SOIL PESTS – CASSAVA AND OTHER CROPS

Introduction

Whitegrubs of the family Melolonthidae and the burrower bug (*Cyrtomenus bergi*) (BB) belong to the most important soil pests in South America. Until about 20 years they were not considered as pests, however, in the recent past they have caused considerable yield losses on many crops that show the necessity of developing efficient and environmentally sound strategies of an integrated control of these insects.

This project is divided in two phases: the diagnostic and strategic research. The first phase is almost coming to its end and has generated important results: we have identified the key pest species in the Savanna of Bogotá, East and North Antioquia, Northern Cauca, Risaralda, and Quindío and we understand their seasonality in Northern Cauca, Risaralda, and Cundinamarca. We also have identified many natural enemies of the most noxious whitegrub species and the BB. Moreover, we developed a communication network on Internet base to facilitate the information exchange of researchers on specialists of the soil pest community (CIAT 2003). In the project's second phase or strategic research these findings will be verified and applied in on farm experiments.

The objectives of the present study were

I. Diagnostic of pest species and understand the life cycle of key pest species in Antioquia and Quindío
II. To identify biological control agents of white grubs and burrower bug
III. To develop biological control tactics against white grubs
IV. To develop biological control tactics against *C. bergi*

References

CIAT. 2003. *Annual Report*. Soil Pests – Cassava and other crops. Pp 53-70.

Activity 1. Identification of key pest species in three regions of Antioquia (Colombia).

Introduction

Key pest species have been collected and identified in the survey's areas of Cauca, Quindío, Risaralda, and Cundinamarca (CIAT, 2003), and presently field experiments are being carried out in such areas. The data of these surveys will be presented by the end of this year.

In Antioquia, the quantity of collected individuals is enormous (CIAT, 2003). Hence, we continued with systematic surveys including the seasonality of the beetles and larvae. The activities in Antioquia are conducted with the collaboration of Corpoica Rionegro ("La Selva"); ICA's program "Epidemiology in Agriculture" in Medellin; the National University Medellin (unit of Postgraduate Studies in Entomology), and the Umata of the municipalities Rionegro, San Vicente, El Carmen de Viboral, and La Union.

Methodology: In Antioquia, we surveyed three zones: The two cold savannas in the North and the East (both 2600-2800 m. a. l. s.), and the moderate cold zone in the East (2100-2400 m.a.l.s). Beetles were collected in a weekly basis from December 2002 to December 2003) and monthly in the case of whitegrubs (in the North where *Ancognatha* dominates we collected all over the year and in the other zones where *Phyllophaga* spp. dominate from August to November) and their natural enemies. We focused our surveys on pasture and potato fields. We installed six light traps in Rionegro (2110 m. a. s. l.), El Carmen de Viboral (2258 m. a. l. s.), San Vicente (2300 m. a. s. l., La Union (2460 m. a. s. l.), Santa Rosa (2486 m. a. s. l.), and Entrerrios (2437 m. a. s. l.). Extensionists of the Umata and in some cases farmers collected and prepared the insects for storage. The collected material was transferred to the laboratory of Corpoica for identification (CIAT, 2003). We have revised approximately 190.000 beetles until December 2003.

Results and Discussion: The collections of adults in the three zones revealed some major peaks during the months of March and June. In the North of Antioquia we captured the greatest number of individuals. Here, the subfamily Dynastinae dominated with 92,589 specimens, followed by Melolonthinae with 669 and a minimal presence of Rutelinae. The collected genera were (order from highest to lowest abundance): *Ancognatha, Cyclocephala, Heterogomphus* (all Dynastinae)*, Astaena* (Melolonthidae)*, Golofa* (Dynastinae)*, Megaceras, Anomala* (both Rutelinae) and *Plectris* (Melolonthinae). We collected 61,103 beetles of *Ancognatha* in Santa Rosa and 12,725 in Entrerrios, followed by *Cyclocephala* with 7,731 and 10,473 individuals, respectively. Among the phytophagous genera *Astaena* dominated with 276 and 417 captures specimens, respectively.

In La Union, municipality in the cold zone of Eastern Antioquia, we captured during February and November 27,366 beetles of Dynastinae, followed by Melolonthinae (49) and Rutelinae (2). The captured genera were *Ancognatha, Astaena, Golofa, Cyclocephala, Heterogomphus, Plectris, Anomala,* and *Isonychus*. The dominance of *Ancognatha scarabaeoides* (27,187 specimens) was again overwhelming, followed by *Ancognatha vulgaris* (139) and *Astaena* (45).

In the moderate cold zone of Eastern Antioquia (Rionegro, San Vicente, and El Carmen de Viboral) 65% of the captured adults were Dynastinae, 33% Melolonthinae, and 2% Rutelinae. The captured genera were *Ancognatha, Cyclocephala, Phyllophaga, Isonychus, Astaena,*

Plectris, Anomala, Golofa, Heterogomphus, and *Macrodactylus*. The presence of the phytophagous genera was surprisingly high in this zone.

It was striking that a small difference of about 150 meters of altitude rigidly changed the species complex. In the lower zone of Rionegro (2100 m. a. s. l.) Melolonthidae (*Phyllophaga, Plectris, Astaena*) was the dominant subfamily, whereas El Carmen de Viboral (2258 m. a. s. l.) Dynastinae (*Ancognatha* and *Cyclocephala*) was the most present group.

Comparing the peaks of seasonality of the beetles with precipitation it was surprising that the population of *A. scarabaeoides* was high during the whole year without pronounced peaks. In contrast, *Cyclocephala sexpunctata* and *Astaena* performed their only peak in April.

In the cold zone of East Antioquia *A. scarabaeoides* performed a similar seasonality as in North Antioquia. In opposite, *A. vulgaris* showed two peaks in April and November, however, population was extremely low compared to *A. scarabaeoides*. *Astaena* has as in the north a pronounced peak in April.

Peaks of flight activity were recorded for *Phyllophaga obsoleta*, *Plectris* sp., and *Cyclocephala sexpunctata* in April in the moderate cold zone, whereas *Astaena* sp. presented a peak in May and *A. scarabaeoides* in June. We captured the greatest number of adults of *Isonycus* sp. in March, a second peak followed in September.

The studies on the taxonomy of larvae are based on three big surveys where they have been individualized for rearing and characterization of their morphology. Additionally, we have successfully recollected some adults in order to obtain a progeny for later description and drawings.

References

CIAT. Annual Report 2003. Integrated pest and disease management in major agroecosystems. Soil pests – Cassava and other crops. P. 53-70.

Contributors: Martha Londoño, Diana Acevedo, Luis Carlos Pardo, Andreas Gaigl.

Activity 2. Development time studies on key pest species under controlled conditions.

Introduction

Although the whitegrubs *Phyllophaga* sp., *P. menetriesi* (subfamily Melolontinae), and *Anomala inconstans* (subfamily Rutelinae) belong to the economically most important pest species (particularly *Phyllophaga menetriesi*) little is known about their biology and behavior. In most of the cases farmers only react when damage has already occurred. The control of the larvae is difficult and expensive. It is necessary to understand the biology of these whitegrubs to be able to develop a successful integrated control.

Methodology: We selected three species of whitegrubs (Melolonthidae) for these studies: *Phyllophaga* sp., *P. menetriesi*, and *Anomala inconstans*. All of them are considered as important soil pests (Morón 1994). Adults were captured by black light traps installed in Pescador (1500 m. a. l. s. Cauca) and Calucé (1637 m. a. s. l., Valle del Cauca). We filled the containers of the traps with sawdust in order to maintain captured adults alive. The captured specimens were transferred to CIAT. The specimens were stored at a temperature of 21 °C and a relative humidity of 70%. Seven pairs of adults were confined in plastic buckets equipped with a transparent plastic cylinder of 40 cm height to allow the beetles to fly. The bucket was filled with a mixture of sterile soil and sand (3:1 soil:sand). Larvae were fed with carrot slices. Eggs were removed once a week. After hatching larvae were individualized. We recorded the day of moulting, and measured the size of the cephalic capsule, diameter and length of larvae during their development. In order to define the duration of each instar we measured the width of the cephalic capsule. A significant increase of the width was the indicator that the larvae have moulted to the next instar. According to Pardo[1] and Quintero[2] (2004) (personal communications) larvae have moulted to the next instar when the cephalic capsule has doubled its size. However, we observed a maximal growing rate of 37.6% from the first to the second instar, and 36% from the second to the third. Hence, when these growing rates were reached we considered moulting as realized.

Results and Discussion: The life table data of the three species evaluated during this study are presented in **Table 1**.

Table 1. Development time of *Phyllophaga* sp., *P. menetriesi,* and *Anomala inconstans.*

Species	N	Mean Development of L1	Max L1	Min L1	Mean Development of L2	Max L2	Min L2	Mean Development of L3	Max L3	Min L3	Pupae	Duration Total
Phyllophaga menetriesi	12	19	19	19	28	15	32	175	201	82	37	259
Phyllophaga sp.	59	24	30	16	29	36	21	171	194	138	30	254
Anomala inconstans	53	27	30	24	29	38	21	163	173	140	30	249

* Development time in days; L1, L2, L3 indicate the instar of the larvae.

[1] Former Research Assistant at CIAT
[2] Former undergraduate student at CIAT

Development time: The development time from egg to adult was similar for all three species (250 – 259 days); however, both *Phyllophaga* species passed a considerably longer period as third instar than *A. inconstans*.

Our results indicate a shorter development time of *P. menetriesi* than previous studies report. This might be due to the fact that they did not carry the experiments under controlled temperature and humidity (Vallejo 1996) or they used elevated temperatures (Hidalgo 1993; Aragon & Pérez 1999). Posterior field samples corroborated these explanation: we found several specimen of *P. menetriesi* in more advanced stages (prepupae or pupae) than expected according to the season while the specimen in the lab still continued as larvae without showing any signs to soon instar change.

Body size: Both *Phyllophaga* species had a wider cephalic capsule than *Anomala inconstans* **(Table 2)**. *P. menetriesi* presented the greatest width and length of the three species. Completing the first instar this species reached a length of 1.51 cm, similar to that of *Phyllophaga* sp. *A. inconstans* was considerably smaller (1.12 cm). The larvae of *P. menetriesi* were also the longest of the three species. Completing the second instar the larvae of *P. menetriesi* reached a width of 0.39 cm, whereas *Phyllophaga* sp. and *A. inconstans* did not pass 0.27 and 0.30 cm, respectively. The body width was the only of the three variables where we could observe significant differences between the species.

Table 2. Body sizes of *P. menetriesi, Phyllophaga* sp., and *A. inconstans* measured at the beginning of the first and the end of the second instar.

Species	Cephalic capsule		Body width		Body length	
	1st initial	2nd end	1st initial	2nd end	1st initial	2nd end
Phyllophaga menetriesi	0.22	0.39	0.22	0.39	1,45	2.24
Phyllophaga sp.	0.18	0.27	0.18	0.27	1.32	1.82
Anomala inconstans	0.18	0.30	0.18	0.30	1.10	1.77

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Contributors: Germán Andrés Calberto, Luis Carlos Pardo, Oscar Yela, Andreas Gaigl.

Activity 3. Search for natural enemies of in Northern and Eastern Antioquia.

Methodology: The physiographical zones where we carried out the surveys and the collaborators were the same as in Activity 1.

Results

- In the moderate cold North (2600 m. a. s. l.) we realized 13 surveys in the municipalities Santa Rosa de Osos, Entrerrios and San Pedro, where we collected 1,150 specimens.
- In the cold North (2600 m. a. s. l.) we realized 10 surveys in the municipality of La Union and collected 862 specimens.
- In the East (2100 m. a. s. l.) we collected 718 individuals in 12 surveys. The municipalities were Rionegro, El Carmen de Viboral, Guarne, and San Vicente.

Most of the collected specimens were larvae, some adults and pupae. These samplings corroborated the surveys described in Activity 1 and are not mentioned here. **Table 1** lists the collected larvae:

Table 1. Genera of collected whitegrub larvae in three ecological zones in Antioquia (Colombia) during 2003.

Region	Municipality	Subfamily	Genera
Savanna North (2600 – 2800)		Dynastinae	*Ancognatha* *Cyclocephala* *Heterogomphus*
		Rutelinae	*Anomala*
		Not determined	sp.
		Melolonthinae	*Phyllophaga*
Cold East (2600 – 2800)	La Union	Dynastinae	*Ancognatha* *Cyclocephala* *Heterogomphus*
		Rutelinae	*Anomala*
		Melolonthinae	*Astaena* *Phyllophaga* *Plectris*
		Not determined	sp.
Savanna East (2100 m. a. s. l.)	El Carmen de Viboral	Dynastinae	*Ancognatha* *Cyclocephala*
		Melolonthinae	*Plectris* *Phyllophaga*
	Rionegro	Melolonthinae	*Phyllophaga* *Plectris*
		Not identified	*Astaena* sp.
		Dynastinae	*Cyclocephala* *Ancognatha* *Heterogomphus*
	San Vicente	Rutelinae	*Anomala*
		Rutelinae	*Anomala*
		Dynastinae	*Ancognatha*

Region	Municipality	Subfamily	Genera
	Guarne	Not identified	sp.
		Melolonthinae	*Plectris*
		Rutelinae	*Anomala*
		Melolonthinae	*Astaena* *Phyllophaga*
		Dynastinae	*Ancognatha* *Cyclocephala*
		Not identified	sp.

Nearly all of 2730 collected larvae died. 56% of them showed signs and symptoms of infections by entomopathogens. These parasitized larvae were transferred to the laboratory in order to isolate and identify the pathogens. **Table 2** shows the mortality of the collected larvae.

Table 2. Mortality of collected whitegrubs in three zones in Antioquia.

Zone		No. of Living Grubs	No. of Dead Grubs	%
North	2600-2800 m. a. s. l.	5	1145	99
East	2600-2800 m. a. s. l.	0	862	100
East	2100-2400 m. a. s. l.	0	718	100

The reasons of the extremely high mortality were mainly bacteria and fungi **(Table 3**).

Table 3. List of isolated entomopathogens from whitegrubs collected in all surveys in Antioquia (Colombia).

	Genus	**Mortality reason**	**%**	**Principal Pathogens**
Savanna North (2600 – 2800 m. a. s. l.)	*Ancognatha*	Bacteria	56	*B. popilliae* *Clostridium sp.* *B. sphaericus*
		Others	34	
		Fungi	6	Ma – 1 Ma – 2 Ma – 3
		Nematodes	1	Small Mermitidae
		Parasitoids	1	Ectoparasites
East (2600 – 2800 m. a. s. l.)				
La Union	*Ancognatha*	Fungi	56	Ma – 1 Ma – 2 *Paecilomyces sp.*
		Bacteria	30	*B. popilliae* *Clostridium sp.* *B. cereus*
		Others	14	
Savanna East (2100 – 2400 m. a. s. l.)				
El Carmen de Viboral	*Ancognatha*	Fungi	42	Ma – 1 Ma – 2
		Others	25	
		Bacteria	17	*B. popilliae* *B. larvae* *B. shaericus*
		Nematodes	14	Small Mermitidae
		Parasitoids	2	Endoparasites

	Genus	Mortality reason	%	Principal Pathogens
Savanna North (2600 – 2800 m. a. s. l.)	*Ancognatha*	Bacteria	56	*B. popilliae* *Clostridium sp.* *B. sphaericus*
		Others	34	
		Fungi	6	Ma – 1 Ma – 2 Ma – 3
		Nematodes	1	Small Mermitidae
		Parasitoids	1	Ectoparasites
Rionegro	*Phyllophaga*	Others	52	
		Bacteria	17	*B. popilliae* *B. larvae* *B. sphaericus*
		Fungi	8	Ma – 1 Ma – 2 Ma – 3
		Parasitoids	3	Ectoparasites
		Protozoa	3	
San Vicente	*Anomala*	Bacteria	39	*B. popilliae* *Clostridium sp.* *B. cereus*
		Others	40	
		Fungi	7	Ma – 3
		Nematodes	7	Mermitidae
		Parasitoids	7	Ectoparasites Endoparasites
Guame	*Anomala and Astaena*	Others	42	
		Bacteria	36	*B. popilliae* *Clostridium sp.* *B. cereus*
		Nematodes	11	small Mermitidae
		Fungi	6	Ma – 3
		Parasitoids	5	Ectoparasites Endoparasites

Since August 2004 we conducted weekly surveys on potato farms in Northern Antioquia in order to identify the pest species among the whitegrub complex.

Conclusions

- The dominating whitegrub species differ in every region.
- The well-defined peaks of flight activity facilitate the development of control strategies.
- The surveyed areas harbor a great diversity of microorganisms that can be a promising tool for the biological control of whitegrubs when following parameters are defined: prevailing whitegrub genus, climate and soil type.

Contributors: Martha Londoño, Elizabeth Meneses (Corpoica Rionegro), and Andreas Gaigl.

Activity 4. Search for natural enemies of Whitegrubs in the Colombian departments of Cauca, Quindío, Risaralda, and Cundinamarca.

Entomopathogenic fungi

CIAT maintains since four years a collection of entomopathogenic fungi and bacteria stored in a ceparium. At this moment our ceparium harbors 411 strains of entomopathogenic fungi (EPF) isolated from spittlebug, whitefly, burrower bug and whitegrubs. In the first two years of this project we have isolated 213 strains of entomopathogenic fungi (EPF) only from whitegrubs and burrower bugs. Of those we have identified 118 strains, belonging to nine genera and 12 species.

On occasion we have isolated two entomopathogenic species from one insect species, without knowing, which one caused mortality and which one is saprophytic. Most EPF are saprophytes and act as facultative entomopathogens.

The ceparium includes of 4 *Aspergillus* sp., 5 *B. bassiana*, 2 *Beauveria* sp., 45 *Fusarium* sp., 13 *Gliocladium* spp., 18 *M. anisopliae*, 6 *Metarhizium* sp., 1 *Mucor* sp., 2 *Paecilomyces fumosoreus*, 13 *Paecilomyces* sp., 5 *Penicillium* sp., and 2 *Trichoderma* sp.

We isolated 126 EPF strains from six (identified) whitegrub species: *P. menetriesi* (55 isolations), *Phyllophaga* sp. (22), *Galleria mellonella* (10), *Cyrtomenus bergi* (35), *Anomala* sp. (1), *A. inconstans* and unidentified whitegrubs (82).

The 213 isolates are from 11 different origins **(Table 1)**. The most frequent isolated fungus was *Fusarium* sp., followed by *Metarhizium* spp. Cauca, Risaralda and Quindío dominate the frequency because these departments were place of our surveys.

Table 1. Origin of CIAT ceparium strains.

Genera	Department							TOTAL
	Caldas	Cauca	C/marca[1]	Quindío	Risaralda	Valle	Others[2]	
Aspergillus	0	0	0	0	3	1	0	4
Beauveria	0	2	0	0	5	0	0	7
Fusarium	0	29	0	7	9	0	0	45
Gliocladium	0	9	0	0	4	0	0	13
Metarhizium	1	10	1	1	12	1	1	27
Mucor	0	0	0	0	1	0	0	1
Paecilomyces	0	10	0	2	1	0	0	13
Penicillium	0	3	0	1	1	0	0	5
Trichoderma	0	2	0	0	0	0	0	2
Unidentified[3]	0	51	0	11	27	3	4	96
TOTAL	1	116	1	22	63	5	5	213

[1]Cundinamarca, [2]One strain from *C. bergi* colony at Hanover University, [3]unidentified.

Entomopathogenic bacteria

We isolated 89 strains of entomopathogenic bacteria (EPB) from whitegrubs collected in the three departments Cauca, Risaralda, and Cundinamarca **(Table 2)**. *Bacillus popilliae* and *B.*

lentimorbus represent the major part of this collection. Other species are *B. larvae, B. sphericus* and two strains of *Serratia* sp.

Table 2. Host and origin of entomopathogenic bacteria isolated from whitegrubs collected in the Colombian departments of Valle, Risaralda, and Cundinamarca.

Genera	Department			Total
	Valle	Risaralda	Cundinamarca	
Clavipalpus spp.	0	0	8	8
Ancognatha spp.	0	0	7	7
H. dilaticollis	0	0	2	2
Anomala	4	1	0	50
P. menetriesi	60	7	0	67
Total	64	8	17	89

Contributors: Sonia Ximena Restrepo, Anuar Morales, Rosalba Tobón, Oscar Yela, Andreas Gaigl.

Activity 5. Search for entomopathogenic nematodes in Colombia and Panama: First description of *Steinernema kraussei* as native entomopathogenic nematode in Colombia.

Introduction

The entomopathogenic nematodes (EPN) of the family Heterorhabditidae and Steinernematidae are used as control agents of a wide range of soil pests. The search of native organisms is realized all over the world, where they have been found in a wide range of soil and crops (Rueda *et al*. 1993). The objective of this study was to identify native EPN species associated with pasture, cassava, onion, groundnut, and other crops in the survey zones.

Methodology: We surveyed different regions in Colombia (Quindío, Risaralda, Caldas, and Cauca) and Panama **(Table 1**. Ten random samples per hectare were taken in a profundity of 15 to 20 cm.) From each hole we took one liter of soil to the lab for further processing. The shovels were disinfected with alcohol before digging a new hole. Once in the lab the samples were stored at 15 °C until processing. We sent 500 g of each sample to the CIAT soil analysis unit to determine pH, humidity, soil organic matter and texture.

300 g of the sample was placed in a plastic cup where 10 larvae of *Galleria mellonella* were confined as baits. This procedure was repeated three times. After five days the infested moth larvae were transferred to 'White traps' (Kaya & Stock 1997) where the EPN abandoned the dead insect and migrated over filter paper to water. In order to test the virulence of the isolated EPN these were transferred into Petri dishes filled with sand harboring *Galleria* larvae (Koch's postulates). The newly extracted EPN were stored in sterile and distilled water. We fixed about 5% of the samples in TAF (Formaldehyde + Triethanoamine + sterile distilled water), the rest were stored alive in sterile distilled water.

The isolated EPN were sent alive for identification to the Department of Biotechnology (DBT), Kiel University, Germany. Molecular techniques were used which were based on Restricted Fragment Length Polymorphism (RFLP) of the Internal Transcribed Spacer Region (ITS). This region was amplified by PCR. PCR was digested by eight restricted enzymes and made visible by electrophoresis.

We evaluated the following parameters:

a. Coloring of dead *G. mellonella* larvae
b. Morphological and behavioral characteristics
c. The response to the test of Koch's postulates
d. Identification of the EPN
e. Soil physical characteristics

Table 1. Survey sites in Colombia and Panama for entomopathogenic nematodes.

Country	Department	Municipality	Date of Sampling	Crop
Panama	***El Valle de Antón***	*Coclé*	Oct-02	Onion-Groundnut
	Ocú	Veraguas	Oct-02	Cassava
	Sioguí	Chiriquí	Oct-02	Cassava
	Cerro Punta	Estación IDIAP	Oct-02	Onion
Colombia	Quindío	Quimbaya	Mar-03	Cassava-Banana-Maize
	Risaralda	Santa Rosa	Mar-03	Onion
		Pereira	Mar-03	Pasture-Cassava-Onion
			May-03	Onion
			Feb-03	Pasture, Onion, Cassava, Pea
		Dosquebradas	Mar-03	
		La Florida	Jun-03	**Pasture**
	Caldas	Manizales	Jun-03	Onion
				Guamo, Breadfruit Tree, Maize, Beans
	Cauca	S/Quilichao	Mar-03	Cassava, Avocado, Banana, Mango, Mandarin, Lemon, Passionfruit, Café
				Groundnut, Pasture, Cassava

Results and Discussion: In total we analyzed 320 soil samples taken from soils of 15 crops **(Table 2)**. 24 of these samples harbored fungi (*Fusarium* sp. and *Metarhizium* sp.), 16 mites (Acardiae and Histiomidae) and about 300 of them nematodes. We classified the majority of the isolated nematodes as saprophagous due to their size, movement and survival. Most of them died during conservation. We used the surviving nematodes for re-infection of *Galleria mellonella* larvae (Koch's postulates). The 21 nematodes with corroborated entomopathogenic characters are listed in **Table 2**. We sent 20 of them to Kiel University for identification where two samples were identified as *Steinernema kraussei* **(Figure 1**). It is the first time that this species was reported in Colombia.

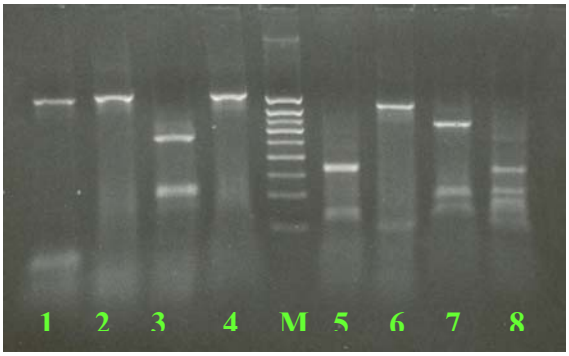

Figure 1. PCR amplified products from the ITS region of *Steinernema kraussei* digested with 8 restriction enzymes. Lanes 1-8 indicate following enzymes: 1. *Desulfovibrio desulfuricans*, strain Norway, 2. *Haemophilus aegytius,* 3. *H. haemolyticus,* 4. *H. influenzae*, M. Molecular weight markers (band sizes 1000, 800, 700, 600, 500, 400, 300, 200, 100 base pairs), 5. *H. influenzae* Rf, 6. *H. aphrophilus,* 7. *Acidiphilium facilis,* 8. *Staphylococcus aureus* 3A.

Table 2. Organisms isolated from soil samples taken in various parts in Colombia and Panama.

Country	Department	Municipality	Crop	No. of Samples (300 g)	Isolated organisms		
					Mites	Fungi	Supposed EPN
Panamá	El Valle de Antón	Coclé	Onion	5	2	2	1
			Groundnut	5	0	2	1
	Ocú	Veraguas	Cassava	10	0	2	1
	Sioguí	Chiriquí	Cassava	6	0	2	0
	Cerro Punta	Estac. IDIAP	Onion	5	1	2	1
Colombia	Quindío	Quimbaya	Cassava	21	1	2	1
			Banana	20	1	2	1
			Maize	20	0	1	1
	Risaralda	Santa Rosa	Onion	5	0	1	0
		Pereira	Pasture	38	1	1	1
			Cassava	33	0	1	1
		La Florida	Onion	24	1	0	1
			Pasture	35	1	0	1
	Caldas	Manizales	Guamo	3	0	1	1
			Breadfruit tree	3	0	0	1
			Maize	3	0	0	1
			Bean	3	1	0	1
			Cassava	4	1	0	1
			Avocado	2	0	0	0
			Banana	2	0	0	0
			Mango	2	1	0	0
			Mandarin	2	1	1	0
			Lemon	3	1	0	0
			Passionfruit	3	0	1	0
			Café	4	0	1	1
			Onion	6	0	1	1
	Cauca	Santander de Quilichao	Pasture	20	1	0	1
			Cassava	31	1	1	1
			Groundnut	2	1	0	1
Total				320	16	24	21 (6,6%)

= Samples with *Steinernema kraussei*

Abiotic factors play an important role in the successful performance of EPN, affecting the searching behavior, finding and invading the host (Parada 2002). EPN can tolerate a wide range of pH: 4 to 8 for *Steinernema* and 4 to 6 for *Heterorhabditis*. Humidity is a key factor for survival, movement, persistence and infectivity of the EPN (Koppenhöfer & Kaya 1996). Sandy and loamy soils harbor more species than clayish (Parada 2001). Our soil analyses showed that we carried out the samplings in zones with a pH range from 6.4 (Cauca) to 5.8 or 5.2 (Caldas). The soil texture varied from loamy (Cauca) to loamy-sandy (Caldas) **(Table 3**).

Table 3. Soil analyses of the surveyed sites.

Site	Crop	pH	SOM *	Texture
Risaralda	Onion	6,2	90,0	Loamy
	Pasture	5,3	77,7	Loamy
	Cassava	5,7	135,3	Loamy
	Pasture	5,9	81,9	Loamy
	Onion	6,0	96,1	Loamy
Quindío	Cassava	5,4	52,5	Loamy
Cauca	Groundnut	4,6	51,5	Clay
	Pasture	5,3	53,4	Clay
	Cassava	6,4	44,9	Loamy
	Fallow land	5,0	51,1	Clay
Caldas	Guamo	5,8	56,3	Loamy-sandy

= Samples with *Steinernema kraussei;* SOM = Soil organic matter.

The fact that our survey sites were in the range of these favorable characteristics we hypothesize that are reasons are responsible for the absence of EPN, for example contamination by pesticides.

Conclusions and recommendations

- *Steinernema kraussei* was described in Colombia for the first time.
- However, *S. kraussei* showed a wide range of soil characteristics like pH (4-8) and texture.
- EPN were present only in 6.6% of all evaluated soil samples, suggesting that much of the area the major part where the survey was realized the use of synthetic insecticides is common. We suggest continuing with these surveys in zones free of insecticide use.

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Contributors: Elsa Liliana Melo, Carlos Alberto Ortega, Andreas Gaigl, Carlos Julio Herrera, Rodrigo Zuñiga, Catalina Ramirez, Carmen Elisa Mendoza, Rómulo Riascos.

Activity 6. Efficiency of entomopathogenic nematodes for whitegrubs control under laboratory conditions.

Introduction

Globally, whitegrubs belong to the most important soil pests of many crops. During the last 20 years their pest status has dramatically increased, especially in the tropics, resulting in considerable yield reductions and environmental degradation: The whitegrub complex is characterized by considerable species richness. Many of these scarab species are important for the decomposition of organic material in the soil. However, the ever increasing reduction of plant biodiversity in many farming systems in the tropics, accompanied by losses of organic soil matter diminishes scarab diversity and leads to a selection of the most resistant and aggressive species. Most often the only control tactics for farmers is the excessive use of synthetic pesticides, with all the inherent health hazards to both farmers and rural and urban consumers. Moreover, synthetic pesticides pollute the environment and through the selection of resistant strains of whitegrubs and burrower bug can aggravate pest status by selecting for resistance.

Entomopathogenic nematodes (EPN) are considered as a promising strategy for the biological control of soil pests (Kaya & Gaugler, 1993). EPNs can be used as microbiological insecticides, suitable for small farmers. Industrial and semi-industrial mass rearing techniques for EPNs have been successfully developed (e.g. Capinera & Epsky, 1992; Ehlers, 1998), and EPNs can be applied using conventional pesticide equipment (Georgis, 1990).

Searching for feasible alternatives for the whitegrub control we tested the effect of ten EPN strains as antagonist of three important pest species: *Phyllophaga menetriesi*, *Phyllophaga* sp., and *Anomala cincta*.

Methodology: The experiment was conducted in the CIAT laboratory (23 ± 2 C, 70 ± 5% RH). We used whitegrubs collected in the fields in Caldono in Cauca (1570 m. a. s. l.) and Calucé in Valle (1637 m. a. s. l.) as target insect. We used larvae in the third instar of *Phyllophaga menetriesi*, *Phyllophaga* sp. (Cauca) and *Anomala cincta* (Calucé). After field collection we quarantined the larvae for four weeks. The larvae were maintained at 19 °C in plastic cups of 100 cm^3 and in a mixture of sand : sterile soil (1:3) and fed with carrots **(Figure 1**).

We tested 10 strains of native and introduced nematodes **(Table 1**). They were multiplied before every experiment in larvae of *G. mellonella*. We evaluated following variables: 1) Infection and mortality rate of whitegrubs and larvae of *G. mellonella* as control. 2) Impact of age of larvae on performance of EPN.

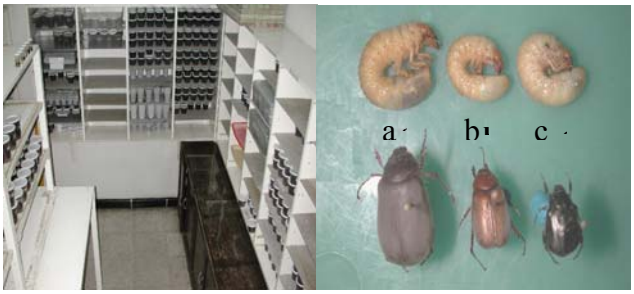

Figure 1. Whitegrub rearing cabins (a. *Phyllophaga menetriesi*, b. *Phyllophaga* sp., and c. *Anomala cincta*) in the CIAT laboratory of Entomology (23 ± 2 C, 70 ± 5% RH, 12 hours photoperiod)

Table 1. Evaluated EPN strains as control agents of three whitegrub species (*Phyllophaga* sp., *P. menetriesi*, *Anomala cincta*) under lab conditions.

Species	Place of Origin			Date of Collection / Arrival	Collector
	CIAT-Code	Country	Institution		
Steinernema riobravis	1	USA	Certis, USA	Jan - 2003	Certis
Steinernema feltiae	2	Colombia	Univ. Nacional Bogotá	Mar - 2003	Parada
Heterorhabditis bacteriophora	3	Italia	CABI/Bioscience	2002	López
Steinernema carpocapsae	4	USA	CABI/Bioscience	2002	López
Steinernema sp.	5	Colombia	Cenicafé	2002	López
Heterorhabditis sp.	6	Colombia	Cenicafé	2002	López
Steinernema arenarium	8	Russia	Kiel University	Jun - 2003	Ehlers
Steinernema feltiae	10	Germany	E-nema	Jun - 2003	E-nema
Heterorhabditis bacteriophora	11	Germany	E-nema	Jun - 2003	E-nema
Steinernema scarabaei	14	USA	Rutgers University	May-2004	Koppenhöfer

The experimental design was completely randomnized, evaluating only one factor (EPN) with a control without any application and another control with *G. mellonella* in order to evaluate the mortality after 48 hours. Every experimental unit consisted of 12 larvae and was repeated four times. We applied 1000 EPN / ml. The experimental unit consisted of plastic cups (56 ml) filled with soil and sterilized sand (3:1). We adjusted the field capacity by aggregating 3 ml water. We applied the EPN one day after the whitegrubs were introduced into the experimental units. The cups were stored in plastic bags in order to avoid the loss of humidity and were maintained in cabins under controlled conditions. We realized these experiments between January and July 2004 with repititions over the time in order to study the impact of the larval development on the eficiency of the EPN. These repititions had to be adjusted according to the quantity of available specimen of whitegrubs.

We used the statistical software Infostat for performing ANOVA and the treatment means were compared by Tukey ($P<0.05$). The non-homogenous data were homogenized by the formula $\sqrt{x+1}$ before analysis.

Results and Discussion: On *Phyllophaga* sp. all *Heterorhabditis* strains were the most efficient EPN. Only the native strain *S. feltiae* from Bogotá showed a similar performance as the imported

H. bacteriophora from E-nema **(Figure 2**). However, mortality and infection rate of EPN were significantly lower when larval age oncreased **(Figure 3**).

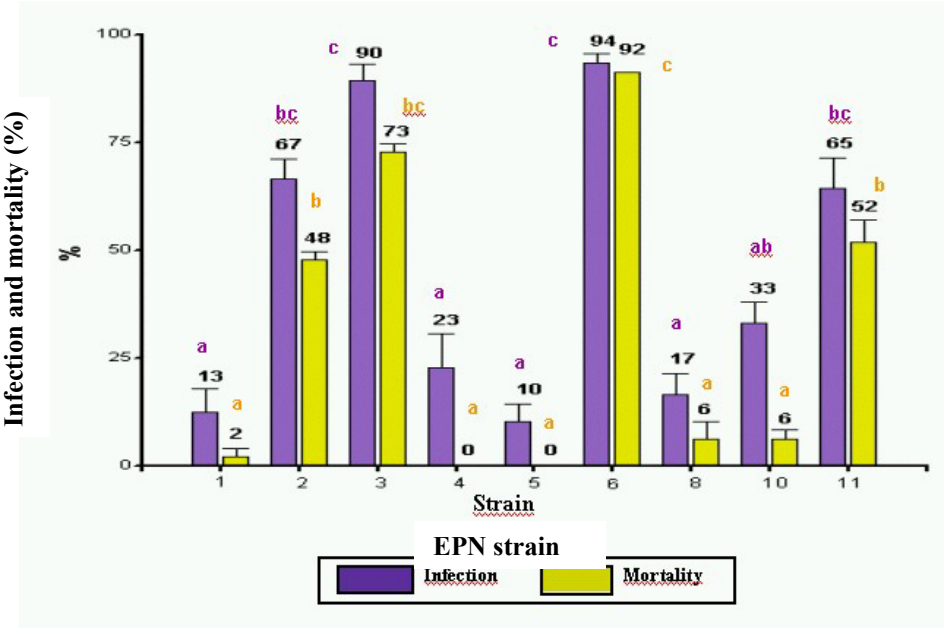

Figure 2. **Infection and mortality rate of nine EPN strains vs. *Phyllophaga* sp. (in %). Strains: 1. *S. riobravis,* 2. *S. feltiae* (UN Bogotá), 3. *H. bacteriophora* (Italia, Cenicafé), 4. *S. carpocapsae,* 5. *Steinernema sp.,* 6. *Heterorhabditis sp.* (Cenicafé), 8. *S. arenarium,* 10. *S. feltiae* (E-nema), 11. *H. bacteriophora* (E-nema).**

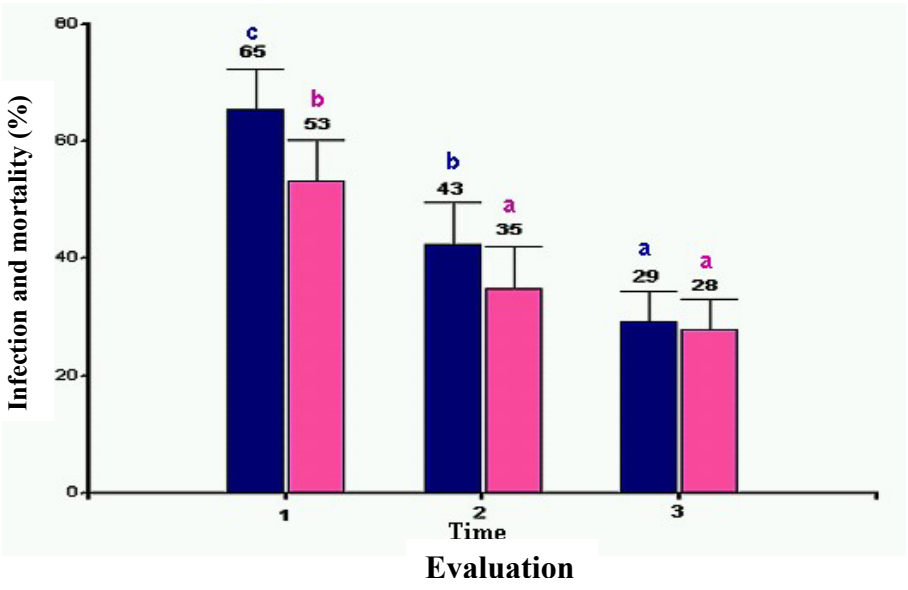

Figure 3. **Means of infection and mortality rate of seven EPN strains vs. the increasing age of the third instar of *Phyllophaga* sp. Evaluations took place in a period of four weeks. Evaluations were subsequently carried out in intervals of four weeks.**

We tested seven EPN strains vs. *P. menetriesi* where EPN were less efficient than vs. *Phyllophaga* sp. Interestingly, *S. carpocapsae* caused the highest whitegrub mortality (29%), *Heterorhabditis* sp. from Cenicafé showed the highest infection rate (40%, but mortality was very low (8%) **(Figure 4**)). After four weeks *H. bacteriophora* (Italia, Cenicafé) and *Heterorhabditis* sp. (Cenicafé) obtained a mortality of 12 and 10%, respectively (**Figure 5).**

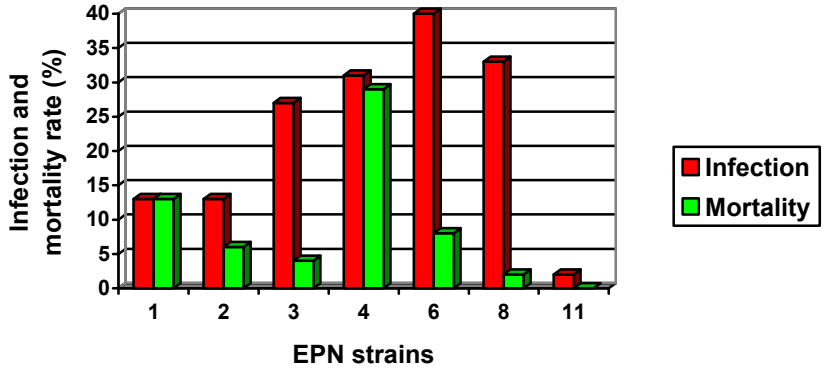

Figure 4. **First evaluation of infection and mortality rate of seven EPN strains vs. *P. menetriesi*. Strains: 1. *S. riobravis,* 2. *S. feltiae* (UN Bogotá), 3. *H. bacteriophora* (Italia, Cenicafé), 4. *S. carpocapsae,* 6. *Heterorhabditis sp.* (Cenicafe), 8. *S. arenarium,* 11. *H. bacteriophora* (E-nema).**

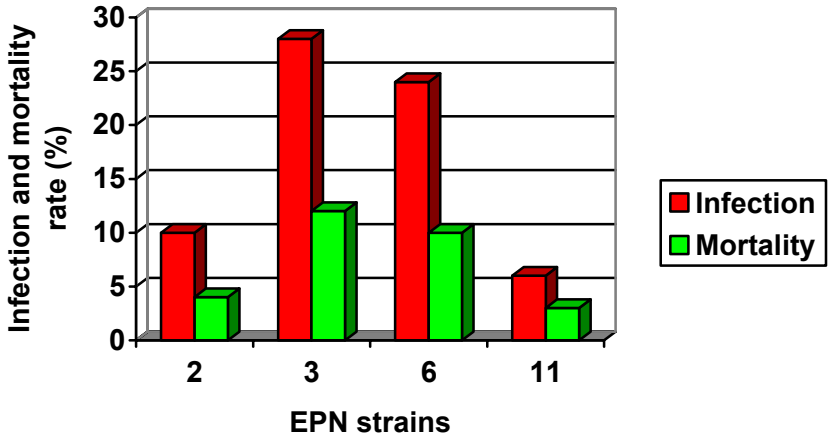

Figure 5. **Second evaluation of four EPN strains vs. *P. menetriesi* (in %). Strains: 2. *S. feltiae* (UN Bogotá), 3. *H. bacteriophora* (Italia, Cenicafé), 6. *Heterorhabditis* sp. (Cenicafé), 11. *H. bacteriophora* (E-nema).**

The EPN performed the best control of *P. menetriesi* during the second evaluation **(Figure 6).**

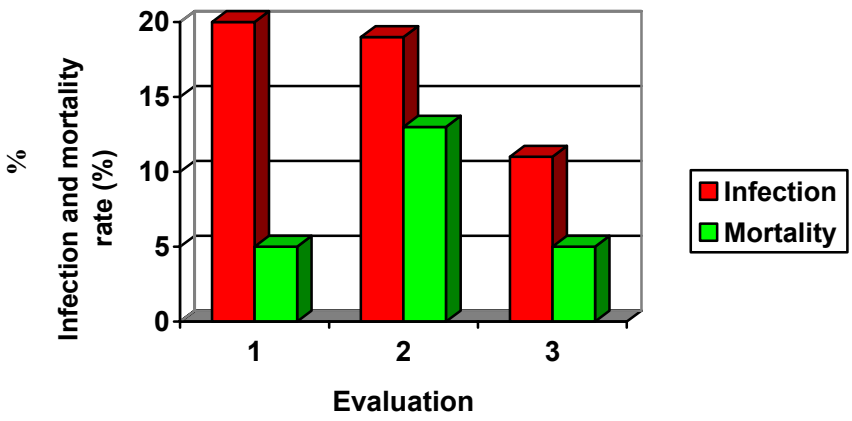

Figure 6. Means of infection and mortality rate of four EPN strains vs. the increasing age of the third instar of *Phyllophaga* sp. Evaluations were subsequently carried out in intervals of four weeks.

The most effective EPN vs. *Anomala cincta* were *H. bacteriophora* (Italia, Cenicafé) and *Heterorhabditis* sp. (Cenicafé) **(Figure 7**). The tested EPN were most efficient vs. *Phyllophaga* sp., followed by *A. cincta*. As mentioned before *P. menetriesi* is the whitegrub with the best defense against EPN: Earlier experiments with EPN (Quintero 2003) and *Metarhizium anisopliae* vs. third instar larvae of *P. menetriesi* (Martha Londoño 2004, personal communication[3]) corroborate this observation.

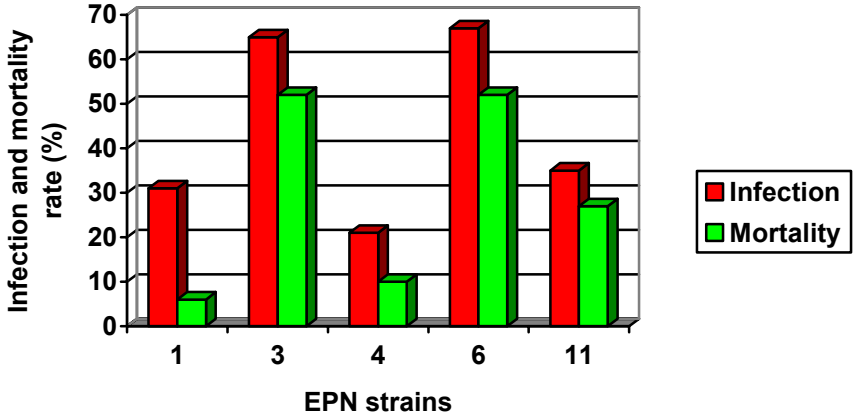

Figure 7. Infection and mortality rate of nine EPN strains vs. *Anomala cincta* (in %). Strains: 1. *S. riobravis* (Certis, USA), 3. *H. bacteriophora* (Italia, Cenicafé), 4. *S. carpocapsae*, 6. *Heterorhabditis* sp. (Cenicafé), 11. *H. bacteriophora* (E-nema).

[3] Martha Londoño is research associate of Corpoica, Rionegro

The evaluations during the first two development phases of *A. cincta* didn't show any significant differences in mortality **(Figure 8**).

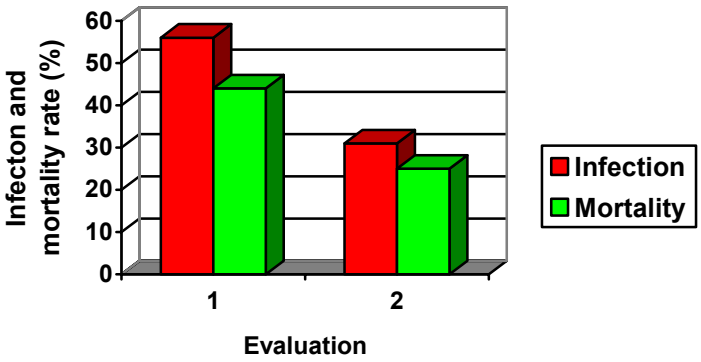

Figure 8. Comparing two consecutive evaluations of infection and mortality rate of four EPN vs. third instar of *Anomala cincta*.

When we compared all tested EPN vs. each whitegrub species we found that the nematodes showed greatest mortality on *Phyllophaga* sp., followed by *Anomala cincta. P. menetriesi* was the most resistant species to EPN infection and mortality **(Figure 9**).

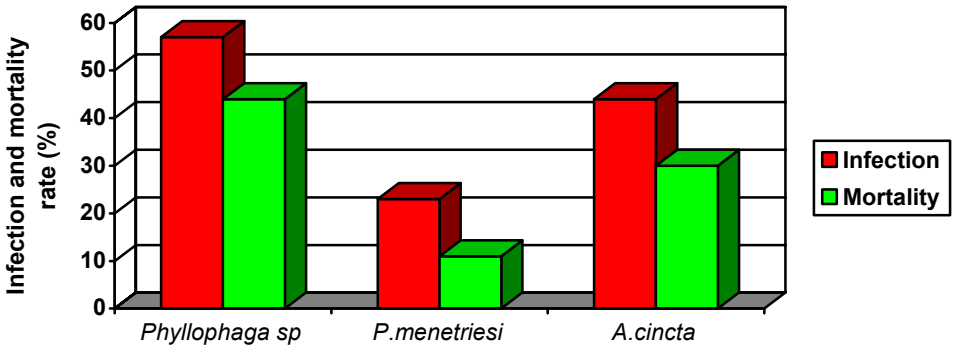

Figure 9. Infection and mortality rates of all EPN strains on three whitegrub species

Conclusions

- *P. menetriesi* was the most resistant whitegrub species to EPN attacks.
- Larval age significantly affects the impact of EPN. The third instar is the most resistant probably due to morphological and functional reasons that still have to be studied and identified.
- *Heterorhabditis* sp. (Cenicafé) was the most promising EPN against *Phyllophaga* sp. and *Anomala cincta*, followed by *H. bacteriophora* from Italia, *H. bacteriophora* (E-nema) and *S. feltiae* (Universidad Nacional).

Recommendations

- Conduct studies with EPN vs. the first two instars of *P. menetriesi.*
- Realize studies to evaluate the optimal concentration of EPN.
- Repeat these experiments under semi-controlled conditions.
- We recommend studying only mortality in order to reduce dissection work; moreover, mortality is the most important variable.

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Contributors: Elsa Melo, Carlos Alberto Ortega, Andreas Gaigl, Carlos Julio Herrera, Rodrigo Zuñiga, Catalina Ramirez, Carmen Elisa Mendoza, Rómulo Riascos.

Activity 7. Infection and mortality rate of *S. scarabaei* vs. *Phyllophaga* sp.

Introduction

Steinernema scarabaei Stock & Koppenhöfer was recently isolated from *Exomala* (= *Anomala*) *orientalis* Waterhouse and *Popillia japonica* Newman in New Jersey, USA (Stock & Koppenhöfer 2003). This EPN reached mortality rates of almost 100% of the whitegrubs *P. japonica, Cyclocephala borealis* and *E. orientalis* under field conditions in New Jersey. *S. scarabei* outperformed clearly other EPN species such as *H. bacteriophora* and the unidentified *Heterorhabditis* sp. (Koppenhöfer & Fuzy 2003). Due to our experience that *P. menetriesi*, the economically most important whitegrub between 1000 and 1500 m. a. s. l., is hard to kill (CIAT 2003, Quintero 2003) we decided to import this EPN species for experiments in the laboratory in spite of the fact, that it has not been possible to mass produce these nematodes in vitro (Ralf-Udo Ehlers 2003, personal communication).

Methodology: We evaluated six concentrations (0, 25, 50, 100, 200, and 400 EPN / ml) of *S. scarabaei* vs. larvae of *Phyllophaga* sp. The further procedure is explained in the previous part A (12 specimen per treatment and four replicates). The nematodes were provided by Albrecht Koppenhöfer, Rutgers University, Brunswick, New Jersey.

Results: *S. scarabaei* obtained greatest infection and mortality rate vs. *Phyllophaga* sp. with the highest concentration. Differences between the concentrations 200 and 400 Dauer Juveniles (DJ) per ml were not significant **(Figure 1**). Interestingly, infection and mortality rate were equal in all treatments. In the control treatment, all *G. mellonella* died after 48 hours.

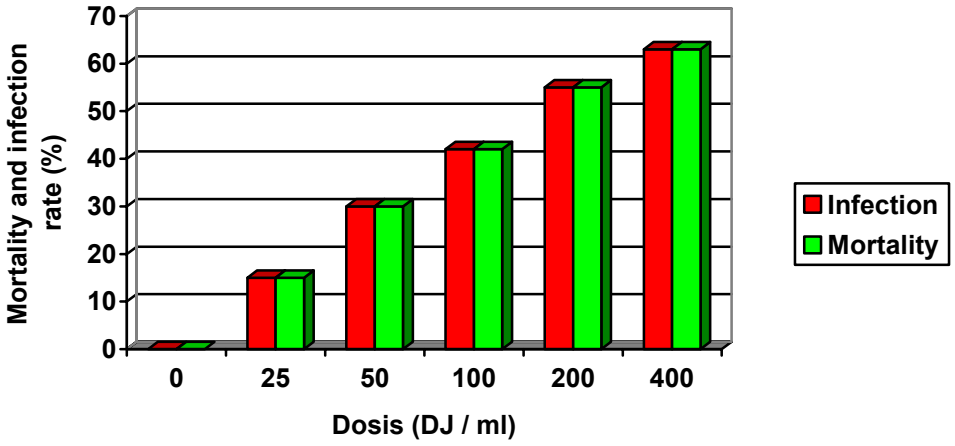

Figure 1. Infection and mortality rate of six concentrations of Dauer juveniles (DJ) of *S. scarabei* vs. *Phyllophaga* sp.

However, in the second experiment the performance of these nematodes was very poor. Infection and mortality was not greater than 5%, the larvae of *G. mellonella* didn't show a high mortality, either. It is possible that the loss of this efficiency was due to the prolonged transport period due to importation problems. Since the mortality in the first experiment wasn't very high and even

100% mortality of *G. mellonella* may not be a reliable indicator of EPN pathogenicity (Albrecht Koppenhöfer 2004, personal communication) this experiment should be repeated with healthier, fresher material.

Conclusions

- Infection and mortality rate of whitegrubs are positively correlated with increasing DJ concentration
- The time of EPN application had an effect on infestation and mortality rate. At the first evaluation using a concentration of 200 and 400 DJ / ml control was greater than 50%

Recommendations

- Reactivate and multiply *S. scarabei* in order to continue these experiments
- Considering the tedious dissection of hosts in order to count the number of pentrated DJ we recommend to include in further trials only the variable "mortality". Moreover, this variable is much more important

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Contributors: Elsa Liliana Melo, Carlos Alberto Ortega, Andreas Gaigl.

Activity 8. Preliminary studies on pathogenicity of entomopathogenic fungi against *Phyllophaga menetriesi.*

Introduction

At this moment, our ceparium harbors 213 strains of entomopathogenic fungi (EPF) that have been isolated from adult beetles or whitegrub larvae. Reactivation of a fungus is the first step of the selection process of the most virulent strains. Here, we describe the process of reactivation of fungi on the third instar of *Phyllophaga menetriesi* generating some important data for selection of the most virulent strains.

Methodology: The larvae of *P. menetriesi* were collected in Pescador (Caldono, Cauca). The larvae were placed in plastic cups of 12 ounces filled with soil and transferred to CIAT. Here, the grubs were confined in sterile soil and observed for at least 40 days in order to detect entomopathogens.

We selected 46 isolates (23 *M. anisopliae*, 9 *B. bassiana*, 4 *Paecilomyces* sp., 3 *Fusarium* sp., 3 *Gliocladium* sp., 2 unidentified strains and 1 *Penicillium* sp.) **(Table 1**). Once they showed a considerable sporulation we applied them to the insects. We confined the grubs in plastic cups filled with soil; three pregerminated maize seeds and one larva of *P. menetriesi* (third instar). Before covering the experimental unit with a perforated lid we added 2 ml of a suspension with spores. The cups were placed in plastic bag filled with wax sheets in order to maintain humidity 70% and stored at 27 °C and 100% darkness.

We counted living and dead larvae once a week during a two months period. The dead insects were transferred to a chamber with a RH of 80% in order to enhance sporulation. Once the growing of the fungus was visible we inoculated it on Agar. We realized the studies on reactivation on larvae using four groups: 7 strains and one control as pilot treatment where we tested the viability of the methodology; the second inoculation with 9 strains; the third inoculation with 20 and the fourth with 26 strains.

Results: Some strains caused mortality of the grubs at 16 days after application. After five weeks the following strains of *M. anisopliae* caused a mortality of 100%: CIAT 323, CIAT 328, CIAT 393, and CIAT 405. In a second experiment the following strains of *M. anisopliae* caused a mortality of 100%: 1p, CIAT 300, CIAT 348, CIAT 418, CIAT 513, and 515.

Recommendations: For the following tests we will use the strains CIAT515 (*M. anisopliae*) and CIAT418 (*M. anisopliae*) due to their high pathogenicity. We also strongly suggest including CIAT 338 and CIAT 405, because those represent the species *Gliocladium* sp. and *Beauveria bassiana*, respectively, and both caused 100% mortality. We plan to test these strains vs. the second instar of *P. menetriesi*. The most pathogenic strain will be tested vs. the third instar of the larvae of the same species.

Table 1. Applied EPF strains vs. larvae of the third instar of *Phyllophaga menetriesi* for reactivation.

	Strain			Host		Origin	
	Code	Genera	Species	Genus	Species	Department	Municipality
1	1p	*Metarhizium*	*Anisopliae*				
2	CIAT014	*Metarhizium*	*anisopliae*	*Aeneolamia*	*varia*	Valle	Palmira
3	CIAT044	*Beauveria*	*bassiana*	*Zulia*	*carbonaria*	Cauca	Sder Quilichao
4	CIAT224	*Metarhizium*	*anisopliae*	*Cyrtomenus*	*bergi*	Valle	Pradera
5	CIAT245	*Metarhizium*	*anisopliae*	*Hypotenemus*	*hampei*	Caldas	Chinchiná
6	CIAT300	*Metarhizium*	*anisopliae*	*Galleria*	*mellonella*	Cauca	Sder Quilichao
7	CIAT302	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
8	CIAT308	*Paecilomyces*	spp.	*Galleria*	*mellonella*	Cauca	Sder Quilichao
9	CIAT309	*unidentified*	*unidentified*	Chisa		Risaralda	Pereira
10	CIAT314	*Gliocladium*	sp	Chisa		Cauca	Caldono
11	CIAT316	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
12	CIAT317	*Beauveria*	*bassiana*	Chisa		Risaralda	Pereira
13	CIAT320	*Fusarium*	spp.	Chisa		Risaralda	Pereira
14	CIAT323	*Metarhizium*	*anisopliae*	Chisa		Cauca	Caldono
15	CIAT325	*Fusarium*	spp.	Chisa		Cauca	Sder Quilichao
16	CIAT328	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
17	CIAT329	*Penicillium*	sp	Chisa		Cauca	Caldono
18	CIAT338	*Gliocladium*	sp				
19	CIAT344	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
20	CIAT345	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
21	CIAT347	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
22	CIAT348	*Metarhizium*	*anisopliae*	Chisa		Risaralda	Pereira
23	CIAT349	*Metarhizium*	*anisopliae*	*Galleria*	*mellonella*	Risaralda	Pereira
24	CIAT351	*Gliocladium*	sp	Chisa		Risaralda	Pereira
25	CIAT354	*Metarhizium*	*anisopliae*	Chisa		Caldas	Manizales
26	CIAT359	*Beauveria*	*bassiana*	*Phyllophaga*	*menetriesi*	Risaralda	Pereira
27	CIAT360	*Beauveria*	*bassiana*	*Phyllophaga*	*menetriesi*	Risaralda	Pereira
28	CIAT363	*Beauveria*	*bassiana*	*Phyllophaga*	*menetriesi*	Risaralda	Pereira
29	CIAT388	*Metarhizium*	*anisopliae*	*Cyrtomenus*	*bergi*	Valle	Florida
30	CIAT389	*Metarhizium*	*anisopliae*	Chisa		Valle	Florida
31	CIAT390	*Metarhizium*	*anisopliae*	*Phyllophaga*	sp	Risaralda	Pereira
32	CIAT393	*Metarhizium*	*anisopliae*	Chisa		Cauca	Sder Quilichao
33	CIAT395	*Paecilomyces*	spp.	*Phyllophaga*	sp	Risaralda	Pereira
34	CIAT401	*Beauveria*	*bassiana*	Chisa		Cauca	Caldono
35	CIAT405	*Beauveria*	*bassiana*	*Trialeurodes*	*vaporariorum*	Valle	Roldanillo
36	CIAT412	*Metarhizium*	*anisopliae*	*Phyllophaga*	sp	Cauca	Sder Quilichao
37	CIAT418	*Metarhizium*	*anisopliae*	*Phyllophaga*	*menetriesi*	Risaralda	Pereira
38	CIAT422	*Paecilomyces*	sp	*Galleria*	*mellonella*	Cauca	Caldono
39	CIAT513	*Metarhizium*	*anisopliae*	*Phyllophaga*	*menetriesi*	Risaralda	Pereira
40	CIAT515	*Metarhizium*	*anisopliae*	*Phyllophaga*	*sp*	Cauca	Sder Quilichao
41	Cuc. CIAT	*Beauveria*	*bassiana*	*Anomala*	Sp	Cauca	Caldono
42	CUN 087A	*Metarhizium*	*anisopliae*				
43	CUN 087B	*Beauveria*	*bassiana*				
44	CUN PER2	*Fusarium*	sp				
45	CUN059	*Metarhizium*	*anisopliae*	*Aeneolamia*	*varia*	Valle	Palmira
46	P.crus.	*Paecilomyces*	*crustaceus*				

Activity 9. Preliminary studies on pathogenicity of *Bacillus popilliae* against *Phyllophaga menetriesi.*

Introduction

Larvae of *Phyllophaga menetriesi* are the economically most important whitegrub species in Colombia in zones from 1200 to 1600 m. a. s. l. (CIAT 2003). The bacteria *Bacillus popilliae* (Bp) has been reported as one of the most important natural antagonists of about 70 species of whitegrubs causing the milky disease (Tanada & Kaya 1993) and is also one of the most frequent pathogen of larvae we collected during this project (CIAT 2003; see also Activity 4 of this report). The objective of this recently initiated undergraduate thesis is to describe pathogenicity and virulence of *B. popilliae* in larvae of *P. menetriesi*. Here we present the results of isolations of native strains of this bacteria and its multiplication.

Methodology: In the first experiment we tested four strains of Bp (LF24, 381, B386, LG) on three species (*P. menetriesi*, *Phyllophaga* sp. and *Anomala cincta*) using 25 insects for each strain and species. Then, we could include two more strains (Bp4 and Bp1) that had been isolated from field-collected whitegrubs. Due to the lack of specimens of *P. menetriesi* we applied these six strains on *Phyllophaga* sp. (100 individuals / strain). For the following experiment sufficient larvae of *P. menetriesi* were available for testing the six Bp strains (75 individuals / strain). We injected in each grub a concentration of 3 x 10^8 spores. The conditions in the laboratory were: 21 °C ± 2, 70% RH ± 5, 965 m. a. s. l., 24 hours darkness.

Results: Figure 1 shows the different mortalities caused by four Bp strains vs. three whitegrub species. LG caused only a mortality of 4% on *A. cincta*, whereas the mortality of the genus *Phyllophaga* varied from 88 to 100%. In contrast, LF24 killed all grubs by 100%. 381 caused a relatively low mortality of *A. cincta* and B386 of *P. menetriesi*. *Phyllophaga* sp. was the species where all bacteria strains caused almost a 100% control. Latter result was repeated when we included the two new strains.

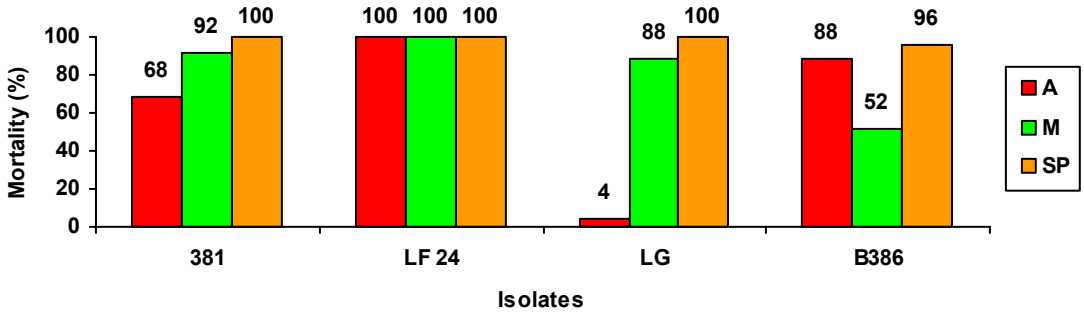

Figure 1. Mortality of three whitegrub species (A=*Anomala cincta*, M= *Phyllophaga menetriesi*, SP= *Phyllophaga* sp.) caused by four strains of *B. popilliae* in the laboratory

The strains LF24 and LG gave the best reproduction in *Phyllophaga* sp. followed by Bp4, Bp1, 381, and B386 **(Figure 2**).

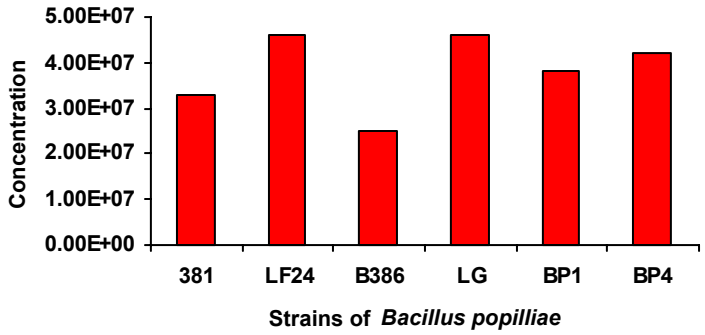

Figure 2. Reproduction of six *Bacillus popilliae* strains in *Phyllophaga* sp. in the laboratory

The control of the *P. menetriesi* by the six *B. popilliae* strains oscillated between 97 and 100% where the strains 381 and Bp4 showed the lowest results (97%) **(Figure 3**). These data suggest that all six strains can be selected for further tests of pathogenicity and virulence.

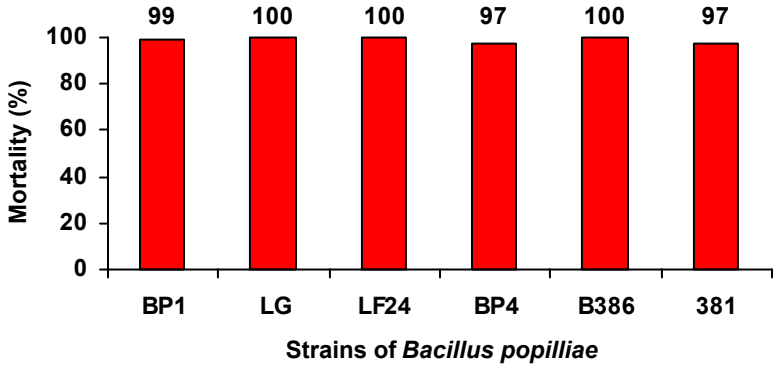

Figure 3 Mortality of *Phyllophaga menetriesi* caused by six strains of *Bacillus popilliae*.

Discussion. Injection of bacteria spores showed good results in the sense of control; however, results indicated that it is not the appropriate method for bacteria multiplication in the insect. This can be explained by the fact that injection is an unnatural way of inoculation. Normally, the larva takes in the bacteria by feeding. For this reason we suggest to use forced alimentation for future studies. All six strains caused a high mortality of all three insect species and are promising candidates as biological control agents of *P. menetriesi* and other whitegrub species.

Recommendations

- We recommend using all six strains for further studies.
- Study pathogenicity of the selected Bp on the *P. menetriesi* using the concentrations of $1x10^5$ and $1x10^8$.
- Morphological, molecular and biochemical characterization of these six strains

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Activity 10. Control of *Cyrtomenus bergi* using entomopathogenic nematodes.

Introduction

During the last 13 years researchers in Colombia tested native and introduced strains for the biological control of the burrower bug *Cyrtomenus bergi* (BB): *Heterorhabditis bacteriophora* as the endemic species and *Steinernema carpocapsae* as introduced strain from the US (Caicedo & Bellotti 1994; Barbarena & Bellotti 1998).

The mentioned research revealed that adults and nymphs at fifth instar are the most susceptible stages to EPN. 60% of the adults and 10 to 50% of the fifth instar nymphs were penetrated by exotic EPN. 65 to 100% of the Dauer Juvenile (DJ) of native strains parasitized the fifth instar, 85 to 100% adults (ibid). Recent greenhouse studies showed that 50% of 25,000 DJ / ml penetrated into the host (CIAT 2003).

The objectives of this study were
1. To determine the efficiency of various strains of entomopathogenic nematodes as antagonists of *Cyrtomenus bergi* under laboratory conditions.
2. To evaluate of the lethal dose of two commercial EPN strains vs. adult burrower bugs in the laboratory.

Methodology: We selected a commercial *H. bacteriophora* strain (E-nema) and two *S. feltiae* strains for these studies **(Table 1**). We obtained some control of *C. bergi* with the commercial strain in greenhouse experiments (see following section). Now, we used this strain as contrast to the native *Steinernema feltiae* (Villapinzón, Cundinamarca). The latter nematode is not only of interest due to its native origin, but also for combining ambusher and cruiser characters (Parada 2003, personal communication). The experiments were conducted with adult *C. bergi.*

Table 1. EPN strains applied for the control of *C. bergi* in the laboratory.

Species	Origin	Source	Date of reception
Steinernema feltiae	Colombia	J.C. Parada*	March / 2003
Steinernema feltiae	Germany	E-nema**	June / 2003
Heterorhabditis bacteriophora	Germany	E-nema**	June / 2003

Supplied by * Julio Cesar Parada, National University Bogotá; ** E-nema, Raisdorf, Germany.

EPN Rearing: We reared EPN "in vivo", using larvae of *Galleria mellonella* as hosts. This method is divided in three parts: infection, harvest, and storage. We maintained the nematodes in the incubator at 10 to 15 °C in complete darkness. The steinernematids are cleaned and reactivated every six months, the heterorhabditids every four months.

Experimental Unit: We filled transparent plastic cups (2 ounces) with 20 g sterile and hydrated (3%) sand. We added one pregerminated maize seed and one BB to each cup and sealed it. We applied a suspension of 1 ml with EPN and 1 ml of distilled and sterile water (DSW). For the control treatments we used only DSW. These units were stored in black plastic bags at complete darkness in order to maintain humidity. Food was renewed once a week. Evaluations were carried out every 14 days according to the periods of moulting, also in the case of the fifth instar

that lasts about 30 days. In the second experiment the evaluation interval for adults was three weeks.

Evaluated Variables

i. Rate of infectivity of EPN. We evaluated dead and live bugs, dissecting them under stereoscope.
ii. Rate of mortality of the BB. We dissected every bug to check the presence of EPN.
iii. Rate of melanization. Melanized EPN in the dissected bugs were counted.
iv. Rate of mortality of larvae of *Galleria mellonella.* In order to have a control for the described experiments we evaluated the mortality of moth larvae after 72 hours.
v. Rate of infectivity and mortality according to EPN strain and EPN concentration. We took the commercial concentration of EPN as base for these experiments and two higher and two lower concentrations, following a geometric progression; the control without EPN was the sixth dose **(Table 2**).

Table 2. Dose of commercial EPN strains (E-nema) for the control of *C. bergi.*

Concentration	Steinernema feltiae	Heterorhabditis bacteriophora
C1	100,000	50,000
C2	10,000	5,000
C3	1,000	500
C4	100	50
C5	10	5
C6	0	0

C3 = commercial dose.

Experimental Design: The experiments were completely randomized, where the factors were the two EPN strains and the six stages of the bug with a check without EPN and another one observing the infectivity of Galleria. In the second experiment we used two EPN strains at six concentrations. We used 10 bugs per treatment with four replicates. As check with *G. mellonella* we used 20 larvae per strain.

Statistical Analysis: An ANOVA and a posterior Duncan Test were realized to establish and evaluate differences between variances. It was necessary to transform the value with the equation $\sqrt{x+1}$. In the figures we used the original data in percentage. Finally, we made a regression analysis to measure the impact of the treatments on the experiments.

Results

Objective 1: Efficiency of various strains of entomopathogenic nematodes as antagonists of *Cyrtomenus bergi* under laboratory conditions

Infectivity of two EPN strains vs. six stages of *C. bergi*: We did not observe any significant differences between the strains but between the stages of the BB. The fourth instar was the most invaded (42%), followed by the adult bug and the fifth instar (28.1% and 25%, respectively) **(Figure 1**).

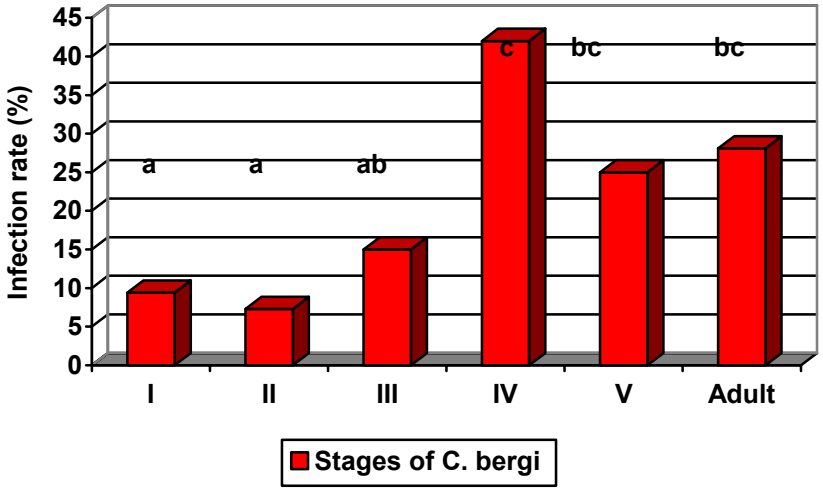

Figure 1. Infection rate of two EPN strains on six stages of the burrower bug (bars with the same letter are not different; $p < 0.05$)

Mortality of two EPN strains vs. six stages of *C. bergi*: Similar to the rate of infectivity of the two EPN strains **(Figure 1)** didn`t differ in mortality of their hosts. For this reason we present here the means of both strains. The fourth and fifth instar were the most suceptible and differed significantly from the the first two instars **(Figure 2)**. All larvae of *G. mellonella* were invaded by the EPN after 48 hours, indicating that the EPN were in good conditions.

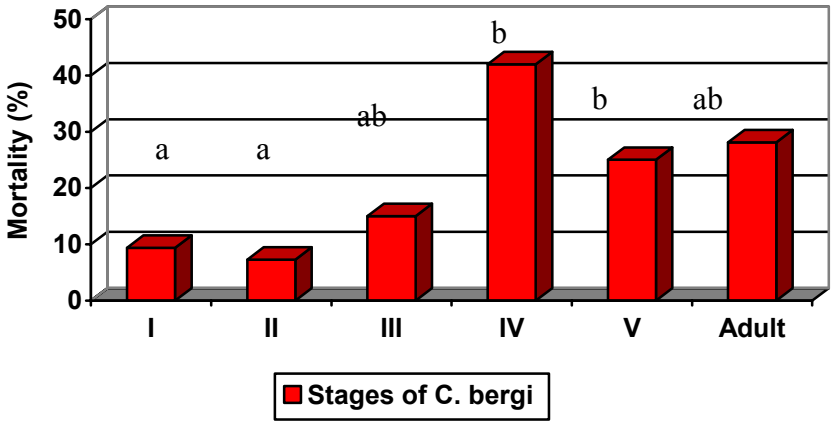

Figure 2. Mortality of six stages of the burrower bug vs. two EPN strains (bars with the same letter are not different; $p < 0.05$)

Objective 2: Evaluation of the lethal dose of two commercial EPN strains vs. adult burrower bugs in the laboratory.

Infectivity rate of two EPN strains applied in six concentrations vs. *C. bergi:* Much more *S. feltiae* penetrated the host than *H. bacteriophora* **(Figure 3)**. The highest penetration was in

correlation with the highest concentration; however, it is difficult to explain, why concentration C2 obtained a penetration of almost 60%.

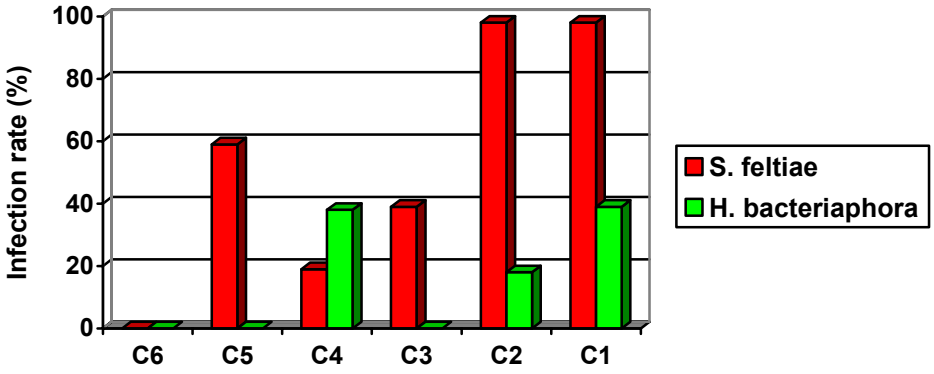

Figure 3. Rate of infection on EPN in six concentrations vs. adults of *C. bergi*

Mortality rate of two EPN strains applied in six concentrations vs. *C. bergi:* Surprisingly, only *S. feltiae* caused mortality of the BB **(Figure 4**). Mortality was correlated with EPN concentration ($r = 0.8293$, $p<0.05$).

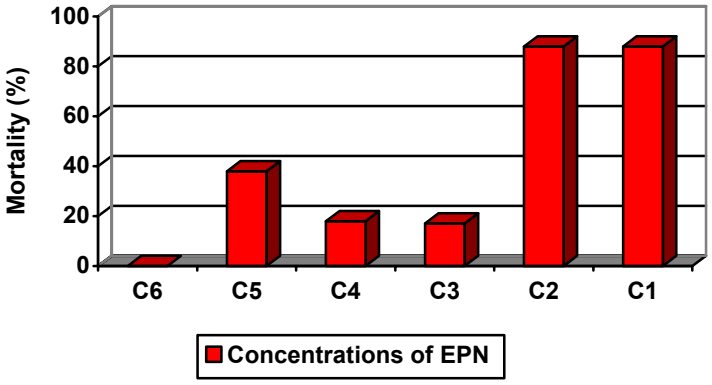

Figure 4. Mortality rate of adult burrower bugs exposed to different doses of *S. feltiae* (in percent) (concentrations see Table 3-Activity 5).

Melanization of EPN: *S. feltiae* showed a higher susceptibility to melanization than *H. bacteriophora* **(Figure 5**). Melanization rate and EPN concentration were correlated ($r = 0.8068$).

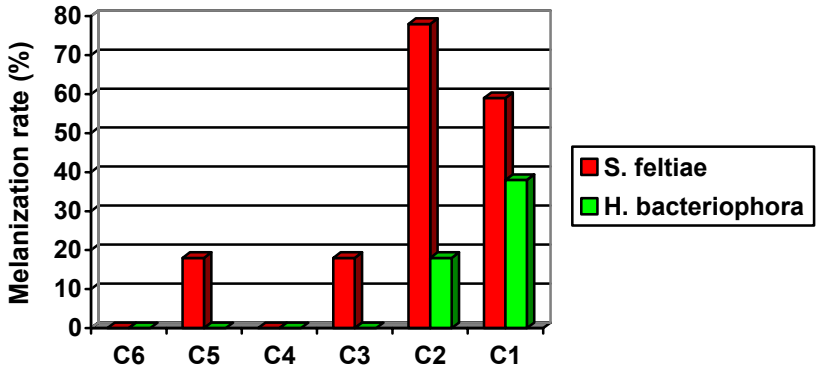

Figure 5. Rate of melanization of EPN as defense mechanism of C. bergi (in percent) (concentrations see Table 3-Activity 5)

Control Treatment: We observed that a native and an introduced strain killed all *G. mellonella* larvae within a period of 48 hours.

Conclusions

- Infectivity and mortality caused by EPN did not differ in any stage of *C. bergi* between introduced *H. heterorhabditis* (E-nema) and native S. feltiae. However, we observed significant differences between host stages. The most susceptible stages were fifth and fourth instar nymphs. This finding should be considered for further attempts of biological control vs. *C. bergi*.
- The two commercial strains of *S. feltiae* and *H. bacteriophora* (E-nema) differed considerably. *H. bacteriophora* didn't cause mortality in any of the six stages. As expected we also observed differences between EPN concentrations.
- It is curious that *S. feltiae* suffered the highest melanization rate but still remained as the more efficient strain.
- We obtained a high mortality of BB when we applied 100,000 EPN / ml. This is an extremely high dose rate and not feasible for a biological control under field conditions. Recognizing the relatively low mortality of even the most efficient strains (S. feltiae)of about 20% and the efficient defense mechanisms of *C. bergi* question if EPN are the appropriate tool for successful biological control. However, the defense mechanism must be understood to be able to make a final conclusion. Moreover, EPN can be combined with sublethal doses of insecticides as we successfully performed with entomopathogenic fungi (see later section) vs. BB or authors vs. whitegrubs (*e.g.* Koppenhöfer *et al*. 2000).
- In opposite to BB, young whitegrubs in the first, second, or even early third instar seem to be susceptible for EPN suggesting to study the set new experiments with these young whitegrub larval stages.

Recommendations: Some values were far out from normal and were are difficult to be explained suggesting to repeat these experiments.

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Contributors: Elsa Liliana Melo, Carlos Alberto Ortega, Carlos Julio Herrera, Rodrigo Zuñiga, Catalina Ramirez, Carmen Elisa Mendoza, Rómulo Riascos.

Activity 11. Evaluation of two strains of entomopathogenic nematodes as natural enemies of *Cyrtomenus bergi* under greenhouse conditions.

Introduction

During recent years there has been an increasing interest in the biological control of pests by means of entomopathogenic nematodes (EPN). As a consequence, it became necessary to describe native species and the interest in exchanging material between institutions has increased. The classical biological control has generated various examples for the successful introduction of exotic EPN as control agents of important pests (Bedding & Akhurst 1975; Parkman *et al*. 1993, Kaya, H.K. 1993). Janssen (1993) mentioned some limiting factors for a successful introduction of EPN: Target host and habitat, nematode suitability and quality, seasonality, application strategy, soil characteristics, compatibility with pesticides, and biotic factors in the soil. The introduction of these organisms may include some risks: range expansion, host range and impact on non-target organisms, effect on community dynamics and displacement, permanence of introduction and vulnerability of target habitat, and the relationship with symbiotic bacteria (ibid.)

The deliberate release of exotic EPN has taken place for more than a century around the globe. The releases have often been ineffective but have very rarely been troublesome. Unlike classical biological control agents, EPN are not normally expected to establish following release. Wild-type EPN have very complex survival repertoires that may be sacrificed in favor of better production and performance of the DJ and in favor of ecological safety in inundative application (Downes 1996).

The objective of this research was to determine the efficiency of *Steinernema feltiae* and *Heterorhabditis bacteriophora* vs. the burrower bug (BB) *Cyrtomenus bergi*. Both are commercial strains and were provided by E-nema, Germany.

Methodology: As continuance of the previously described laboratory experiment we realized this work in the greenhouse at CIAT experimental station. The temperatures oscillated between 18 and 28 °C and RH between 55 and 85%. The BB were taken from the lab colony. We used adults and the fifth instar, which are the most vulnerable stages to EPN according to earlier experiments presented in an earlier section of this report.

The experiment was designed as a completely randomized block with the factors 2x2+1 and three replicates. We evaluated two factors: the two ages of the BB and the two EPN strains. As the first control treatments we applied distilled water to the bugs. As second control we infested larvae of *G. mellonella* with both EPN strains. We evaluated EPN infection and mortality rates of the insects after 15 and 30 days after the application of nematodes (daa).

We used groundnuts (*Arachis hypogaea*) as host plant which were sowed 15 days before we released 20 bugs /pot. Each unit had six pots (15 x 15 x 10 cm), which were combined with transparent acetates as cages **(Figure 1**).

Figure 1. Experimental units with acetate cages where host plants and insects (adults and fifth instar) were confined

We applied the EPN according to the provider's instructions: Nemaplus (*S. feltiae*) with 1000 DJ / ml water and 7.7 ml / pot and Nematop (*H. bacteriophora*) with 417 DJ / ml water and 18.47ml / pot. After the application we watered each pot with 40 ml water in order to facilitate the propagation of the EPN.

The data were subjected to ANOVA and the treatment means were compared by Tukey-Test ($P<0.05$). The data were homogenized by $\sqrt{x+1}$ before analysis. We used the original percentage values for the figures.

The evaluated variables were:

1. Infectivity of EPN (Infection). The bugs were dissected and checked for daa. Only the infected bugs were considered counted independently of the quantity of penetrated EPN.
2. Mortality rate.
3. Melanization rate.
4. We verified the virulence of EPN in a control treatment using larvae of *G. mellonella* and "White Traps". We applied simultaneously in a multiple well dish BB and moth larvae and applied a solution of 300 µl with EPN in each well that contained 3 g sand. Moreover, we checked the external characteristics of larvae of *G. mellonella* five daa of EPN as additional control of nematode virulence.

Results: Both EPN strains did not show any significant differences in rate of infection. There was almost no penetration of EPN into fifth instar nymphs; however, adults were infected by 90% and 75%. Infestations between days after application were not significant ($p<0.01$) **(Figure 2**).

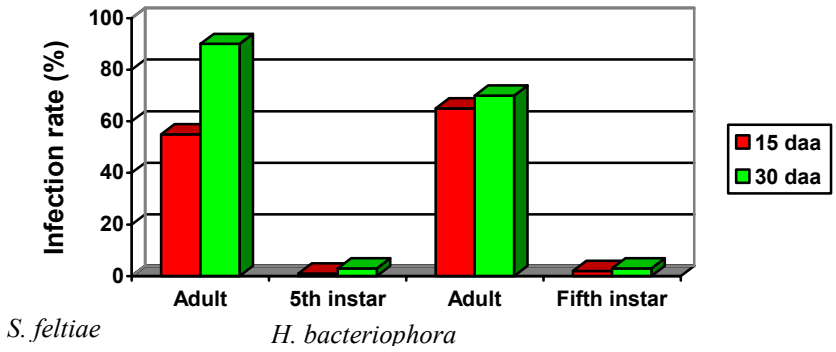

Figure 2. Infection rate of two commercial EPN strains on two stages of *C. bergi* and after 15 and 30 days of application (daa)

Both EPN strains achieved a higher mortality on adults than on fifth instar (p<0.01). We also observed a higher mortality caused by *H. bacteriophora* at 15 daa **(Figure 3**). After 30 daa differences were not significant.

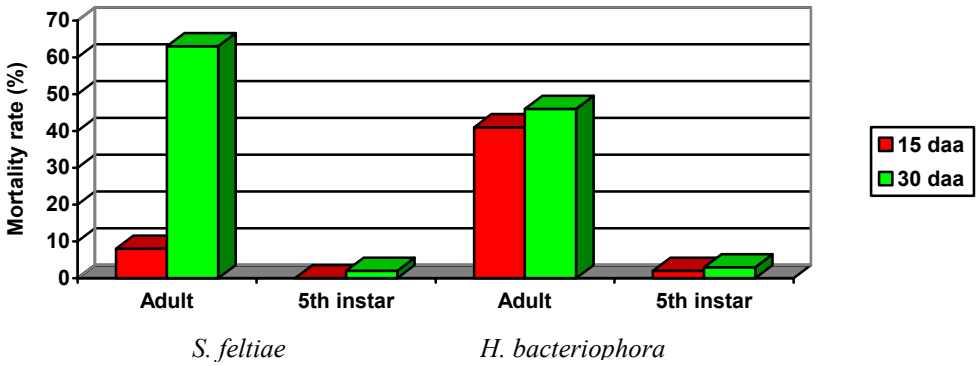

Figure 3. Mortality rates of two commercial EPN strains on two stages of *C. bergi* and after 15 and 30 days of application (daa)

Only EPN in adult showed symptoms of melanization. *H. bacteriophora* caused a higher rate of this defense mechanism after 30 days (37.5%) than *S. feltiae* (13.2%). Of all 180 evaluated adult bugs 120 specimens were invaded by EPN after 15 days and 160 after 30 days **(Figure 3**). 45 individuals (28%) of these bugs harbored melanized EPN after 30 days (dead, rigid, and dark brown coloring) **(Figure 3**). The period of harboring (15 and 30 daa) EPN did not show significant differences. The low number of BB with melanized EPN and the low mortality indicate that this insect possesses more defense mechanism than only melanization. This conclusion is strengthened by the observation that the utilized EPN killed all *G. mellonella* larvae. More research is needed to understand the defense mechanisms of the BB.

All inoculated EPN maintained their viable character confirmed by the 100% mortality of *Galleria* larvae. However, we didn't find any penetration of EPN in the fifth instar. Moreover, *S. feltiae* didn't cause any mortality in adults, whereas *H. bacteriophora* obtained a mortality of 49%. This

result is contradictory to the experiment in the lab (see **Figure 4**) where *S. feltiae* obtained the highest mortality.

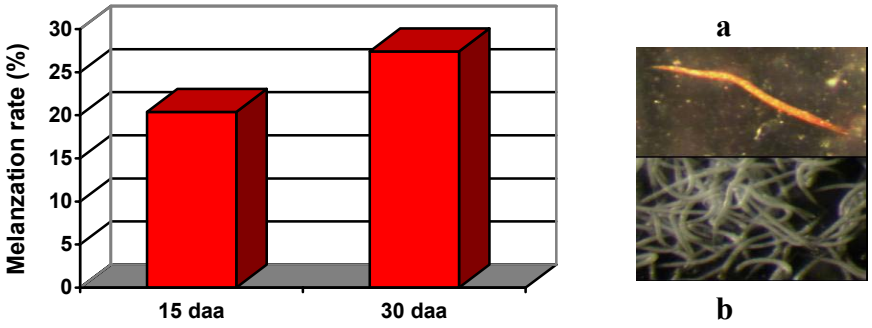

Figure 4. Percentage of BB harboring melanized EPN; a. melanized EPN, b. healthy EPN after 15 and 30 days of application (daa)

Conclusions

- The adult BB is more susceptible to EPN than the fifth instar
- Infectivity can be evaluated 15 daa; however, mortality should be determined after 30 dda
- *S. feltiae* was the most affected strain by melanization
- The applied commercial products remained viable during the entire experiment

Recommendations

- These experiments should be repeated in order to verify the obtained results and include other EPN strains.
- Experiments should be realized in order to understand the defense mechanisms of BB

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Contributors: Elsa Liliana Melo, Carlos Alberto Ortega, Carlos Julio Herrera, Rodrigo Zuñiga, Catalina Ramirez, Carmen Elisa Mendoza, Rómulo Riascos.

Activity 12. Control of *Cyrtomenus bergi* using entomopathogenic fungi.

M.Sc. Thesis. Faculty of Horticulture. University of Hannover, Germany.

In this study we investigated the pathogenicity of four strains of entomopathogenic fungi (EF) against the subterranean burrower bug *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae) under laboratory and greenhouse conditions. In order to select the most promising EF species/strain for *C. bergi* control, in the first part of the study the pathogenicity of three native Colombian strains of *Metarhizium anisopliae* (CIAT 224, CIAT 230, CIAT 245) and one of *Paecilomyces* sp. (CIAT 308) were tested against fourth instar nymphs of *C. bergi* in sand bioassays under laboratory conditions. In the initial screening as well as in the re-infection host passage experiments, comparatively low levels of corrected mortality were obtained never exceeding 50% (twenty days after treatment). *M. anisopliae* strains CIAT 224 and CIAT 245 caused the highest corrected mortality (34.7 and 49.3%, respectively) at the end of the initial screening. Based on these results the efficacy of combined applications of *M. anisopliae* strain CIAT 224 and a sub-lethal dose of imidacloprid (Confidor® SC 350) was evaluated under laboratory and greenhouse conditions. Initially, the *in vitro* fungitoxic effects of three different concentrations of imidacloprid on the germination and vegetative growth of *M. anisopliae* colonies were studied. After 24 hours germination ranged from 97-98.6% and was not significantly affected by any dose rate of imidacloprid present in the Potato Dextrose Agar (PDA) culture medium. On the other hand, the vegetative growth of the fungus was affected by the presence of imidacloprid in the medium. Significantly greater *M. anisopliae* colony size was found when the fungus was cultured in PDA medium amended with 300 ppm of imidacloprid. Subsequently, *C. bergi* nymphal mortality was assessed in a sand bioassay. Significantly higher *C. bergi* nymphal mortality was always recorded when *M. anisopliae* was applied in combination with imidacloprid compared to sole applications of the fungus. A *M. anisopliae* treatment (1E+07 conidia/gram of sand) in combination with 30 ppm of imidacloprid resulted in 87.2% nymphal mortality compared to 34.2% when the fungus was applied alone at the same dose rate 20 days after application. Sub-lethal doses of imidacloprid not only enhanced the efficacy of the fungus but also lead to a reduced quantity of inoculum needed to cause high levels of nymphal mortality. For instance 80.3% mortality was recorded 25 days after application of 1E+06 conidia/gram of sand of *M. anisopliae* strain CIAT 224 and a sub-lethal dose of imidacloprid; this level of mortality did not differ significantly from a 10-fold decreased concentration (i.e., 1E+05 conidia/gram of sand) and the same imidacloprid dose. Combined applications of *M. anisopliae* and a sub-lethal dose of imidacloprid also resulted in high *C. bergi* mortality when applied to a native Colombian soil under greenhouse conditions. Mortality levels in nymphs in the combined treatment 30 days after application were 87.1 and 82.6% in sterile and non-sterile soil, respectively, compared to 66.5 and 32.4% in the same soil types following a sole application of *M. anisopliae*. In conclusion, the results of this thesis indicate that the use of combinations of *M. anisopliae* and a sub-lethal dose of imidacloprid might become an important component within an IPM strategy against *C. bergi* and possibly also other subterranean pests in Colombia, thereby enabling farmers to reduce the use of highly toxic synthetic insecticides like chlorpyrifos and carbofuran, currently the most common control strategy for *C. bergi*. However, in a next step combination of *M. anisopliae* and a sub-lethal dose of imidacloprid need to be tested under field conditions.

Contributors: Juliana Jaramillo-Salazar, Christian Borgemeister, Lemma Ebssa (IPP, University Hanover), Gisbert Zimmermann (BBA, Darmstadt), Rosalba Tobón, Sonia Ximena Restrepo, Rodrigo Zúñiga, Carmen Elisa Mendoza, Andreas Gaigl (CIAT), and Esther Cecilia Montoya (Cenicafé).

Activity 12. Behavior of *Cyrtomenus bergi* as response to the presence of entomopathogenic fungi by means of radiography.

Introduction

Soil insects like whitegrubs and crickets are able to avoid the sites where their natural enemies are present. using a methodology that includes the creation of a microcosm and the use of radiography it was possible to corroborate this behavior for larvae of the Japanese Beetle (*Popillia japonica)*, European Chafer (*Rhizotrogus majalis*), Oriental Beetle (*Anomala orientalis*) and nymphs or adults of crickets. Determining the soil insect's behavior is important for the selection of a natural enemy. Moreover, biochemical studies should identify responsible metabolites emitted by the entomopathogenic fungi (EPF) that have this repellent effect.

Methodology

Experiment 1: We used white PVC tubes of ¾ and ½ inches diameter and filled 60g soil type Cornell with 10.5% humidity from both ends with 100 in order to have an equal density. Then, we introduced one 5th instar of the BB and a pregerminated maize grain via an aperture of 0.6 cm diameter in the middle of the tubes **(Figure 1)**. We took radiographies of different potential (45, 50, 55, 60, 65, and 70 Hz) and exposition periods (5, 10, 15, and 20 sec). We realized eight replicates of each treatment. The treatments are presented in **Figure 2**.

The units were placed in an incubator (25 °C, 70% RH, 100% darkness). We took radiographies after 0, 1, 2, 3, 4, 5, 6, 12, 24, 36, 48, 60, 72, and 96 hours after introducing the insect into the experimental unit.

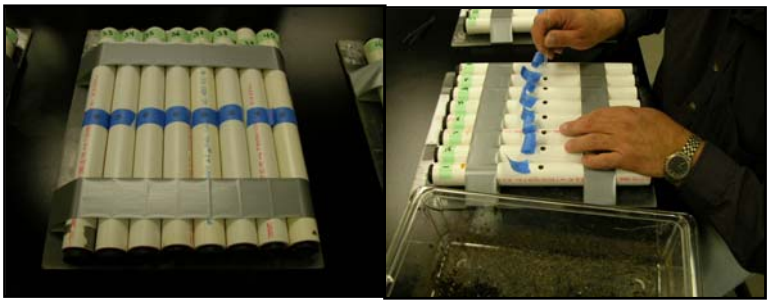

Figure 1. Experimental units and assembling of experiment. The left photo shows the tubes for taking radiography; the right the introduction of bugs of the 5th instar.

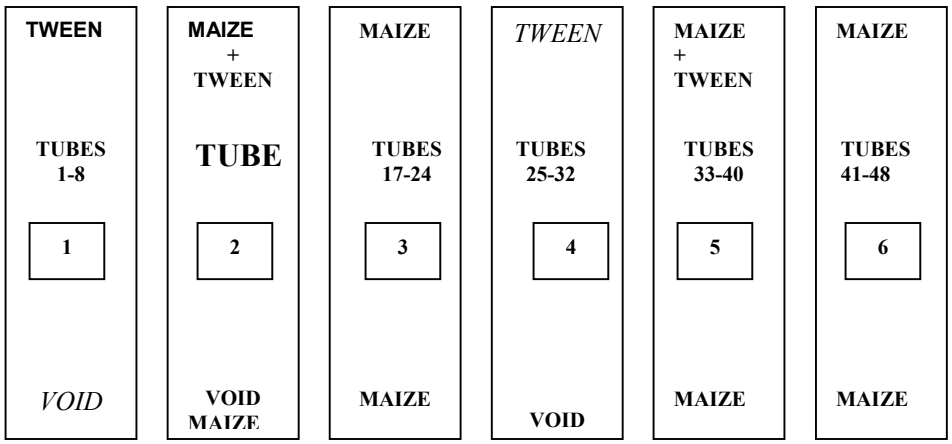

Figure 2. Treatments of Experiment 1: movement detection of *C. bergi* by means of radiography.

Experiment 2: The experimental setup was the same as in Experiment 1 with some modifications: we used only 60 Hz and exposed the film only 15 sec. The treatments are displayed in **Figure 3**.

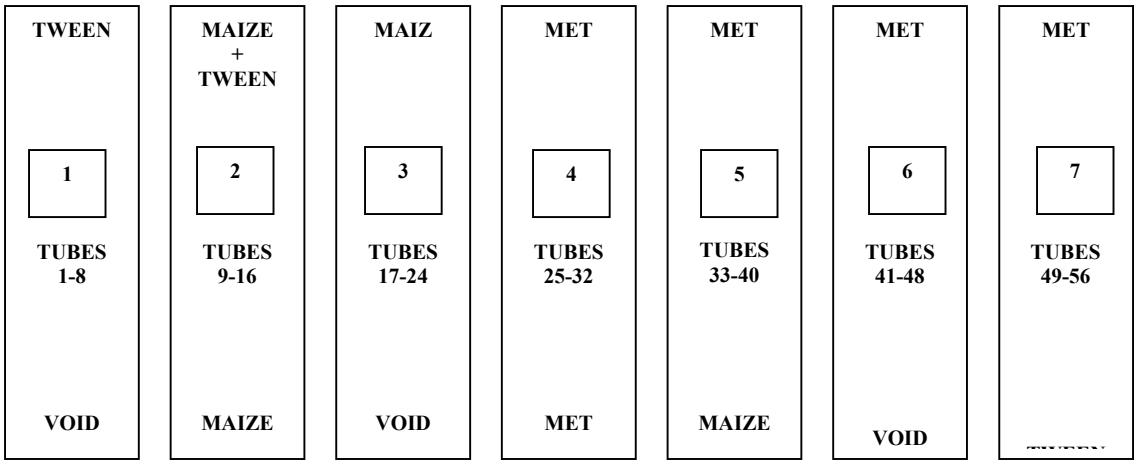

Figure 3. Treatments of Experiment 2: movement detection of *C. bergi* by means of radiography. MET= *Metarhizium anisopliae* (USDA collection, this strains was isolated from the Japanese Beetle).

Results

Experiment 1: It was not possible to detect the insect in the tubes of ¾ inches because the photography became blurred. Resolution was much better with the tubes of ½ inches. Hence, we could define that the potential of 60 Hz and an exposition of 15 seconds were the optimal

adjustments of the camera. Moreover, we decided to replace the maize by peanuts due to the fast growing roots of the maize that probably allowed the insect not to move.

Experiment 2: The fungus didn't cause any reaction of avoidance by the bug; however, we could observe the insect moving within the tube. The insect moved less when the fungus was present at both ends of the tube compared to the tubes where this pathogen was absent.

Discussion: Following conclusions can be drawn from this preliminary experiment: The deployed methodology is useful to detect movements of the BB; however the lack of a clear tendency of the bug behavior needs to be reflected. One reason may be that the fungus wasn't directly isolated from the BB and it might be possible, that it is not even pathogenic to the insect. Another explanation might be that the spore concentration was too low compared to former experiments realized with whitegrubs and crickets. Hence, we recommend continuing these interesting studies deploying higher concentrations and volumes of spores and use strains with known pathology to *C. bergi*.

Contributors: Anuar Morales (Cornell University), Daniel Peck, Andreas Gaigl.

Activity 13. Production of *Galleria mellonella* within 44 days for studies with entomopathogens.

The larvae of *Galleria mellonella* are commonly used in entomological studies on biological control, such as tests of infection and mortality by entomopathogenic organisms such as nematodes, fungi or bacteria, capturing entomopathogens from the soil, and mass production of these organisms. For this reason we need a high and rapid production of healthy larvae of the last instar. Here we present the 7 steps of our new technology.

Step 1
Sterilize all recipients with hypochlorite and UV-radiation.

Step 2
Introduce glass paper with paraffin bended like an accordion into recipient, then, adults (10 males and 20 females) are confined in these pots and kept during five days in darkness.

Step 3

100 g artificial diet per recipient.
Normal: 500g wheat bran, 145g yeast, 72g bee wax, 150ml glycerin, 270ml bee honey, 1% formaldehyde of all liquids.

Alternative: 400g wheat bran, 100g yeast, 70g bee wax, 400g glycerin, 300g honey, 200g milk powder, 200g maize mill, 100g wheat germs.

The paraffin paper is introduced into the trays. Once the larvae have hatched, the food has been consumed and the larvae have moulted into the third instar the trays have to be moved to bigger trays and added more food. Moreover, the paraffin paper has to be removed in order to prevent eggs from hatching and to obtain a greater homogeneity of size and age of the larvae.

Step 4
The larvae should be permanently revised for infection by fungi or bacteria. Is this the case then the tray has to be removed immediately. If the infection is limited to only some individuals it is sufficient to replace them by healthy ones.

Step 5
Diet should be provided twice a week. The alternative diet should be given twice a month. Larvae have to be divided when the tray is overcrowded. Larvae climbing the walls of the trays in spite of sufficient food is available is an indicator that the tray is overcrowded. Then, a part has to be transferred to a new tray.

Step 6
When the first larvae start to web the cocoon and reduce food consumption it is time to select larvae for experiments. The not considered larvae can be left in their trays and stored in an incubator in order to delay moulting to pupae and adults.

Step 7
It is recommended to carry the whole time a big tray with paper towels in order to transfer potential pupae into this tray. The future adults that are necessary for the colony will be obtained in these trays.

Contributors: Catalina Ramirez, Elsa Liliana Melo, and Andreas Gaigl.

Activity 14. Publications, Conferences, Workshops, Training, Students.

Publications

Accepted

Struck, E., L. Ebssa, R.-U. Ehlers, H.-M. Poehling, A. Gaigl and C. Borgemeister. 2004. Interactions between host plants, the subterranean burrowing bug, *Cyrtomenus bergi*, and the entomopathogenic nematode *Heterorhabditis megidis.* Nematology (July 2, 2004).

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Zuluaga, C. A., M.S. Serrano, and A. Gaigl. 2004. Distribución espacial, fluctuación poblacional y enemigos naturales de chizas en pasto kikuyo en Cundinamarca, Colombia. Submitted to Rev. Colombiana Entomol. (Spanish) Revista Colombia de la Sociedad de Entolomolgia).

In Preparation

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Zuluaga, C.A., M.S Serrano, L.C. Pardo & A. Gaigl. 2004. Identification of larvae of Melolonthidae associated to "Kikuyo" grass and potatoes in Cundinamarca, Colombia.

Zuluaga, C.A., M.S. Serrano, A. Gaigl & L. C. Pardo. 2004. Natural enemies of whitegrubs from potatoes and "Kikuyo" grass in Andean regions of Colombia.

Presentations

Calberto, G.A.; L.C. Pardo y A. Gaigl. 2004. Observaciones preliminares sobre el crecimiento y ciclo de vida de la chisa *Phyllophaga menetriesi* (Coleoptera: Melolonthidae) en condiciones de laboratorio. Memorias XXXI Congreso Sociedad Colombiana de Entomología, SOCOLEN. July 28-30, Bogotá, Colombia. p. 42 (Spanish).

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Gaigl. A. 2004. Investigación del CIAT en el chinche de la viruela. Capacitation of asparagus farmers associated with Erupción. June 15, 2004.

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Cydnidae). Memorias XXXI Congreso Sociedad Colombiana de Entomología, SOCOLEN. July 28-30, Bogotá, Colombia. p. 98 (Spanish).
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Pardo, L.C., M.A. Morón, A. Gaigl y A.C. Bellotti. 2003. Los complejos regionales de Melolonthidae (Coleoptera) rizófagos en Colombia. In: Aragón, G.A.; M.A. Morón, and A. Marín (Editors): Estudios sobre coleópteros del suelo en América. Benemérita Universidad Autónoma de Puebla, Mexico, pp. 45-63. (Spanish).
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Zuluaga, C.A.; M.S. Serrano; L.C. Pardo; A. Gaigl. 2003. Fluctuacion poblacional, identificacion de larvas y enemigos naturales de chisas en pasto Kikuyo en Cundinamarca. Memorias XXX Congreso Sociedad Colombiana de Entomología, SOCOLEN. July 17-19, Cali, Colombia. p. 73 (Spanish).
Zuluaga, C.A.; M.S. Serrano; J. Parada; A. Gaigl. 2003. Registro de nematodos entomoparasitos asociados a chisas en Cundinamarca. Memorias XXX Congreso Sociedad Colombiana de Entomología, SOCOLEN. July 17-19, Cali, Colombia. p. 73 (Spanish).

Posters

Melo, E.L.; C.A. Ortega; A. Gaigl; A.C. Bellotti; C. Borgemeister, R.-U. Ehlers, and A. Susurluk. 2004. Búsqueda de poblaciones nativas de nematodos entomopatógenos en regiones de Colombia y Panamá. Memorias XXXI Congreso Sociedad Colombiana de Entomología, SOCOLEN. July 28-30, Bogotá, Colombia. p. 98 (Spanish).

L.C. Pardo and A. Gaigl. 2004. Complejo chisa en Colombia (Coleoptera : Melolonthidae). Generalidades y avances en identificaciones. Presented on Field Days in Pescador on the farm Manuel Trujillo, April 6, 2004 and the Hacienda Córcega, Quimbaya (Quindío), February 2, 2004.

Complejo de chisas identificadas en el municipio de Quimbaya (Quindío), Finca Córcega. Presented at SENA, Armenia, June 2, 2004.

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Telepacífico (Mayo 12 and 19, 2004). Mirando al Mundo con Ojos de Mujer.

Awards

Zuluaga, C.A., M.S. Serrano, L.C. Pardo and A. Gaigl. 2004. Fluctuacion poblacional, identificacion de larvas y enemigos naturales de chisas en pasto Kikuyo en Cundinamarca. Presented at XXX Congreso Sociedad Colombiana de Entomología, SOCOLEN. July 17-19, Cali, Colombia. Third place of Premio Francisco Luis Gallego, July 30, 2004.

Training

- Training in taxonomy of scarabs Melolonthidae for the assistants of CIAT Cassava Entomology. September 6–10, 2004. The course was held by Jhon Cesar Neita, taxonomist of the Universidad Nacional, Bogotá.
- Training of farmers and extensionists in Pescador (Cauca), Quimbaya and Armenia (Quindío) in biology and basic taxonomy of whitegrubs. In both sites we realized the capacitating twice. The first course was to evaluate farmer's knowledge of whitegrubs, in the second course farmers learned to identify the most important whitegrub species and they got familiar with the project objectives and activities (February 17 and June 2, 2004, in Quimbaya and Armenia, respectively) and April 6 in Pescador (Cauca).
- Field day organized by Erupcion, Manizales, an association of asparagus producers, where the latest results of research on the control of the burrower bug were presented. (June 15, 2004).

Thesis Students

Carlos Alberto Ortega (M.Sc., Escuela Politécnica del Ejército, Quito). Evaluation of whitegrub damage (Coleoptera: Melolonthidae) after natural infestation on cassava *Manihot esculenta* in Pescador (Cauca).

Carolina Buitraga Aya (Universidad del Valle). Evaluación de la patogenicidad y virulencia de *Bacillus popilliae* Dutky sobre larvas de tercer instar de *Phyllophaga menetriesi* Blanchard (Coleoptera: Melolonthinae).

Thesis Completed

German Andreas Calberto (Universidad Autonoma de Occidente, Cali). Estudio del ciclo de vida de *Phyllophaga menetriesi* B. (Col: Melolonthidae) en condiciones de cría artificial.

Catalina Ramirez (Universidad Autonoma de Occidente, Cali). Obtencion de *Galleria mellonella* (Lepidoptera: Pyralidae) en un periodo de 44 días para estudios con entomopathogenos.

Project Staff List

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