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BIOLOGICAL CONTROL OF *RHYNCHOPHORUS FERRUGINEUS*

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SUMMARY
The invasive red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is one of the most destructive pests of palms in the world. To date, the control of this pest has been mainly based on the use of insecticides. However, this control method can result in environmental, social and economic problems. To improve management options against the weevil, the efficacy of the entomopathogenic nematode *Steinernema carpocapsae* Weiser (Nematoda: Steinernematidae) and the potential of a strain of the entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Clavicipitaceae) were evaluated in laboratory, semi-field and field assays. The use of these entomopathogenic microorganisms resulted highly efficient against *R. ferrugineus*.

Key words: *Steirnernema carpocapsae*, *Beauveria bassiana*, entomopathogenic microorganism.

RÉSUMÉ
CONTROLE BIOLOGIQUE DE *Rhynchophorus ferrugineus*
Le charançon rouge du palmier, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), est l'un des ravageurs les plus destructeurs des palmiers dans le monde. A ce jour, le contrôle de ce ravageur a été principalement basé sur l'utilisation d'insecticides. Cependant, cette méthode peut entraîner des problèmes environnementaux, sociaux et économiques. Afin d'améliorer les options de gestion contre le charançon, nous avons évalué l'efficacité du nématode entomopathogène *Steinernema carpocapsae* Weiser (Nematoda: Steinernematidae) et le potentiel d'une souche du champignon entomopathogène *Beauveria bassiana* (Ascomycota: Clavicipitaceae) comme agents de lutte biologique contre le charançon à la fois dans des essais de laboratoire, en conditions semi-naturelles et sur le terrain. L'utilisation de ces microorganismes entomopathogènes a montré une très bonne efficacité contre *R. ferrugineus*.

Mots-clés : *Steirnernema carpocapsae*, *Beauveria bassiana*, micro-organisme entomopathogène.

INTRODUCTION

The invasive red palm weevil, *Rhynchophorus ferrugineus*, is one of the most destructive pests of palms in the world. Biological pest control relies on the use of natural enemies, either entomophagous arthropods (predators and parasitoids) or entomopathogenic microorganisms (nematodes, bacteria, fungi and viruses). Few studies have been conducted on the natural entomophagous enemies of *R. ferrugineus* or other *Rhynchophorus* species (Murphy & Briscoe 1999; Faleiro, 2006). The predatory earwig *Chelisoches morio* (Fabricius) (Dermaptera: Chelisochidae) was studied under laboratory and field conditions in India (Abraham and Kurian, 1973). However, it did not provide a measurable impact on the weevil. Although various mites have been reported in India as parasites of *R. ferrugineus* (Nirula, 1956; Peter, 1989), their impact on the population has not been determined yet. Gopinadhan *et al*. (1990) reported that a cytoplasmic polyhedrosis virus infected all stages of the weevil in Kerala (India). Infected mature-larval stages resulted in deformed adults and severe suppression of the host population. The entomopathogenic nematode *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae) proved effective against *R. ferrugineus* in semi-field including both preventive and curative assays (Llácer *et al.*, 2009). Likewise different strains of *Metarhizium anisopliae* (Metschnikoff) Sokorin (Ascomycota: Clavicipitaceae) and *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Clavicipitaceae) were tested against *R. ferrugineus* (Gindin *et al.*, 2006). The former proved more virulent than *B. bassiana*. However, none of the strains tested was originally obtained from diseased *R. ferrugineus* specimens. More recently, in preliminary studies, Sewify *et al.* (2009) successfully reduced the incidence of *R. ferrugineus* under field conditions in Egypt using a native strain of *B. bassiana* isolated from a *R. ferrugineus* cadaver.

ENTOMOPATHOGENIC NEMATODES

An interesting alternative to the chemical control of *R. ferrugineus* is the use of entomopathogenic nematodes (EPNs) (Abbas *et al.,* 2001a, b; Elawad *et al.,* 2007; Saleh and Alheji, 2003, Llácer *et al.,* 2009; Dembilio *et al.,* 2010a). EPNs are safe for non-target vertebrates and for the environment, and production costs have been significantly reduced in recent times as they are mass produced in liquid media (Ehlers 2001, 2003). The infective third juvenile stages (Dauer Juvenile, DJ) survive outside the insect and can actively search for the host. DJs enter the insect host through any natural opening (mouth, anus or spiracles) and grow into the parasitic stage. The death of the insect due to nematode parasitism is caused by Gram-negative bacteria which are carried within the gut of the DJs (Forst and Clarke, 2002). *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae), which is mutualistically associated with the bacterium *Xenorhabdus nematophila* (Enterobacteraceae), is the most studied, available, and versatile of all EPNs. Although field experiments in date palms, *Phoenix dactylifera* L., conducted several years ago, produced inconsistent results (Abbas *et al.,* 2001b), recent assays carried out using *S. carpocapsae* in a chitosan formulation were successful (Llácer *et al.,* 2009; Dembilio *et al.,* 2010a). Semi-field trials including both preventative and curative assays proved the potential of this nematode to control *R. ferrugineus*. Efficacies around 80% were obtained in a curative assay, and up to 98% in a preventative treatment in *Phoenix canariensis* Hort. ex. Chabaud (Llácer *et al.,* 2009). This treatment proved highly efficient in *Phoenix theophrasti*, as well (Dembilio *et al.,* 2011). Under field conditions, treatments using imidacloprid and *S. carpocapsae*, either alone or in combination, were not significantly different, with efficacies ranging from 73 to 95% (Dembilio *et al.,* 2010a). Therefore, EPNs should not be neglected when developing guidelines for treatments against *R. ferrugineus*.

ENTOMOPATHOGENIC FUNGI

In addition to EPNs, entomopathogenic fungi (EPF) can also provide an excellent alternative to chemical control. Unlike EPNs, EPF infect the host by contact, then germinate and penetrate the insect cuticle. The host can be infected both by direct treatment and by horizontal transmission from infected insects or cadavers to healthy insects. Subsequently, infection can occur via the new generation of spores (Lacey *et al.*, 1999; Quesada-Moraga *et al.*, 2004). These unique characters make EPF especially important for the control of concealed insects such as *R. ferrugineus*. Different strains of *Metarhizium anisopliae* and *Beauveria bassiana* were tested against *R. ferrugineus* (Gindin *et al.*, 2006). The former proved more virulent than *B. bassiana*. However, none of the strains tested was originally obtained from diseased *R. ferrugineus* specimens. More recently, in preliminary studies, Sewify *et al.* (2009) successfully reduced the incidence of *R. ferrugineus* under field conditions in Egypt using a native strain of *B. bassiana* isolated from a *R. ferrugineus* cadaver.

In 2007, *R. ferrugineus* pupae presumed to be infected with EPF were collected in a date palm grove in Spain (Dembilio *et al.*, 2010). The *B. bassiana* strain isolated from these pupae proved to infect eggs, larvae and adults of *R. ferrugineus*. Furthermore, *B. bassiana* infection reduced adult lifespan from one half to almost one tenth. Adults of either sex inoculated with the fungus efficiently transmitted the disease to untreated adults of the opposite sex conferring rates of transmission between 55% and 60%. In addition, treatment with this *B. bassiana* strain significantly reduced fecundity (up to 63%) and egg hatching (33%). Likewise, 30–35% increase in larval mortality was observed in larvae obtained from eggs from fungus inoculated females or from untreated females coupled with inoculated males, resulting in an overall 78% progeny reduction compared to an untreated control. This strain was subsequently tested in semi-field preventive assays on potted 5-year old *P. canariensis* palms. Efficacies up to 86% were obtained, and these results are indicative that contact infection of adults actually occurred and confirm the potential of this strain as a biological control agent against *R. ferrugineus*. Consequently, adults should be considered as the targets of any treatment involving this entomopathogenic fungus because they are actually the only free-living stage.

Strategies aimed at attracting and infecting adult weevils could prove the most effective way to spread the disease, and this is one of the works that our group is developing at this moment. The main difficulty in the implementation of the above mentioned approach is pest accessibility to these biocontrol agents. Being mostly hidden within the host tissue, a systemic distribution of the agent is highly desirable. It has been proved that fungal endophytes play an important role in protecting plants against herbivorous insects (Jallow *et al.,* 2004; 2008) and plant pathogens. *Beauveria bassiana* has been reported as an endophyte in a variety of plants (Bills and Polishook, 1991; Evans *et al*., 2003; Quesada-Moraga *et al.,* 2006; Posada *et al.,* 2007), including monocotyledonous species such as maize (Bing and Lewis 1991; 1992a; b; Wagner & Lewis, 2000) and *Pinus monticola* (Ganley and Newcombe 2005). In some cases these endophytic fungi have resulted in complete control of the target pest (Quesada-Moraga *et al.,* 2006). However, the use of fungal strains of EPF with endophytic behaviour is still a poorly explored tool for systemic protection of palms against *R. ferrugineus*. Therefore, it is urgent to perform sound open field experiments with *B. bassiana* strains to explore their biocontrol potential.

ENTOMOPATHOGENIC BACTERIA

The efficacy of the entomopathogenic bacterium *Bacillus thuringiensis* to control *R. ferrugineus* has been tested in laboratory conditions (Manachini *et al*., 2009; Alfazariy, 2004). Although there were evidences indicating midgut damage and feeding inhibition among larvae that survived the treatments, results showed that the activity of *B. thuringiensis* against *R. ferrugineus* immature stages was low.

CONCLUSION

Steirnernema carpocapsae in a chitosan formulation is highly effective against *R. ferrugineus* in the field. Different timings and product combinations were studied, and high efficacies were obtained in all cases. *Steinernema carpocapsae* was applied on a monthly basis and therefore resulted more expensive and time consuming than chemical applications. However this invertebrate biological control agent could be most suitable for ornamental palms in public areas.

An indigenous strain of *B. bassiana* found naturally infecting pupae of *R. ferrugineus* resulted highly virulent against all developmental stages of the weevil in the laboratory. Additionally, adults of either sex inoculated with the fungus efficiently transmitted the disease to healthy adults of the opposite sex. Furthermore, *B. bassiana* infection resulted in reduced fecundity and egg hatching. Semi-field preventive treatments on *P. canariensis* palms with this strain were highly effective against *R. ferrugineus* and this result confirmed the potential of *B. bassiana* as a biological control agent against this pest.

Overall, to improve *R. ferrugineus* control by entomopathogenic microorganisms, a more deep investigation on the interaction between them and *R. ferrugineus* immune system is required. Studies, such as that of Manachini *et al*. (2011), who studied the response of *R. ferrugineus* larval hemocytes to infection by *B. thuringiensis* and *Saccharomyces cerevisiae* could contribute to this final goal.

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