SHORT COMMUNICATION

ENTOMOPATHOGENIC NEMATODES IN SUGARCANE IN SOUTH AFRICA

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Abstract

Entomopathogenic nematodes (EPNs) and their symbiotic bacteria have been the focus of research for many years due to the possibility of their use as biocontrol agents. In the South African sugarcane industry, their use against the main insect pest, the stalk borer *Eldana saccharina*, was documented in the 1980s. This study re-investigates the use of EPNs for their effect against *E. saccharina* and other pests of sugarcane, including thrips *(Fulmekiola serrata)* and white grubs (Coleoptera: Scarabaeidae). EPNs were isolated from soils under sugarcane cultivation from 10 locations in KwaZulu-Natal. These were subsequently cultured *in vivo* on *E. saccharina* larvae and then identified as being either *Heterorhabditis* or *Steinernema* using PCR-based molecular methods. Results showed of the 10 isolates, five were *Heterorhabditis* and five were *Steinernema* species. Two *Steinernema* isolates (EST3D and GING13G) were used to conduct pathogenicity tests and thereafter in pot and field trials. Results of the pathogenicity tests showed that 100% mortality was achieved with both isolates within 48 hours. The isolates were then used at a high rate (2000 IJs/m^2) and a low rate (1000 IJs/m^2) in a field trial to determine their efficacy against thrips. Results from the first sampling, three weeks after application, showed that isolate EST3D at the high rate resulted in significantly less thrips in sugarcane leaves. A pot trial was also conducted using white grubs identified as *Hypopholis* sp. Susceptibility of the white grub to the infective juveniles was seen when application involved the addition of an insecticide containing the active ingredient, imidacloprid.

Keywords: entomopathogenic nematodes, bio-control, sugarcane, pathogenicity

Introduction

Entomopathogenic nematodes (EPNs) and their symbiotic bacteria are present in a variety of soils and insect hosts throughout the world. Two genera in particular, *Heterorhabditis* and *Steinernema*, and their symbiotic bacteria *Photorhabdus* and *Xenorhabdus*, respectively, have been studied in great detail. This relationship entails the release of bacteria from the nematodes into the host once an insect has been infected. The bacteria cause death via septicaemia and aid in the breakdown of nutrients which help the nematodes feed and reproduce. The bacteria are then passed on to the next generation of nematodes. These nematodes have been documented in sugarcane research previously, specifically against the stalk borer, *Eldana saccharina* (Spaull, 1988, 1990). The results from those studies showed that EPNs are capable of decreasing *E. saccharina* numbers in sugarcane when applied correctly at an appropriate rate. However, the costs of production and application were considered exorbitant at that stage. This study re-investigates the effects of EPNs on *E.*

saccharina, as well as other important sugarcane pests which result in great losses in the sugar industry.

Materials and Methods

EPNs were isolated from soil collected from various sugarcane trial sites in KwaZulu-Natal. The EPNs were baited by placing 10 *E. saccharina* larvae into jars of the collected soil and those larvae which were found dead after three days were put onto white traps (White, 1929). The isolates obtained, known as infective juveniles (IJs), were maintained *in vivo* (Poinar, 1979) and stored at room temperature in 250 ml culture flasks. Identification of the isolates was carried out using molecular methods. DNA was extracted from a single adult by digesting the nematode at 25ºC in 20µl 0.25M NaOH overnight. The suspension was then heated to 99ºC for three minutes before the addition of 4µl 1M HCl, 5µl 1M Tris-HCl and 5µl 2% Triton X-100. This was then heated to 99ºC for three minutes and thereafter left overnight at minus 20ºC before use. The internal transcribed space (ITS) region was amplified using the universal 18s forward and 5.8s reverse primers. Genus specific identification was conducted using the 18s forward primer and specifically designed genera-specific reverse primers (denominated 'Steiner R' and 'Hetero R'). Amplified fragments were then purified and sequenced using the BigDye sequence terminator kit, v3.1 (Applied Biosystems). Pathogenicity tests were conducted by inoculating Eldana larvae with EPNs at rates of 250, 500, 1000, 2000 and 4000 IJs/ml. The rates were replicated six times and the results were then used to calculate LT_{50} and LD_{50} values. The two isolates found to be most pathogenic were used in a field trial against thrips and in a pot trial against white grub.

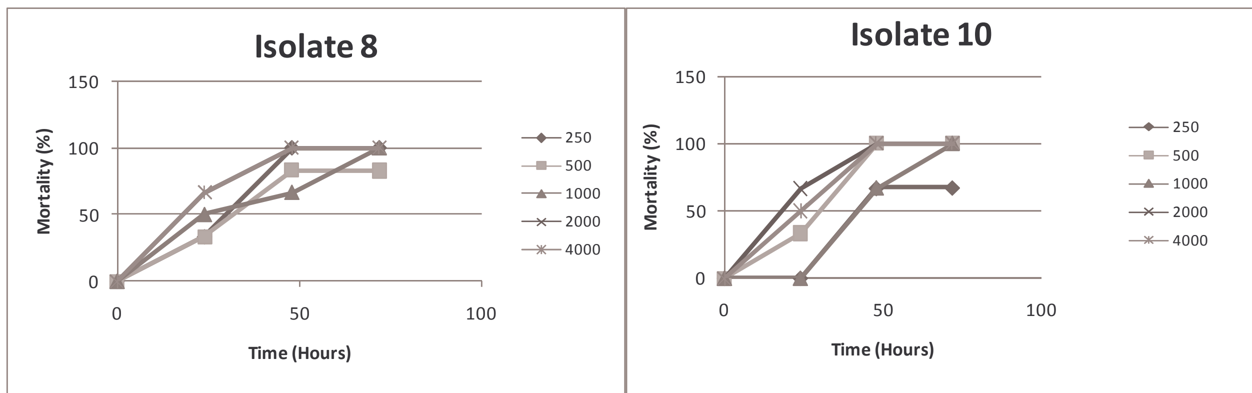
The field trial contained six treatments, including a low (1000 IJs/ml) and high (2000 IJs/ml) rate of each isolate, a control (water) and a chemical treatment (Bandit®). These treatments were replicated six times, in two sugarcane fields, resulting in two field trials consisting of 36 plots each. Each plot was composed of 3 lines, 8 m long. The treatments were applied onto the foliage of the sugarcane plants in 20 L of water using watering cans. The fields were sampled weekly by cutting five spindles per line from each plot. The spindles were cut and rinsed in a container containing water to remove any thrips present on the leaves. The leaves were discarded and the water poured through a fine mesh in order to remove the liquid whilst retaining any insects which were present. The thrips present were counted using a dissecting microscope and the results subjected to statistical analysis.

The white grub pot trial consisted of eight treatments, which included the six treatments used in the previous trial, as well as the high rate of each isolate with imidacloprid (insecticide) included in the mixture. The treatments were replicated six times resulting in 48 pots in a random arrangement. The white grubs were placed into pots of sterile soil and treatments were applied in 50 ml of water. After six days, the dead and living white grubs were collected from the soil and the former placed onto white traps and monitored for nematode emergence.

Results and Discussion

Of the 20 sites sampled, 10 isolates were obtained and further cultured in the laboratory. These EPN cultures were refreshed monthly to prevent contamination and to maintain viability and pathogenicity. PCR using universal primers resulted in amplification for all 10 isolates and the amplified bands were of the expected size (~550 bp). Further molecular identification with genera-specific primers showed that of the 10 isolates, five were *Heterorhabditis* and five were *Steinernema.* Sequencing of the isolates has shown them to be

different species within these two genera. The pathogenicity tests of all of the isolates revealed that isolate 8 (EST3D) and isolate 10 (GING13G) were the most pathogenic, resulting in 100% mortality within 48 hours (Figure 1a,b).



Figures 1a,b. Results from pathogenicity tests showing the percentage mortality for two isolates at rates ranging from 250 to 4000 IJs/ml.

A rate of 2000 IJs was chosen for application during trials due to the LT_{50} and LD_{50} calculated from the results depicted above. Isolate 8 and 10 both showed a LT_{50} of 24 hours and LD_{50} of 1621 IJs and 3299 IJs, respectively.

Three weeks after application, thrips adult and nymph numbers were significantly reduced using isolate 8 at the high rate (8H) compared to the control (Figure 2). However, this trend was not that obvious in samplings at other time intervals. This could be due to the cryptic nature of the thrips (hiding in the leaf whorls) and/or the need for multiple applications of EPNs for longer control. Future trials may therefore benefit from repeat applications of EPNs.

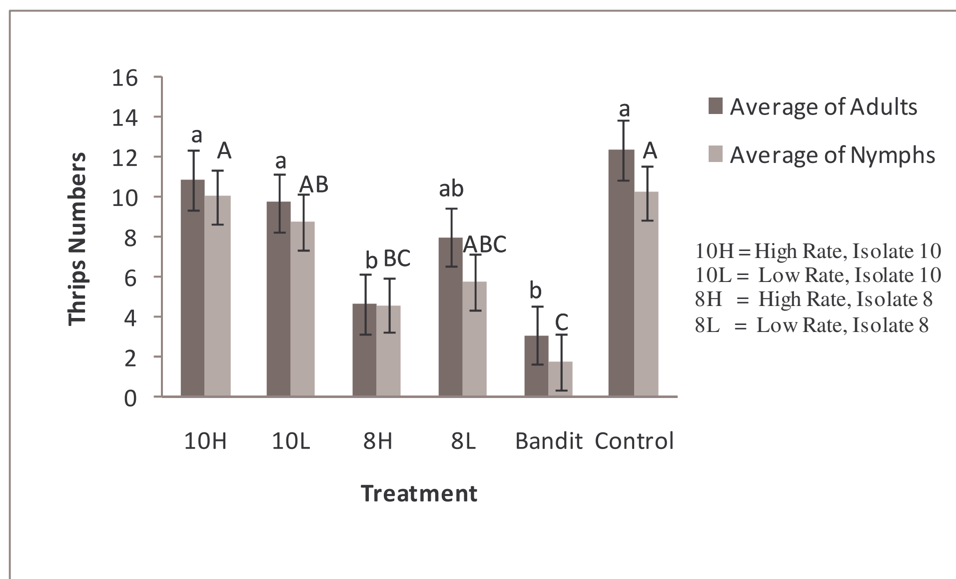


Figure 2. Thrips numbers under differing treatments three weeks after inoculation. Bars indicate standard error of the means and letters indicate significant differences.

Results from the white grub pot trial were largely inconclusive; however, EPNs were recovered from a few white grub samples, specifically, in the treatments which included the

insecticidal active ingredient, imidacloprid. It is known that white grubs have adopted a variety of behavioural traits which help them avoid infection from EPNs. These include grooming, rolling up into a ball and movement away from nematodes. Imidacloprid presumably helps to immobilise the white grub thereby enabling infection by EPNs (Koppenhofer *et al.*, 2000). These effects were observed in the pot trial, although not consistently throughout the replicates. Further trials will be conducted, varying the rates of EPN application in order to determine the full effects of this relationship.

During this study, various isolates of EPNs have been obtained from sugarcane soils from various parts of the industry. Of these 10 isolates, two have been found to be highly pathogenic and show promise for further work. Further research will now entail refining application methods and timing of application and testing these agents in field trials against eldana in stubble of ratoon cane and white grubs in the soil. In addition, work will continue on the isolation of the symbiotic bacteria and investigating these bacteria in the absence of their nematode host.

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