On behalf of the Rector of the University of Ghent and the Dean of the Faculty of Agricultural and Applied Biological Sciences, you are cordially invited to the public defence of the doctoral thesis of

Minshad Ali Ansari

entitled

Biological control of *Hoplia philanthus* (Coleoptera: Scarabaeidae) with entomopathogenic nematodes and fungi

Biologische bestrijding van *Hoplia philanthus* (Coleoptera: Scarabaeidae) met entomopathogene nematoden en schimmels

The defence will take place on 18th May 2004 at 17.30h in room 20 (Block A) of the Faculty of Agriculture and Applied Biological Sciences, University of Ghent, Coupure links 653, Ghent.

Summary

The white grub, *Hoplia philanthus* Füessly, (Coleoptera: Scarabaeidae) became an economically important pest of lawns, turf, pastures and ornamentals in Belgium. The overall objective of this study was the search for an alternative control method for the recently emerged pest insect. The strategy we used along this study was to search for two biological control agents (BCAs) (i.e. entomopathogenic nematodes and fungi) to learn more about their activity and mode of action when they are applied alone. In addition, we examined whether the combined application of both BCAs could result in a better control of *H. philanthus* larvae.

Several series of laboratory, greenhouse and field experiments were conducted. In a first series the nematode species, *Heterorhabditis megidis*, *Steinernema feltiae* and *S. glaseri* were tested against *H. philanthus* in order to select a virulent nematode species for further study. In a dose response experiment, 0, 1, 10, 100, 1000 and 10,000 infective juveniles (IJs) were inoculated in wells of 24-well plates containing sand along with a single *H. philanthus* larva. *Steinernema glaseri* was most effective (LC_{50} = 4.6 and LC_{90} = 79.3 IJs/larva after 7 days exposure) compared to *H. megidis* (LC_{50} = 9.7 and LC_{90} = 511.8 IJs/larva). The lethal concentration of *S. feltiae* could not be determined because of the strain's low virulence against *H. philanthus*. In a second experiment, the pathogenicity of the symbiotic bacteria *Photorhabdus luminescens*, *Xenorhabdus bovienii* and *X. poinarii* was studied. Three microlitres of a bacterial suspension (containing 0, 25, 250, 2500 or 25,000 cells) were injected into the haemoceel of *H. philanthus* and late instars of *Galleria mellonella*. *Photorhabdus luminescens* and *X. bovienii* killed 100% of the larvae of both species after 72 h; *X. poinarii* caused lowest mortality for both insect species. In a third experiment, 3 µl of a cell-free filtrate of *P. luminescens*, *X. bovienii* and *X. poinarii* were injected into the haemoceel of *H. philanthus* and *G. mellonella*. The filtrates of *P. luminescens* and *X. bovienii* caused 100% mortality after 24 h to both insects. The *X. poinarii* filtrate was least toxic to both insect species. In pot trials, *H. megidis* and *S. glaseri* caused more than 80% mortality of *H. philanthus* larvae infesting potted perennial ryegrass (*Lolium perenne*) 42 days after application of 2.5-7.5 billion nematodes/ha. The mortality was greater than the grub mortality caused by either *S. feltiae* (16%) or the control (10%).

In order to select virulent fungal species for further study, 34 isolates from the genera *Metarhizium*, *Beauveria* and *Paecilomyces* were tested in bioassays by dipping larvae in 1×10^{7} conidia/ml suspensions. Two isolates of *M. anisopliae* (CLO53 and CLO54) caused maximally 90% mortality 10 weeks post-inoculation while other isolates only caused mortalities between 10 and 62%. The virulence of *M. anisopliae* CLO53 was further tested by exposing *H. philanthus* larvae to conidial serial concentrations of 10^{4} to 10^{9} conidia/g sandy soil for up to 11 weeks at 15, 20 or 25°C. Mortality was dependant on the fungal concentration, exposure time and temperature. Eleven weeks after inoculation, the LC_{50} values for this isolate ranged from 1.3 to 4.0 × 10^{6}, 1.0 to 3.2 × 10^{5} and 2.5 × 10^{4} to 10^{5} conidia/g soil at 15, 20 and 25°C, respectively. The LT_{50} values for this isolate ranged from 3.5 to 21.7, 2.4 to 18.7 and 2.9 to 16.1 weeks at concentrations of 10^{9} and 10^{4} conidia/g soil at 15, 20 and 25°C, respectively. In a glasshouse pot experiment with perennial ryegrass, the isolate CLO53 caused mortalities of 50 and 88% of *H. philanthus* larvae 10 weeks after application of 1× 10^{4} and 1 × 10^{6} conidia/cm² soil surface, respectively. The results suggest that the Belgian isolate CLO53 has excellent potential for biological control of *H. philanthus*.

The interaction between *M. anisopliae* CLO53 and *H. megidis* and *S. glaseri* against *H. philanthus* was studied in a third series of experiments. Insect larvae were exposed to various concentrations of both *M. anisopliae* and nematodes and larval mortality was assessed weekly. Nematodes were added simultaneously or 2, 3 and 4 weeks after application of *M. anisopliae*. Throughout the experiments, the combined application of *M. anisopliae* with nematodes increased larval mortality either in an additive or in a synergistic way. To achieve strong synergistic effects, larvae had to be exposed to *M. anisopliae* for at least 3 or 4 weeks before the addition of nematodes. This interaction was observed between *M. anisopliae* and both nematode species. The nematode reproduction in insect larvae exposed to the *M. anisopliae*-*H. megidis* combination was not significantly higher than in larvae exposed to only *H. megidis*. The combination of higher concentrations of *M. anisopliae* with *H. megidis* resulted in an antagonistic effect on nematode reproduction. In a greenhouse trial, *H. philanthus* larvae were placed in three-litre pots with perennial ryegrass treated with *M. anisopliae*, the nematodes *H. megidis* and *S. glaseri*, or the combination of fungus and nematode. Combinations of *M. anisopliae* and nematodes generated a synergistic effect at all application rates (1 × 10^{12} and 1 ×

10^{13} conidia/ha). This effect was observed when *M. anisopliae* was applied first and followed by the nematodes 4 weeks later.

Finally, these interactions were also tested in field conditions. Three field experiments were conducted in September to November 2003 against natural populations of *H. philanthus* consisting of late second and early third-instars. In all experiments, the combination of *M. anisopliae* with *H. bacteriophora* resulted in additive mortality but not in synergistic effects. The *H. bacteriophora* and *M. anisopliae* combination is likely to improve control of *H. philanthus* larvae. Combinations of *M. anisopliae* and nematodes may offer a powerful and reliable tool for biological control of *H. philanthus* or other insect pests. These combinations may not only relieve of the use of broad-spectrum chemical insecticides, but also provide between season controls because of their ability to recycle in field populations.

Curriculum vitae

Minshad Ali Ansari was born on December 31, 1968 in Setlapur (Mahrajgung), Uttar Pradesh, India. In 1990, he obtained the degree of Bachelor of Science in Agriculture at University of Gorakhpur, India. In 1993, he obtained his MSc degree in Agriculture Nematology from the Institute of Agriculture at Aligarh Muslim University, India. Afterwards, in 1996, he was employed as a Research Associate (Crop Protection Division) at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, India. During the academic year 1998-1999 he got a grant from the VLIR to follow the International Nematology Course organised at Ghent University. Immediately after he obtained his MSc degree in Nematology he started his PhD research at the CLO-Department of Crop Protection, Merelbeke, under the guidance of Prof. Dr ir. M. Moens and Prof. Dr ir. L. Tirry. He published more than 10 papers in peer review international journals.

Promoters

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FACULTY OF AGRICULTURAL AND APPLIED BIOLOGICAL SCIENCES

Invitation

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Tuesday 18th May, 2004, 17.30 h

Room A20
Faculty of Agricultural and Applied Biological Sciences, University of Ghent