MICRO-ORGANISMS AS POTENTIAL BIOLOGICAL CONTROL AGENTS OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

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Abstract

The bacterium *Bacillus thuringiensis* produces a toxic crystal which is lethal to lepidopterous insects. Local *B. thuringiensis* isolates caused higher mortalities in the laboratory than did Thuricide (R), a commercially available *B. thuringiensis* preparation. *B. thuringiensis*, however, is of limited efficacy in the field because the crystals are degraded by sunlight. A genetic engineering project has therefore been started to transfer the gene coding for the toxic crystal from *B. thuringiensis* to another bacterium, a fluorescent pseudomonad, which has been selected for its ability to colonise sugarcane plants. Once the genetic transfer has been completed, the fluorescent pseudomonads should produce the toxic crystal on sugarcane plants, where it is likely to be ingested by foraging eldana larvae.

Other micro-organisms which are potential candidates for biological control are fungi and viruses. The fungus *Beauveria bassiana* has been isolated from eldana larvae at Mount Edgecombe. The fungal spores are susceptible to desiccation, so various methods of application are being investigated to try to overcome this shortcoming. Feral eldana larvae are being screened for a baculovirus and larvae are being stressed in the laboratory to see whether a latent viral infection can be activated.

Introduction

Eldana saccharina Walker (Lepidoptera: Pyralidae) larvae bore into the stem of sugarcane plants, damaging stalks and causing a decrease in sucrose content. The potential of mirco-organisms as biological control agents of eldana is being investigated. The three broad divisions and main areas of interest are bacteria, fungi and viruses.

Bacteria

Bacillus thuringiensis, when it sporulates, produces a toxic crystal which is lethal when ingested by lepidopterous insects. A commercially available preparation of *B. thuringiensis* var *kurstaki* spores and crystals, known as Thuricide (R), has been used against a number of insect pests, but offers limited control of eldana because it is not persistent in the field (Krieg[8]). Fluorescent pseudomonads are bacteria which are able to colonise plants without any deleterious effects. They have been selected as the recipient bacteria in a genetic engineering project in which the gene coding for the *B. thuringiensis* toxin will be transferred from *B. thuringiensis* to the fluorescent pseudomonad. When the genetically engineered fluorescent pseudomonad colonises sugarcane plants, it should produce the toxin and this can be expected to be ingested by foraging eldana larvae.

Isolations of both *B. thuringiensis* and fluorescent pseudomonads have been made locally and the most toxic *B. thuringiensis*, as determined by bioassays, and the best fluorescent pseudomonad, as determined by colonising ability, have been selected for the toxin transfer.

Fungi

The entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuil., has been isolated locally from feral eldana larvae. The development of a suitable formulation is essential to the successful use of mycoinsecticides. An attempt is being made to formulate *B. bassiana* so that the ineffective units (spores) maintain viability and infectivity during storage and application.

Viruses

The baculoviruses, which comprise the Nuclear Polyhedrosis Virus (NPV) and Granulosis Virus (GV), are potential biological control agents (Payne[11]), because they have a high degree of host specificity and produce relatively stable infectious forms which can persist for long periods. No virus has yet been observed in the eldana population in South Africa, so a system for screening larvae for a virus has been implemented. Healthy larvae are stressed in the laboratory using factors such as overcrowding, unsuitable diet (David, *et al*[3]), and extreme hot and cold temperatures (Longworth and Cunningham[9]), in the hope of activating a latent virus, which could perhaps be exploited as a biological control agent.

Materials and Methods

Bacteria

Eldana larvae infected with *B. thuringiensis* are characteristically black in colour and very soft. Cadavers displaying these symptoms were homogenised, and the homogenate subjected to a heat-shock treatment of 65°C for 10 minutes to try to eliminate contaminants. The homogenate was then plated out onto a selective medium, called PEMBA (Holbrook and Anderson[4]). *B. thuringiensis* was distinguished by its colony morphology on PEMBA and microscopically by the presence of spores and crystals. Cells of the bacterium were grown up on Lablemco agar/broth (Oxoid) and then incorporated into petri dishes containing synthetic eldana diet at rates of 10^4, 10^6 and 10^8 cells/g diet. Synthetic eldana diet was a modification of that developed by Atkinson[2]. Second instar larvae were allowed to feed on each concentration of *B. thuringiensis*-contaminated diet for 7 days. Mortality was then assessed and remaining larvae were transferred to uncontaminated diet, where mortality was assessed weekly or until larvae pupated (Kalfon and de Barjac[5]; Mohd-Salleh and Lewis[10]). Mortality caused by local *B. thuringiensis* isolates was compared with that of Thuricide (R) and B401 (*B. thuringiensis* var *aizawai*).

Fluorescent pseudomonad bacteria were isolated from leaves, stalks and eldana borings on sugarcane plants using a selective medium, King's medium B (KMB) (King, *et al*[6]). The bacteria were identified with an API NE20 kit (Path Ident, Johannesburg). Double antibiotic resistance to rifampicin and nalidixic acid was induced so that colonisation studies on sugarcane using antibiotic-resistant bacteria could

be carried out. Bacteria applied to plants could then be recovered on KMB containing the above antibiotics at various time intervals, and their numbers assessed.

Sugarcane setts were dipped into liquid bacterial suspensions containing 0,1% gum xanthan, or rolled in a dry formulation of bacterial inoculum, using 0,1% gum xanthan and talc, and then planted out in pots (Kloepper and Schroth[7]; Suslow and Schroth[14]).

Fungus

B. bassiana was isolated from infected feral eldana cadavers, which were covered with white mycelium. *B. bassiana* was maintained in culture on Sabauroud dextrose agar (Biolab) plus 1% yeast extract.

Reinfection of eldana larvae by *B. bassiana* in the laboratory is carried out by spraying larvae with a spore suspension. The larvae are then placed in a bottle containing crushed sugarcane, which serves as food for the larvae and provides an ideal climate for infection by *B. bassiana* spores.

Production of large numbers of spores for use in germination and pathogenicity testing and for future field trials, is carried out according to Rombach, *et al*[13].

Prior, *et al*[12] found that *B. bassiana* spores maintained viability for longer periods in coconut oil than in water. Spore viability and virulence of *B. bassiana* isolated locally are being monitored when stored in coconut oil, and once this has been determined, the feasibility of using coconut oil to apply *B. bassiana* spores in the field will be investigated.

Viruses

A programme to screen local eldana larvae for a virus was initiated. Symptoms of cadavers possibly infected with a virus were noted, larvae homogenised and the homogenate added to eldana diet, on which healthy eldana were allowed to feed (Whitlock pers. comm.). Dead larvae resulting from this treatment were viewed for viral particles under the Transmission Electron Microscope (TEM) (Phillips 301). Secondly, to test for the presence of a latent viral infection, larvae were stressed in the laboratory. They were subjected to overcrowding, to half the normal amounts of sucrose and casein in their diet (David *et al*[3]), and to extreme hot (37°C) and cold (4°C) temperatures. Mortalities were recorded and dead larvae viewed under the TEM for virus particles.

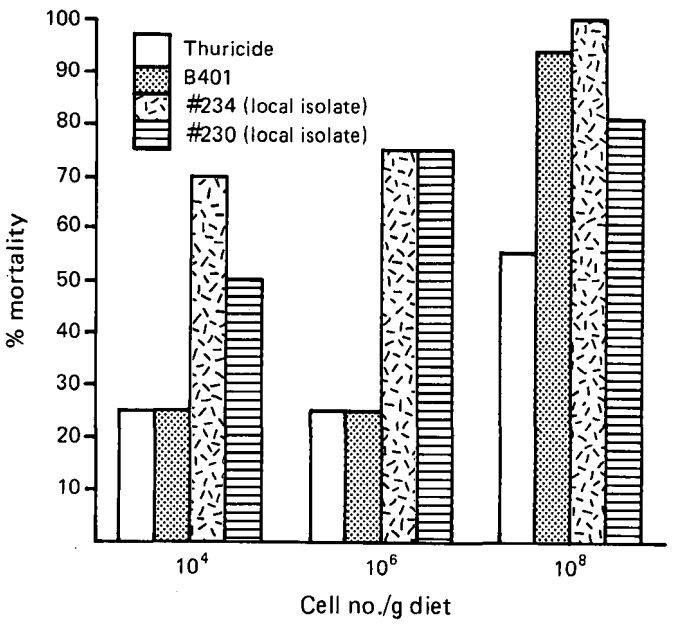
Larvae were prepared for viewing under the TEM in two ways. The first involved homogenising larvae and centrifuging (Beckman L5–50 ultracentrifuge) on a glycerol density gradient. Bands were removed from the gradient and stained with 2% phosphotungstic acid, before being viewed on carbon-coated G200 grids. The other method involved the use of tissue most likely to harbour NPV or GV replication, eg mid-gut and fat cells. The areas were dissected out of cadavers and fixed, embedded and sectioned before viewing (Adams, *et al*[1]).

Results

Bacteria

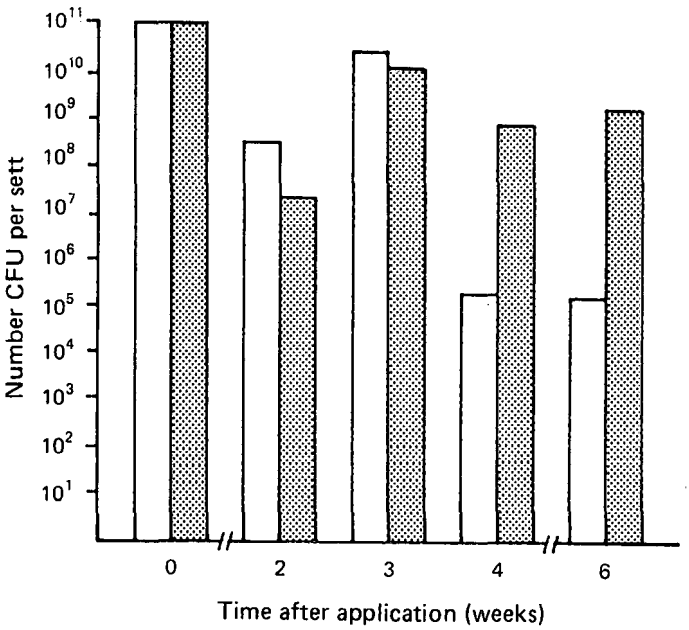
Some of the local *B. thuringiensis* isolates apparently are more toxic than Thuricide or B401 to eldana larvae (Figure 1). The results presented are the means of 3 replicates; but because the standard deviations are larger than 10% of the mean, they are not presented. Evidently some refinement of the bioassay technique is necessary.

Figure 1 Mortality of eldana larvae caused by *B. thuringiensis* isolates at different concentrations.



The fluorescent pseudomonads which were isolated from sugarcane were identified as either *Pseudomonas putida* or *P. fluorescens*. Figure 2 presents the numbers of colony forming units (cfu)/sett of two *P. fluorescens* isolates immediately after application in a liquid suspension, and 2, 3, 4 and 6 weeks after planting in pots. The numbers of cfu/sett declined after the initial application, but began to stabilise after a period of about 3–4 weeks. Bacteria prepared in the dry formulation maintained viability for 4 months when stored at room temperature, and sugarcane setts could be easily rolled in the powder before they were planted.

Figure 2 Changes with time in number of colony forming units per sett of two *P. fluorescens* isolates (□ 14, ⊠ 13), applied to setts in a liquid suspension, with 0,1% gum xanthan.



Virus

A small proportion of larvae which were allowed to feed on diet contaminated with cadaver homogenate died, and were viewed under the TEM. No virus-like particles were observed.

Larvae which were subjected to stress tests in the laboratory were found to be most adversely affected by overcrowding, and cadavers often displayed symptoms which indicated an infection by *B. thuringiensis*. This was confirmed by microscopic examination of the homogenate. No virus-like particles were observed in these cadavers.

Some larval mortality occurred when larvae were fed on diet containing half the normal amounts of sucrose and casein, and there seemed to be an increased amount of cannibalism, but again no virus was evident.

Exposure to a temperature of 37°C resulted in most of the larvae dying, but no agent responsible for the deaths could be identified. Larvae appeared to survive a short exposure to 4°C relatively well, and only a few deaths occurred as a result of such exposure.

Discussion

Bacteria

It is encouraging that some of the local *B. thuringiensis* isolates have proved to be more toxic than Thuricide or B401. The bioassay results suggest that there might be local strain differences in toxicity, because of the different mortalities when the same cell numbers were incorporated into diet, and perhaps this study emphasises the importance of the choice of *B. thuringiensis* strain for use against a specific insect. The variability that occurred in the bioassays suggests that some refinements need to be made to obtain more accurate results, eg using larvae from a more uniform culture, and presenting individual larvae with small amounts of *B. thuringiensis*-contaminated diet.

The colonisation studies with the fluorescent pseudomonads suggest that an initial decrease in cfu/sett occurs, but that numbers stabilise after approximately 3–4 weeks. The smaller numbers attained by *P. fluorescens* 14, does not imply that it is a poorer coloniser than *P. fluorescens* 13, but that the two organisms have different thresholds at which they colonise plants. At present, it is not known what the optimal numbers of *P. fluorescens* are on sugarcane plants, and this will probably depend on how much toxin the coloniser can produce and how much toxin is required to effectively reduce the eldana population.

Fungus

The natural spread of fungal pathogens is limited largely by climatic conditions. Oil-based formulations of fungal pathogens potentially could reduce dessiccation of spores and also aid in adhesion of the spores to the insect cuticle. Accordingly, the use of coconut oil (Prior *et al*[12]) for the formulation and application of *B. bassiana* to control eldana is being investigated.

Viruses

The methods used to screen the eldana population for a virus have not yet been successful. There are, however, other avenues that could be pursued. One is the exposure of eldana to foreign insect viruses, which might stimulate the activation of a latent virus.

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