formulation was sprayed onto palm seedlings which were subsequently introduced to insects placed in large cages. We established that the spores of all three fungal isolates were still viable in the formulation after application, but there were no significant (or even noticeable) differences in insect mortality between these and control treatments, in either experiment. Although this result is interesting from a biological perspective, it is of course disappointing for the management of this important oil palm pest.

INTRODUCTION

Major pests of both commercial and smallholder oil palm plantations in Papua New Guinea (PNG) are large long-horned grasshoppers or Katydids (Orthoptera:Tettigoniidae), locally called 'sexava'. In West New Britain province these insects are *Segestes decoratus* Redtenbacher and *Segestidea defoliaria* Uvarov. Heavy attacks by these insects result in almost total defoliation ("broomsticks") of the oil palms and subsequent serious reduction of fresh fruit production. This, in turn, seriously effects the livelihoods of smallholder growers and milling companies Pest damage can result in greatly reduced productivity for up to 5 years (OPRAtive Word 20).

Insecticide compounds that are used against sexava must be applied under strict conditions, since they are hazardous to handle (belonging to WHO/EPA class I). The standard method of insecticide application is targeted trunk injection (TTI) of a systemic organophosphate (OP) methamidophos; at 10ml per palm trunk. Many OP insecticides have been withdrawn in the Organisation for Economic Co-operation and Development (OECD) countries, and the future availability of remaining permitted compounds is a matter of considerable debate.

The spraying of broad spectrum insecticides, undertaken for many oil palm pests in other countries, is not an option in the PNG oil palm industry, because it is both difficult and unacceptable under the strict health, environmental and sustainability standards imposed (RSPO 2008). Trunk injection with a specified OP insecticide works well and is not overly persistent, however from a pest management point of view, reliance on a single mode-of-action insecticide to control a major pest is highly unsatisfactory in the long-term.

There has been much enthusiasm and acceptance for the use and encouragement of natural enemies which is currently undertaken by PNGOPRA, however these interventions are time consuming and the insects are difficult to rear and release in the quantities that are required.

An alternative option is the use of mycoinsecticides as an inundative biological control method. Entomopathogenic fungi are part of the naturally occurring microbial biodiversity in tropical and temperate agro-ecosystems, and have been recovered from the soils in WNB (PNGOPRA 2000). These fungi are important members of natural enemy communities regulating arthropod populations typically by the action of many isolates of a particular species infecting and killing a proportion of the population. Occasionally, epizootics of highly virulent isolates, cause population crashes of insect pests. Previous work has shown that the *Metarhizium anisopliae* isolates which successfully infect Orthoptera belong to a specialised clade of *M. anisopliae* var *acridum*, and there are recognised Tettigoniid specific strains recently identified (St.Leger 2009 *in litt.)*.

Entomopathogenic fungi in the genera: *Metarhizium, Beauveria* and *Paecilomyces* have been found on sexava in New Britain (R. Caudwell, pers. comm., and Fig.1). Caudwell carried out preliminary experiments to evaluate locally-obtained isolates (41 *Metarhizium*, 8 *Paecilomyces*) against sexava, but results were marred by high control mortality (60% at day 5, 100% at day 7).

Figure 1: *Male S.defoliaria covered with entomopathogenic fungus growth.*

Three Tettigoniid isolates were tested, belonging to different entomopathogenic fungal species that may be found naturally in PNG:

1. *Beauveria bassiana* ARSEF 6234
2. *Metarhizium anisopliae* ARSEF 0727
3. *Paecilomyces reniformis* Samson & Evans ARSEF 0484

METHODS

a. Mass Production: First Stage

This was a two phase production process that took place at CABI Europe-UK, starting with a liquid medium, of sterile water, 2% yeast extract and 2% sucrose, which was inoculated with the lyophilised cultures (as above).

Flasks were placed on a rotary shaker (approx. 175 rpm) for 4 days.

In the early stages of growth distinct clumps of mycelium may be formed within an otherwise clear broth. This form of growth is less productive than homogenous colonisation of the culture broth by evenly dispersed mycelium and hyphal fragments.

Both *M. anisopliae* and *B. bassiana* were taken through to a secondary stage; however the 1st run of *P. reniformis* showed no growth. As only one ampoule of fungus was supplied, it was decided to leave two flasks on the shaker to see if any growth did occur.
After 14 days a useable mycelial broth was produced. (Fig. 2).

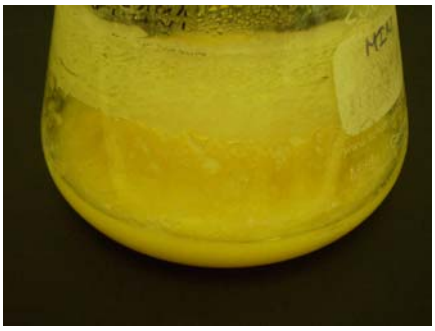

Figure 2: *P. reniformis* (ARSEF 0484) after 14 days showing mycelial growth.

Figure 3: *M.anisopliae* (ARSEF 0727) showing pelleting at the bottom of the flask.

b. Spore Production: Second stage

Dry, unpolished rice was placed in an automatic rice cooker with a measured amount of water (300ml/Kg *B. bassiana*; 600ml/Kg *M. anisopliae* & *P. reniformis*) and cooked. The rice was then divided into autoclave bags (600 x 700mm - 500g rice per bag), autoclaved for 30mins at 121°C, 103kPa (15psi), and placed in a laminar flow cabinet to cool.

Each 500g bag of rice was inoculated with 75ml of inoculum. Prior to inoculation the flasks containing *M. anisopliae* & *P. reniformis* were diluted with 75ml sterile water. The *B. bassiana* was not diluted due to the pelleting, also seen in *M. anisopliae* (Fig.3).

Once inoculated, the tops of the bags were loosely folded once and the contents massaged to evenly distribute the inoculum. The bags

Figure 4: Bags of *M. anisopliae* & *B. bassiana* in mass production suite at CABI Europe-UK

were then transferred to an incubator room containing multi-tiered shelves 300mm apart (Fig.4).

c. Spore separation

With the *B. bassiana* and *M. anisopliae* bags, it was necessary to re-massage the substrate to break up mycelial clumps. All bags were opened for 2 days, to surface dry the substrate (and encourage further conidiation), before extraction using the MycoHarvester (version 5 prototype) at International Pesticide Application Research Centre, UK (IPARC), (Fig.5).

Following extraction, the conidial powder was further dried in a dessicator, using indicating silica gel before sealing in tri-laminate aluminium sachets for shipment. During the drying-spore extraction process, there was a strong odour produced (similar to sour milk) from the *P. reniformis* isolate.

Figure 5: MycoHarvester: version 5

The production and extraction processes were not optimised for any of the isolates but, to indicate the considerable differences in productivity, Table 1 shows the weights of conidia obtained from the primary cyclone cylinder of a MycoHarvester.

Table 1:

Fungus	ARSEF	Production (g. per 1 kg rice)
Beauveria bassiana	6234	1.6 (2 kg)
Metarhizium anisopliae	727	32.4 (2 kg)
Paecilomyces reniformis	484	5.5 (large quantity of mycelium in secondary)

APPLICATION AND TESTING

A simple technique for spraying at ultra-low volume (ULV) formulation rates in oils is described below. Formulated fungal isolates were plated out on PDA shortly after application; and all three samples of conidia were >95% viable which went on to produce characteristic growths of the respective fungal species. The *P. reniformis* again showed secondary metabolite formation, with a purple colouration of the agar.

METHODOLOGY IN PNG (WNB)

Treatment and assessment of cage tests, supplemented by insect bioassay, was carried out in the Quarantine Facility of the PNG-OPRA Entomology Section (PEQCS4).

In these experiments populations of parthenogenetic females of *Segestes decoratus* Redtenbacher were used. Two experiments were performed according to the following protocols:

A. Sprayed oil palm seedlings: cage test

1. 30 adult female *S.decoratus* were introduced into each of 10 large cages (1805 x 1050 x 1100 mm) - the day before introducing treated seedlings (any moribund insects were replaced before the treatment).
2. Treatments were randomised in 2 blocks and cages were clearly labelled (Fig.6).
3. A "mini-Ulva lite" 9000 rpm, 65 ml/min (red restrictor) sprayer was calibrated.
4. Formulation was prepared as follows:
   - 50% groundnut oil / kerosene mixture as the stock carrier
   - 2.5g spores in 125ml of carrier for each isolate;
5. Control was blank groundnut oil with UV tracer.
   - Isolates were not characterised, but the formulation contained approximately 10^9 conidia/ml.
6. 2 seedling palms (growing in pots and about 6 months old) were sprayed for each replicate. Each palm was sprayed for approximately 10 seconds on both sides, in 2 pairs. With a 25% estimated droplet capture, delivery was estimated to be 3 x 10^9 conidia/seedling.
7. Seedlings that were sprayed with UV tracer (astral pink) were examined in the dark: and showed >1 droplet mm^{-2}.
8. Before carefully introducing the seedlings into the correctly labelled cage, the insects were checked for any handling mortality. Numbers were adjusted if necessary.
9. A fresh oil palm seedling was placed in each cage after 7 days to maintain insect welfare.
10. At 24 hour intervals the cages were checked carefully (ground, crevices and foliage) for cadavers.
11. Any cadavers found were placed on moist tissue paper in 80 x 70 x 40mm plastic boxes for assessment of mycosis. (They were kept for 4-5 days at room temperature and if nothing found, they were thrown away).
12. Data were recorded on record sheets, and entered onto an excel spreadsheet at the end of the trial.

Recording was continued until control mortality was >50%.

B. Topical application bioassay

1. Adult *S. decoratus* were randomly selected from stock rearing cages, and 12 individuals were carefully released into each of 5 300 x 300 x 600mm cages covered with shade netting (Fig.6).
2. Insects were inoculated with 5µL suspension of isolates using 'Drumond Microcaps' (Broomall, PA USA). The five treatments consisted of: 3 formulations (of fungal suspension), blank groundnut oil control and untreated control. Estimated delivery was approximately 2.0 x 10^5 conidia/insect.
3. Foodplant (oil palm plants in pots) was introduced into cages; it was replaced every 5 days.
4. Assessments were made at 24 hour intervals - checking the foliage floor of the cage, corners and crevices and carefully for cadavers.
5. Any cadavers found were placed on moist tissue paper in 80 x 70 x 40mm plastic boxes for assessment of mycosis (they were kept for 4-5 days at room temperature and disposed of if no mycelial growth was observed).
6. Data were recorded on a record sheet.

Figure 6: Recording mortality in one of the walk-in cages.

RESULTS

In the cages, untreated control mortality was reduced to 10% at the end of the first week, and 50% by the end of the second week as compared to 100% by day 7 in Caudwell's experiments. There were no significant (or noticeable) differences in insect mortality between these control treatments in either topical bioassay or the sprayed oil palm seedlings/cage experiments. The final mortality with the four treatments was 76% for *B. bassiana*, 65% for *P. reniformis*, 70% (oil control) and 72% for *M. anisopliae* (Fig 7).

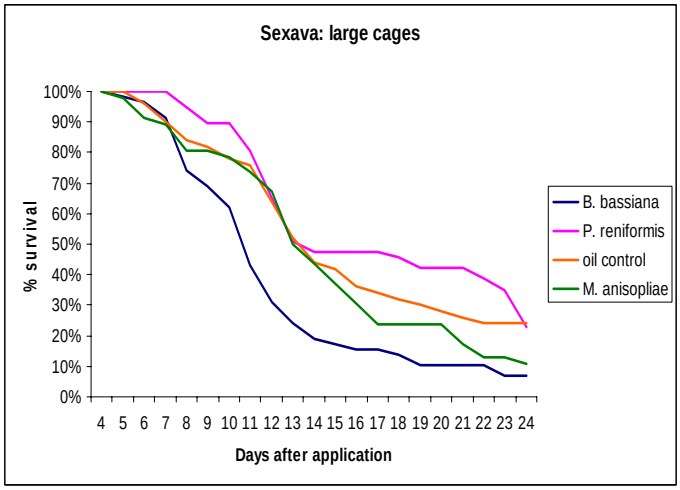

Figure 7: *S. decoratus* mortality over time using different fungi.

DISCUSSION

The original 49 isolates obtained in Caudwell's study, could not be traced, therefore isolates obtained from other Tettigoniidae were used.

From experience gained from work at the Lutte Biologique contre les Locustes et Sauteriaux (LUBILOSA) project in West Africa, the apparent complete absence of any biological activity with all 3 isolates was surprising (Bateman *et al*. 1996), although the environmental conditions in the Sahelian and West New Britain habitats are totally different. Although operationally very disappointing, from a biological perspective this is an interesting "negative result", and raises important questions about disease resistance by insects living in humid environments.

Experimental procedures reduced untreated control mortality to an acceptable level, while a protocol and infrastructure has been put in place for assessing fungal isolates in the future.

The important question of medium and long term control techniques for sexava remains to be addressed.

It would be appropriate to continue monitoring field infestations for local isolates - preferably epizootics of diseased sexava, which might have co-evolved.

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For further information please contact:

Head of Entomology
Papua New Guinea Oil Palm Research Association Inc.
P.O. Box 97, Kimbe, West New Britain Province, Papua New Guinea
Tel: +675 9854009 ▫ Fax: +675 9854040 ▫ Email: info@pngopra.org.pg