POTENTIAL OF BIOCONTROL OF SIX ENTOMOPATHOGENIC NEMATODES SPECIES AGAINST *Cyrtomenus bergi* IN THE LABORATORY

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INTRODUCTION

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* together with their symbiotic bacteria, *Xenorhabdus* and *Photorhabdus* respectively (Fig. 1A), represent a unique insect biological control "system". Laboratory and field studies have shown that insects from over 17 orders and 135 families are susceptible to some degree to them (Akhurst & Smith, 2002).

Figure 1 A. *Heterorhabditis sp* infective juvenile

Figure 1 B: *C. bergi* adult infected with nematodes

C. bergi Froeschner (Hem: Cydnidae) is a polyphagous pest and has been reported on cassava, maize, peanuts, potatoes, sorghum, onions, African oil palm, coffee, sugarcane, beans, peas, coriander, asparagus, pasture and weeds. Since its first description as a pest on cassava in Colombia in 1980, it has been considered a serious problem throughout the neotropics. The potential of entomopathogenic nematodes to control pests has been evaluated under laboratory and greenhouse conditions with commercially available and native nematodes (Belotti, 2002).

MATERIALS AND METHODS

Nematodes and *C. bergi* insects

The nematodes used in the present work are listed in Table 1. All nematodes were cultured in the final instar larvae of the greater wax moth, *Galleria mellonella* L. at 23°C according Kaya & Stock's (1997) methodology. Infective juveniles were stored in 0.01% formaldehyde solution at 10°C for 5-7 days and one day before use they were acclimatised to room temperature for at least 24 h before inoculation.

C. bergi fifth and adult stages were selected from the colony that has been established in the entomology laboratory of the cassava program at CIAT, Cali-Colombia.

Table 1. Entomopathogenic nematodes species and origin

Species	Origin
***Steinernema riobrave* (Sr)**	**United States**
***Heterorhabditis bacteriophora* (Hb)**	**United Kingdom**
***Steinernema* sp SNI-0100 (SNI)**	**Colombia**
***Heterorhabditis* sp HNI-0198 (HNI)**	**Colombia**
***Steinernema feltiae* strain Villapinzon (Sf)**	**Colombia**
***Heterorhabditis* sp- CIAT 2003(HCIAT)**	**Colombia**

Assays: Fifth and adult stages of *C. bergi* were exposed to 5000 infective juveniles per millilitre of each nematode species in plastic cups containing 10 grams of sand (4% w/w) with one insect and one germinated corn seed (Caicedo & Bellotti, 1994). The experiment was replicated five times in randomised complete blocks with twelve replications. The control groups were exposed to one millilitre of distilled water. Parasitism and mortality were recorded after 10 days and all insects were dissected under a stereoscope microscope.

In a second test, three species of nematodes were applied in lots of 2000, 4000, 6000, 8000 and 10,000 nematodes per millilitre against the adult stage of *C. bergi*. The experiment was replicated four times in randomised complete blocks. The evaluation period and method were the same as described previously.

Statistical analysis

The data were statistically analysed by ANOVA (GLM) for mean separation by the Duncan test and Probit analyses respectively.

RESULTS AND DISCUSION

The results obtained in this evaluation showed that both *C. bergi* stages were parasitised by all entomopathogenic nematode species. *Steinernema* sp-SNI 0100 was the species that showed the highest parasitism in the fifth and adult stage of *C. bergi* with 77 and 100% parasitism respectively and the lowest percentage was showed by *Heterorhabditis* sp HNI with 28 and 49% parasitism in the fifth and adult stages respectively (Figure 2) after 10 days of inoculation.

Although higher percentage parasitism was shown in the fifth stage, no correlation was observed with mortality, only 22% mortality compared with 77% parasitism. The lowest mortality was observed with *Heterorhabditis* sp HNI with only 4% (Figure 3).

This low mortality observed in both *C. bergi* stages with all the entomopathogenic nematodes, could be because *C. bergi* shows a very strong immune response to all the entomopathogenic nematodes species evaluated.

Koppenhöfer et al. (2003) mentioned that different species/strains of nematode differ in their efficacy significantly in controlling the same insect pest. This can be influenced by the penetration rate of the infective juveniles into the host, the release time of the bacteria, and the virulence degree of killing the insect.

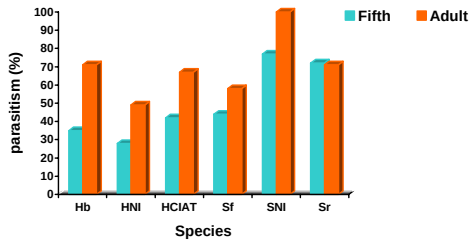

Figure 2. Parasitism of two *C. bergi* stages with six nematodes species

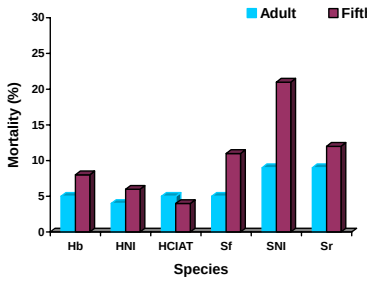

Figure 3. Mortality of two *C. bergi* stages with six nematodes species

When *C. bergi* adults were exposed to different dosages of three nematode species, no significant differences were observed between the lowest and the highest dosages. The results obtained were similar to the above-mentioned, all three nematode species causing parasitism of 65-100% in the adult stage but only able to cause low mortality of 3-40%.

This confirms the results obtained by Caicedo & Bellotti (1994) with *Steinernema carpocapsae* in all the *C. bergi* stages, the adult was the most susceptible to being parasitised with 60%, but very low mortality, 3% after 10 days of inoculation and the youngest instars were least susceptible with 3-17% parasitism. These results are also comparable with those obtained by Barberena & Bellotti (1998).

At this point we can only speculate about the factors responsible for the interactions among nematode species and *C. bergi* stages. It could be that *C. bergi* has coevolved with entomopathogenic nematodes and other pathogens in the soil but at this time we do not know whether the defence mechanisms reported in other species, such as white grubs which present infrequent CO_2 output, sieve plates covering the spiracles, frequent defecation, other defensive and evasive behaviours and strong immune a response, operate here.

For this reason we consider that is very important to understand the innate immune response of *C. bergi* and to determine if a correlation exists in the insect species between levels of cellular and humoral response of the different species/strains used as a challenge for the ultimate choice of effective species/strains for its control (CIAT, 2003).

CONCLUSIONS

All the entomopathogenic species parasitised both *C. bergi* stages, fifth and adult.

Steinernema sp SNI 0100 showed the highest parasitism in both stages, fifth and adult, 77 and 100% respectively, but this high parasitism was not correlated with the mortality, only 9 and 21% mortality respectively was observed.

It is a priority to initiate basic studies into understanding the innate immune response of *C. bergi* and to determine if a correlation exists in the insect species between levels of cellular and humoral response of the different species/strains used as a challenge for the ultimate choice of effective species/strains for its control.

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