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Review

An overview on the natural enemies of *Rhynchophorus* palm weevils, with focus on *R. ferrugineus*

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HIGHLIGHTS

- *Rhynchophorus* species are polyphagous and some are pests of several palm species.
- We reviewed their natural enemies in both their native and introduced regions.
- More than 50 natural enemies have been reported to attack *Rhynchophorus* species.
- Fungi are the most promising ones for biological control.

GRAPHICAL ABSTRACT

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ABSTRACT

Rhynchophorus palm weevils are large insects belonging to the family Dryophthoridae. All *Rhynchophorus* species are polyphagous and have a similar life history but some are major pests because of the serious economic damage they cause, in particular to several species of the family Arecaceae. Here we review the natural enemies of *Rhynchophorus* species in both their native and introduced regions of the world, to assess the possibility of biological control of this taxon. Moreover, particular attention is paid to the well-studied and harmful species *Rhynchophorus ferrugineus*, about which more information is available, and to its natural enemies in the Mediterranean region, because the impact of this pest in this recently colonized area is particularly remarkable and also the recent trend in species management is looking for indigenous natural enemies.

More than 50 natural enemies have been reported to attack *Rhynchophorus* species, even if most of them are associated to *R. ferrugineus* (Olivier), highlighting the lack of information on the other species of the genus. Pros and cons of all the biological control agents are then discussed: among the considered organisms, fungi are noteworthy to be considered for inclusion in integrated pest management programs.

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Overall, our overview underlines the need to increase knowledge on natural enemies of all the species of the genus *Rhynchophorus*, to isolate more virulent strains and to determine the optimum conditions for the actions of the biocontrol agents.

1. Introduction

Rhynchophorus palm weevils are large insects belonging to the family Dryophthoridae, subfamily Rhynchophorinae, and tribe Rhynchophorini (Bouchard et al., 2011). The genus *Rhynchophorus* was erected by Herbst in 1795 and has undergone several extensive revisions (Wattanapongsiri, 1966; Hallett et al., 2004). Nowadays, ten species are recognized: three from the New World [*Rhynchophorus cruentatus* (Fabricius), *Rhynchophorus palmarum* (L.) and *Rhynchophorus richteri* (Wattanapongsiri)], two from Africa [*Rhynchophorus phoenicis* (Fabricius) and *Rhynchophorus quadrangulus* (Queden)] and five from tropical Asia [*Rhynchophorus bilineatus* (Montrouzier), *Rhynchophorus distinctus* (Wattanapongsiri), *Rhynchophorus ferrugineus* (Olivier), *Rhynchophorus lobatus* (Ritsema) and *Rhynchophorus vulneratus* (Panzer)] (Wattanapongsiri, 1966; Hallett et al., 2004; Rugman-Jones et al., 2013). The taxonomic status of some species is still uncertain (e.g. *R. lobatus*), pending a comprehensive analysis combining morphological and biomolecular approaches, which could result in synonymization and clarify the relationships between species (see also Wattanapongsiri, 1966; Murphy and Briscoe, 1999). In this context, very recently, Rugman-Jones et al. (2013) provided conclusive evidence for the reinstatement of *R. vulneratus* as a valid species, distinct from *R. ferrugineus*.

All *Rhynchophorus* species are polyphagous and have similar life histories: females are usually attracted by palm volatiles and lay several eggs in dying or damaged parts of palms, although undamaged palms could also be attacked. After a few days, eggs hatch into larvae which develop within the trunks of palms, frequently leading to the plant death (Wattanapongsiri, 1966; Giblin-Davis et al., 1996; Murphy and Briscoe, 1999).

Few or no data on host plants and other biological information are available for four of these species (*R. distinctus*, *R. lobatus*, *R. quadrangulus* and *R. richteri*: Wattanapongsiri, 1966; Murphy and Briscoe, 1999). The other species (*R. bilineatus*, *R. cruentatus*, *R. ferrugineus*, *R. palmarum* and *R. phoenicis*) are well known and are major pests because of the serious economic damage they cause, in particular to several species of the family Arecaceae (Wattanapongsiri, 1966).

Due to the difficulty in timely detection of the damage caused by *Rhynchophorus* species during the early stages of infestation (particularly because of the cryptic habits of the larvae), integrated pest management (IPM) is mainly considered with an attempt to combine public awareness, precautionary measures and efficiency of control methods (Murphy and Briscoe, 1999; Faleiro, 2006a,b). Nowadays, control methods revolve around treatments based on chemicals, biotechnological systems using semichemicals or the development of the Sterile Insect Technique (hardly sustainable at this time) (e.g. Paoli et al., 2014), and biological control (Wattanapongsiri, 1966; Murphy and Briscoe, 1999; Faleiro, 2006a,b). Since chemical applications elicit serious concerns related to environmental pollution and insects' resistance, and affect human health, eco-friendly biological control has now attracted high interests. Biological control (or biocontrol) is a broad term including a variety of management actions based on the use of natural enemies of the invader. However, these enemies are not always specific to the target organism and may instead attack native organisms. Control agents should thus be thoroughly checked for specificity and for non-target effects before their release into the wild (De Clercq et al., 2011).

Reginald (1973) suggested that natural enemies cannot play a decisive role in controlling the world's worst pest of palm trees, *R. ferrugineus* (RPW) and few studies have been conducted on natural enemies of *Rhynchophorus* (Murphy and Briscoe, 1999; Faleiro, 2006a,b). Here, we review the natural enemies associated to palm weevils (Table 1) by considering their pitfalls and potentialities in order to pinpoint management techniques to be considered in the development and integration of biological control strategies. Compared to previous reviews focusing on RPW (e.g. Murphy and Briscoe, 1999; Faleiro, 2006a,b), this contribution gives particular emphasis to the Mediterranean area, where the peculiar traits of its invasion differed from those registered in most countries infested by the pest until about ten years ago (Jacas et al., 2011) and where, recently, many research studies have been performed because of the importance acquired by the pest since that time. In addition, this review extends the analysis to the entire set of *Rhynchophorus* enemies.

2. Viruses

Entomopathogenic viruses belong to 11 families and have been isolated from more than a thousand species and at least 13 different orders of insects (see Flexner and Belnavis, 2000). Virus diseases of insects have long been recognized, although only in the last 40 years has there been increasing interest in the use of these agents to control insect pests (Flexner and Belnavis, 2000).

The highly potent cytoplasmic polyhedrosis virus (CPV) is the only one recorded from RPW. After its first record in India, where it infected all stages of the insects (Gopinadhan et al., 1990), the virus was detected in Egypt from dead RPW (El-Minshawy et al., 2005). Infection in the late larval stage resulted in deformed adults and drastically reduced insect population (Gopinadhan et al., 1990).

3. Bacteria

Pathogenic entobacteria mostly belong to the families Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae and Micrococaceae (Tanada and Kaya, 1993). Although many bacteria can infect insects, only members of two genera of the order Eubacteriales, *Bacillus* and *Serratia*, have been registered for the control of insects (Tanada and Kaya, 1993).

For the genus *Rhynchophorus*, bacteria have only been isolated from RPW: Dangar and Banerjee (1993) discovered some belonging to *Bacillus* sp., *Serratia* sp. and the coryneform group in larvae and adults in India, while Alfazairy et al. (2003) and Alfazariy (2004) isolated *Bacillus thuringiensis* Berliner and *Bacillus sphaericus* Meyer and Neide from larvae and adults in Egypt. Alfazariy (2004) reported successful control of RPW in laboratory conditions by infection with *B. thuringiensis* subspecies *kurstaki* isolated from larvae in Egypt. In contrast, other authors showed a different susceptibility of RPW to *B. thuringiensis* (Manachini et al., 2008a,b, 2009). *Pseudomonas aeruginosa* (Schroeter) was isolated from infected larvae collected in Kerala, India (Banerjee and Dangar, 1995). Laboratory assays demonstrated that this bacterium was pathogenic for weevils when ingested through force-feeding or

Table 1
Natural enemies of *Rhynchophorus* species.

Biocontrol agents	Genus	Species	Attacked stage (s)	*Rhynchophorus* species	Location (s) of record	Reference
Viruses	*Cypovirus*	sp.	All stages	*R. ferrugineus*	Egypt, India	Gopinadhan et al. (1990), Alfazariy et al. (2003), Alfazariy (2004), El-Minshawy et al. (2005)
Coryneform group of bacteria			Larvae and adults	*R. ferrugineus*	India	Dangar and Banerjee (1993)
Bacteria	*Bacillus*	*laterosporus*	Larvae	*R. ferrugineus*	Egypt	Salama et al. (2004)
		megaterium	Larvae and adults	*R. ferrugineus*	Egypt, Italy	Salama et al. (2004), Francesca et al. (2008)
		sp.	Larvae and adults	*R. ferrugineus*	India	Dangar and Banerjee (1993)
		sphaericus	Larvae and adults	*R. ferrugineus*	Egypt, Italy	Alfazariy et al. (2003), Alfazariy (2004), Salama et al. (2004), Francesca et al. (2008)
		thuringiensis	Larvae and adults	*R. ferrugineus*	Egypt, Italy	Alfazariy et al. (2003), Alfazariy (2004), Francesca et al. (2008)
	Pseudomonas	*aeruginosa*	Larvae	*R. ferrugineus*	India	Banerjee and Dangar (1995)
	Serratia	*marcescens*	Eggs, larvae and adults	*R. ferrugineus*	China	Jing et al. (2011)
		sp.	Larvae and adults	*R. ferrugineus*	India	Dangar and Banerjee (1993)
Fungi	*Aspergillus*	sp.	Larvae, pupae and adults	*R. ferrugineus*	Italy	Torta et al. (2009)
	Beauveria	*bassiana*	Larvae, pupae and adults	*R. ferrugineus*	Egypt, Iran, Italy, Spain, United Arab Emirates	Ghazavi and Avand-Faghih (2002), El-Sufty et al. (2009), Sewify et al. (2009), Torta et al. (2009), Dembilio et al. (2010a), Güerri-Agulló et al. (2011), Francardi et al. (2012)
		sp.	Adults	*R. ferrugineus*	India	Shaju et al. (2003)
	Fusarium	sp.	All stages	*R. ferrugineus*	Italy	Torta et al. (2009)
	Metarhizium	*anisopliae*	Several stages, mainly adults	*R. ferrugineus*	Iran, Italy, Egypt	Ghazavi and Avand-Faghih (2002), Merghem (2011), Francardi et al. (2012)
			Adults	*R. bilineatus*	New Guinea	Prior and Arur (1985)
		pingshaense	Adults	*R. ferrugineus*	Vietnam	Cito et al. (2014)
		sp.	Adults	*R. ferrugineus*	Italy	Torta et al. (2009)
	Penicillium	sp.	Larvae, pupae and adults	*R. ferrugineus*	Italy	Torta et al. (2009)
	Trichothecium	sp.	Larvae and adults	*R. ferrugineus*	Italy	Torta et al. (2009)
Yeasts			Larvae and adults	*R. ferrugineus*	Egypt, India	Dangar (1997), Salama et al. (2004)
Nematodes	*Heterorhabditis*	*bacteriophora*	Larvae, pupae and adults	*R. ferrugineus*	Turkey	Atakan et al. (2009a)
		indica	Larvae, pupae and adults	*R. ferrugineus*	Egypt, India, United Arab Emirates	Abbas et al. (2001a), Sosamma and Rasmi (2002), Banu and Rajendran (2002, 2003), Banu et al. (2003)
	Praecocilenchus	*rhaphidophorus*	Adults	*R. bilineatus*	Papua New Guinea	Poinar Jr (1969)
		ferruginophorus	Larvae and adults	*R. ferrugineus*	India	Rao and Reddy (1980)
	Steinernema	*abbasi*	Larvae and adults	*R. ferrugineus*	Egypt, United Arab Emirates	Abbas et al. (2001a)
		glaseri	Larvae and adults	*R. ferrugineus*	India	Banu et al. (2003)
		sp.	Larvae, pupae and adults	*R. ferrugineus*	India, Egypt, Saudi Arabia	Sosamma and Rasmi (2002), Banu et al. (2003), Shamseldean and Atwa (2004), Saleh et al. (2011)
Mites	*Aegyptus*	*alhassa*	Eggs, pupae and adults	*R. ferrugineus*	Saudi Arabia	Al-dhafar and Al-Qahtani (2012)
		rynchophorus	Pupae and adults	*R. ferrugineus*	Egypt	Hassan et al. (2011)
		zaheri	Pupae and adults	*R. ferrugineus*	Egypt	Hassan et al. (2011)
	Fascuropoda	*marginata*	Pupae and adults	*R. ferrugineus*	Egypt	Hassan et al. (2011)
	Hypoaspis	*sardoa*	All stages	*R. ferrugineus*	Egypt	El-Sharabasy (2010)
	Hypoaspis	sp.	Adults	*R. ferrugineus*	India	Peter (1989)
	Iphidosoma	sp.	All stages	*R. ferrugineus*	Egypt	El-Sharabasy (2010)
	Parasitis	*zaheri*	Larvae and pupae	*R. ferrugineus*	Egypt	El-Sharabasy (2010)

(*continued on next page*)

Table 1 (*continued*)

Biocontrol agents	Genus	Species	Attacked stage (s)	*Rhynchophorus* species	Location (s) of record	Reference
	Rhynchopolipus	*rhynchophori*	Larvae	*R. ferrugineus*	India	Peter (1989)
			Adults	*R. palmarum*	Central and South America, Costa Rica	Husband and OConnor (1999)
		brachycephalus	Adults	*R. phoenicis*	Cameroon	Husband and OConnor (1999)
		swiftae	Adults	*R. ferrugineus*	Indonesia, Malaysia, Philippines	Husband and OConnor (1999)
Insects	*Anisolabis*	*maritima*	Eggs, larvae and pupae	*R. ferrugineus*	Saudi Arabia	Abbas (2010)
	Chelisoches	*morio*	Eggs and larvae	*R. ferrugineus*	India	Abraham and Kurian (1973)
	Euborellia	*annulipes*	Eggs	*R. ferrugineus*	Italy	Massa and Lo Verde, 2008, Mazza (Unpub. Data)
	Platymeris	*laevicollis*	Unknown	*R. ferrugineus*	Sri Lanka	Reginald (1973)
	Xylocorus	*galactinus*	Eggs, larvae and pupae	*R. ferrugineus*	Saudi Arabia	Abbas (2010)
	Xanthopygus	*cognatus*	Eggs and larvae	*R. palmarum*	Ecuador	Quezada et al. (1969)
	Sarcophaga	*fuscicauda*	Adults	*R. ferrugineus*	India	Iyer (1940)
	Billea	*rhynchoporae*	Pupae	*R. palmarum*	Brazil	Moura et al. (2006)
		maritima	Pupae	*R. ferrugineus*	Italy	Lo Verde and Massa (2007)
		menezesi	Pupae	*R. palmarum*	Brazil	Moura et al. (1993)
	Megaselia	*scalaris*	Pupae	*R. ferrugineus*	Italy	Mazza (Unpub. Data)
	Scolia	*erratica*	Larvae	*R. ferrugineus*	Malaysia	Burkill (1917)
Vertebrates	*Centropus*	*sinensis*	Unknown	*R. ferrugineus*	India	Faleiro (2006b)
	Dendrocitta	*vagabunda parvula*	Adults	*R. ferrugineus*	India	Krishnakumar and Sudha (2002)
	Pica	*pica*	Unknown	*R. ferrugineus*	Italy	Lo Verde et al. (2008)
	Apodemus	*sylvaticus*	Pupae and adults	*R. ferrugineus*	Italy	S. Longo (Pers. Com.)
	Rattus	*rattus*	Larvae and pupae	*R. ferrugineus*	Italy	Lo Verde and Massa (2007)

when insects were forced to wade through a suspension of bacterial cells. Mortality occurred eight days after inoculation and small larvae were more susceptible than larger larvae (Banerjee and Dangar, 1995), probably due to lack of antimicrobial cuticular compounds (Mazza et al., 2011a).

Salama et al. (2004) isolated three potent spore-forming bacilli from larvae in Egypt. The three bacteria belonged to the genus *Bacillus* and were identified as variants of *B. sphaericus*, *Bacillus megaterium* de Bary and *Bacillus laterosporus* Laubach. Under laboratory conditions, the mortality of larvae ranged between 40% and 60%. The most active culture was that of *B. sphaericus* which produces spherical endospores and crystalline endotoxins, probably responsible for the observed activity against RPW.

In Italy, *B. thuringiensis*, *B. sphaericus* and *B. megaterium* were isolated from dead RPW from Sicily, but preliminary bioassays with these bacteria performed on eggs showed a weak pathogenic effect (Francesca et al., 2008) despite lack of antimicrobial compounds on the eggs (Mazza et al., 2011a).

4. Fungi

Many entomogenous fungi are relatively common, often inducing epizootics, and thus can be considered a significant factor in the control of insect populations. Most species attacking terrestrial insects belong to the Hyphomycetes and Entomophthorales. Unlike other insect pathogens, fungi usually infect the host by contact, penetrating the insect cuticle (Butt and Goettel, 2000). The host can be infected by: (a) direct treatment, (b) horizontal transmission from infected insects or cadavers to untreated insects, and (c) vertical transmission to subsequent developmental stages via the new generation of spores (Lacey et al., 1999; Quesada-Moraga et al., 2004).

Beauveria bassiana (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin are two of the most commonly studied species of entomopathogenic fungi. Both *Beauveria* and *Metarhizium* are cosmopolitan anamorphic genera of soil-borne facultative necrotrophic arthropod-pathogenic fungi (Bischoff et al., 2009; Rehner et al., 2011). Both have provided encouraging results for the microbial control of certain economic crop pests (Jaronski, 2010). The most important aspects of exposure to entomopathogenic fungi include sublethal effects such as alteration of feeding behavior (e.g. Tefera and Pringle, 2003) and of survival and reproductive potential of the progeny (e.g. Dembilio et al., 2010a).

El-Sufty et al. (2009) studied the pathogenicity of an indigenous strain of *B. bassiana* isolated from pupae and adults of RPW from the United Arab Emirates. They found that most adults died between the first and second weeks after treatment and that young larvae were more susceptible than old ones; this agrees with Mazza et al. (2011a) who found a lack of antimicrobial cuticular compounds in small larvae. This strain was also efficaciously used in traps for auto-dissemination in date palm plantations (El-Sufty et al., 2011).

Dembilio et al. (2010a) evaluated the potential of an indigenous strain of *B. bassiana* obtained from a naturally infected RPW pupa in Spain, both in the laboratory and in semi-natural assays. In addition to laboratory results showing that this strain can infect RPW eggs, larvae and adults, the fungus efficiently transmitted the disease to untreated adults of both sexes and reduced fecundity and egg hatching. Therefore, *B. bassiana* treatments against RPW should reduce its populations both by (a) mortality of the primarily infected weevils and by (b) sublethal effects on reproduction and on the offspring of these adults and those in contact with them (Dembilio et al., 2010a; Llácer et al., 2013). Sewify et al. (2009)

and Besse et al. (2011) reported interesting results, including a considerable reduction of the palm weevil population, from the use of an indigenous strain of *B. bassiana*, confirming the role of this fungus as a promising biocontrol agent. A *B. bassiana* solid formulation with high RPW pathogenicity and persistence could be applied both as a preventive treatment and as a curative one for RPW control (Güerri-Agulló et al., 2011). However, Abdel-Samad et al. (2011) reported that a commercial oil formulation of *B. bassiana* had little effect on RPW, and thus was not a good candidate since it is expensive for field application. Moreover, polar extracts from adults inhibited the germination of *B. bassiana* spores obtained from a commercial product Naturalis® (Mazza et al., 2011a).

Francardi et al. (2012, 2013) showed that *M. anisopliae* isolated from RPW in Italy had the highest efficacy against RPW larvae and adults. This is in agreement with Gindin et al. (2006) who compared the entomopathogenicity of *B. bassiana* and *M. anisopliae* strains obtained from different sources and also showed the higher virulence of *M. anisopliae*. The infecting method seems to influence the mortality of both larvae and adults, with a higher efficacy of dry spores with respect to the application of aquatic spore suspension (Gindin et al., 2006).

M. anisopliae was also isolated from *R. bilineatus* in New Guinea after an accidental infection during treatment against the scarabaeid *Scapanes australis* with a formulation based on *M. anisopliae* spores (Prior and Arur, 1985). However, *M. anisopliae* was discovered in naturally infected RPW in Egypt and this strain caused a high mortality rate for larval and adult stages only under laboratory conditions (Merghem, 2011). Cito et al. (2014) very recently reported the first recovery of *Metarhizium pingshaense* associated to RPW in Vietnam, a native area of this pest: this entomopathogenic fungus was able to kill adults in few times thanks to an efficient protease activity and toxin production.

Colonies of *B. bassiana*, *Aspergillus* sp., *Fusarium* sp., *Metarhizium* sp., *Penicillium* sp. and *Trichothecium* sp. were isolated from several stages of RPW in southern Italy (Torta et al., 2009; Tarasco et al., 2008). One of the last indigenous strains of *B. bassiana* proved useful for the control of small larvae in the laboratory (Torta et al., 2009).

5. Nematodes

Interest in the use of nematodes as biological pest control agents has increased exponentially over the past two decades. Indeed, researchers are exploring the potential of nematodes to manage harmful insects, mollusks, plant nematodes and even soil-borne plant pathogens (Grewal et al., 2005).

The nematode fauna associated with *R. palmarum* has been studied because of its practical importance concerning red ring disease, but one of the other *Rhynchophorus* species has not been effectively surveyed. In addition to *Bursaphelenchus cocophilus* (Cobb) Baujard (the red ring nematode, causal agent of red ring disease of palm trees in the neotropics), (Giblin-Davis, 1993), *B. gerberae* Giblin Davis et al., *Caenorhabditis angaria* Sudhaus, Kiontke and Giblin-Davis, and *Mononchoides* sp. have been reported from *R. palmarum* (Gerber and Giblin-Davis, 1990a,b; Giblin-Davis et al., 2006; Kanzaki et al., 2008; Sudhaus et al., 2011). Other nematodes with apparently no adverse effects on palm weevils are known from three *Rhynchophorus* species: *Acrostichus rhynchophori* Kanzaki et al. (named *Diplogasteritus* in older publications; Kanzaki et al., 2009) and *Teratorhabditis palmarum* Gerber and Giblin-Davis were isolated from *R. palmarum* and *R. cruentatus* (Gerber and Giblin-Davis, 1990b), while *Teratorhabditis synpapillata* Sudhaus was isolated from RPW in Japan and India (Kanzaki et al., 2008). Recently, *Mononchoides* sp., *Teratorhabditis* sp. and *Koerneria* sp. were isolated from RPW pupae and adults in southern Italy, but studies are in progress to identify the species, clarify their biology, the type of association with RPW and their possible effects as biocontrol agents (Oreste et al., 2013).

Among parasitic species, *Praecocilenchus rhaphidophorus* Poinar was isolated from *R. bilineatus* in New Britain and Papua New Guinea (Poinar, 1969), while *P. ferruginophorus* Rao and Reddy was isolated from RPW in India (Rao and Reddy, 1980). The latter species was found in the trachea, intestine and fat tissue of larvae and in the uterus and hemocoel of RPW adults. The nematodes are most likely released when infected females lay eggs, but they may also pass into the feces through the intestine. The ovaries of parasitized weevils are damaged, with possible consequences for the production of eggs (reviewed by Triggiani and Cravedi, 2011). Since there are only a few slight morphological differences between *P. rhaphidophorus* and *P. ferruginophorus*, further analyses are necessary to establish whether they are conspecific (Rao and Reddy, 1980).

Entomopathogenic nematodes (EPNs) are lethal obligatory parasites of insects. They are ubiquitously distributed and comprise the families Heterorhabditidae and Steinernematidae. The families are not closely related phylogenetically but share similar life histories through convergent evolution. These nematodes carry specific pathogenic bacteria, *Photorhabdus* by Heterorhabditidae and *Xenorhabdus* by Steinernematidae, which are released into the insect hemocoel after penetration of the insect host into the infective stage of the nematode. Few entomopathogenic nematodes have been recorded as naturally infecting *Rhynchophorus* species. *Steinernema* sp. was extracted from naturally infected RPW adults in the Eastern Province in Saudi Arabia (Saleh et al., 2011). Laboratory bioassays and semi-natural trials showed high virulence of this nematode against RPW adults; in particular, the results in the field highlighted the importance of temperature since entomopathogenic nematodes are highly sensitive to high temperatures *in primis*, high ultraviolet radiation and low relative humidity (Saleh et al., 2011).

Encouraging results were obtained with another *Steinernema* sp. isolated from RPW pupae and adults in Egypt; this strain was tested with two other local isolates of the same genus against larvae and adults both in the laboratory and in the field (Shamseldean and Atwa, 2004). In the laboratory, most nematodes were pathogenic to larvae, pupae and adults, while in the field the use of the EPN failed to control the pest, particularly due to hot weather and sap flooding of the infested site (Abbas et al., 2001b).

The frequent association between RPW and other organisms can interfere with management techniques: predation by mites such as *Centrouropoda almerodai* Wisniewski and Hirschmann, closely associated with RPW particularly in Italy (Mazza et al., 2011b), can reduce the efficacy of the nematode *Steinernema carpocapsae* (Weiser) (Morton and Garcia-del-Pino, 2011). The use of chitosan as adjuvant can protect nematodes, particularly *S. carpocapsae*, from environmental conditions, increasing the length of efficacy period of this natural enemy (Llácer et al., 2009; Dembilio et al., 2010b).

However, significant differences have been observed in the mortality of various life stages of RPW when associated to different concentrations and species of nematodes. For example, *Heterorhabditis bacteriophora* Poinar was the most virulent against larvae and adults of RPW, in laboratory trials performed in Italy, and was the only nematode that always developed new generations in RPW adults (Triggiani and Tarasco, 2011). This agrees with the results for *H. bacteriophora* isolated from RPW specimens in Turkey, which caused considerable mortality rates of larvae (69%) and pupae (80%). Nevertheless, few adults were attacked, restricting its potential as a biocontrol agent (Atakan et al., 2009a). Recently Manachini et al. (2013) discovered that *S. carpocaspsae* was not encapsulated by RPW hemocytes. Therefore, it is necessary to investigate the lack of reproduction of some EPN species in the larvae and adults of RPW.

6. Mites

Nowadays, mites are used in various ways for biological control, with an increasing number of species commercially sold throughout the world. They prey on all the development stages, and several species are mass reared commercially for the management of mite, insect, nematode and mollusk pests (as well as noxious weeds) in greenhouses and in field-grown crops (Orr and Suh, 2000).

In 1966, Wattanapongsiri reviewed the mites associated with palm weevils of the genera *Dynamis* and *Rhynchophorus*. These mites belonged to several families such as Acaridae, Anoetidae, Blattisociidae, Diplogyniidae, Macrochelidae and Uropodidae.

After recent RPW colonizations, particularly in the Mediterranean area, several authors reported the presence of mite species of several families associated with this pest in Egypt (El-Sharabasy, 2010; Hassan et al., 2011), Italy (Longo and Ragusa, 2006), Malta (Porcelli et al., 2009), Saudi Arabia (Al-Dhafar and Al-Qahtani, 2012), Turkey (Atakan et al., 2009b) and the United Arab Emirates (Al-Deeb et al., 2012). The most common species are phoretic and belong mainly to the Uropodina (Wattanapongsiri, 1966; Longo and Ragusa, 2006; Atakan et al., 2009b; Porcelli et al., 2009; El-Sharabasy, 2010; Al-Deeb et al., 2012). Phoretic mites were also recorded for *R. phoenicis* (Kontschán et al., 2012) and *R. palmarum* (Rodríguez-Morell et al., 2012).

Some parasitic mites can be associated with *Rhynchophorus* species, including ectoparasitic podapolid mites (reviewed in Husband and OConnor, 1999) such as *Rhynchopolipus rhynchophori* (Ewing) on *R. palmarum*, *Rhynchopolipus brachycephalus* Husband and OConnor on *R. phoenicis*, and *Rhynchopolipus swiftae* Husband and OConnor on *R. ferrugineus.*

Hypoaspis sp. and *R. rhynchophori* have been found in association with *R. ferrugineus*, but the status of these species as parasites, *sensu* Peter (1989), remains uncertain (Nannelli R, pers. obs.). Instead, Abdullah (2009) reported the successful use of *R. rhynchophori* as a biocontrol agent against RPW. Other species (e.g. *Iphidosoma* sp.) reported by El-Sharabasy (2010), Hassan et al. (2011) and Al-Dhafar and Al-Qahtani (2012) require more in-depth studies for their correct identification and elucidation of their true relationship with RPW.

7. Insects

Parasitoid and predator insects have been employed in the management of insect pests for centuries since they are natural enemies of various life stages. However, their use as biological control has largely gone unexploited for several reasons (e.g. limited number of non-target species, poor taxonomic information, many regulations concerning the collection and application) despite being the primary solution in any pest management program after assessment of balancing of risks and benefits associated with the introduction of alien biological control agents (De Clercq et al., 2011). Natural insect enemies of *Rhynchophorus* include several species belonging to the orders Dermaptera, Heteroptera, Coleoptera, Diptera and Hymenoptera (Murphy and Briscoe, 1999).

Among earwigs, *Chelisoches morio* (Fabricius) was reported as common predator of RPW eggs and larvae in the canopy of coconut plantations in India (Abraham and Kurian, 1973), and *Euborellia annulipes* (Lucas) was found in RPW-infested palms in Sicily (Massa and Lo Verde, 2008). The latter cosmopolitan earwig is an important insect predator of various preys such as caterpillars, beetle larvae (also weevils), leafhoppers (Klostermeyer, 1942) and RPW eggs in the laboratory (Mazza G, unpublished). Reginald (1973) reported a casual occurrence of the predatory bug *Platymeris laevicollis* Distant on RPW; this hemipteran, imported into Sri Lanka against *Oryctes rhinoceros* (L.), seems to prefer RPW.

A few predatory coleopterans are associated to *Rhynchophorus* species. For example, larvae and adults of the rove beetle *Xanthopygus cognatus* Sharp. are egg and larval predators of *R. palmarum*; yet in spite of its feeding preference for the coconut weevil it is a facultative monophagous and can eat other preys when weevils are unavailable (Quezada et al., 1969).

Parasitoids occur in five orders of holometabolous insects, but Diptera and Hymenoptera parasitoids are the best known, including respectively 20% and 78% of all estimated species (Feener and Brown, 1997). Among Diptera, a few species of Sarcophagidae and Tachinidae exploit *Rhynchophorus* species as hosts (Murphy and Briscoe, 1999). *Sarcophaga fuscicauda* Bottcher attacked RPW adults in South India (Iyer, 1940). These predaceous and parasitic flies are mostly larviparous, usually feeding on the host larvae and adults. In contrast, Tachinidae larvae are all internal parasitoids, mainly of larvae of Lepidoptera and Coleoptera. As they mostly have a very narrow host range, several species have been successfully used in classic biological control programs (Murphy and Briscoe, 1999). *Billaea menezesi* (Townsend) is a gregarious parasitoid of *R. palmarum* (with an average number of ca. 18 pupae per beetle host) and was recorded in oil palm plantations in Brazil (Moura et al., 1993). The level of parasitism was around 50% and this tachinid fly was observed throughout the year, which is encouraging for its use in integrated pest management (Moura et al., 1993). Another tachinid fly, *Billaea rhynchophorae* (Blanchard), was reported by Guimarães (1977) as a parasitoid of *R. palmarum* in Brazil, with a mean parasitism of 40% (Moura et al., 2006). Unfortunately, mass rearing of this fly was not possible due to the lack of information about its biological cycle. However, the positioning of a large number of *R. palmarum* cocoons in a close-mesh net cage allowed the collection of parasitoids and their subsequent release (Moura et al., 2006). Laboratory assays performed in Colombia with another two species of tachinid flies, *Paratheresia claripalpis* Van der Wulp and *Metagonistylum minense* Townsend, revealed no use of *R. palmarum* larvae as hosts after 12 days (Blandón and Viáfara, 2008). In Sicily, Lo Verde and Massa (2007) reported, for the first time in Italy, the presence of an autochthonous parasitoid of cetonid beetles, *Billaea maritima* (Schiner), on RPW pupae, although no studies have been conducted on this fly. In addition, the generic phorid, *Megaselia scalaris* (Loew), was recently discovered in RPW pupae from Sicily (Mazza G, unpublished).

Among Hymenoptera, *Scolia erratica* Smith was found in Malaysia as a parasitoid of RPW (Burkill, 1917), but no biological data have been published on this wasp. Further studies are so necessary because scoliid wasps have been successfully used as classic biological control agents since the larvae feed ectoparasitically on the larvae of Scarabaeidae and, less commonly, of large Curculionidae (Murphy and Briscoe, 1999). More in-depth studies are necessary to investigate if the autochthonous *Megascolia flavifrons* (Fabricius), commonly found in infested palms in Sicily, can use RPW larvae as hosts (Longo S, pers. obs.).

8. Vertebrates

Besides the classic biocontrol agents such as bacteria, fungi and nematodes, some vertebrates (birds and mammals) are reported to eat RPW. Krishnakumar and Sudha (2002) noticed that the Indian treepie bird, *Dendrocitta vagabunda parvula* (Whistler and Kinnear), preys on RPW adults. The crow pheasant bird, *Centropus sinensis* Stephens, an opportunistic feeder, was also reported to take RPW (Faleiro, 2006b). In Italy, only the Eurasian magpie, *Pica pica* L., is known to eat RPW (Lo Verde et al., 2008).

Several RPW pupae and adults were preyed on by two mammals, *Rattus rattus* and *Apodemus sylvaticus*, in infested palms in Sicily (Lo Verde and Massa, 2007; Longo S, pers. obs.). However, the role of these fortuitous predators against RPW is very limited and it is more a biological curiosity than one real opportunity of use.

9. Conclusion and future outlook

Even though the many natural enemies of *Rhynchophorus* species have failed to stop the spread of the pest, anyway biological control of *Rhynchophorus* species should be considered among the promising tools of an IPM strategy to manage some of these palm weevils. Although new knowledge about the relationship between pests and their potential enemies has been acquired in recent years and has provided new avenues of research to find novel strategies to control the pests, much remains to be done before biological control can begin to show results.

Extensive field surveys will be necessary to identify biocontrol agents both in the native and in the introduced ranges and also those related to apparently non-harmful *Rhynchophorus* species. On the basis of the screening reported here, particular attention should be paid to the fungal biocontrol agents. For example, the recent discovery of *M. pingshaense* associated to RPW in Vietnam, native area of this insect, showed this fungus is able to kill 100% of adults in few days in lab trials (Cito et al., 2014). Obviously, due to the concern about import and release of alien natural enemies, and the increased evaluation and registration demands, there is a trend nowadays to first look for indigenous natural enemies when a new alien pest establishes. As evidenced by the increased biological control, several popular alien biological control agents have recently been replaced by indigenous ones (van Lenteren, 2012). Nevertheless, as found by Cito et al. (2014), also an indigenous fungus such as *M. anisopliae* MET 08/I05 associated to RPW in Italy, an area of recent introduction, showed a similar mortality rate against adult insects in lab trials, with dissimilar enzymatic and toxicological profiles with respect to *M. pinghshaense*, suggesting a different virulence activity, but both effective against this pest.

More in-depth studies are necessary for the identification and practical use of viruses, particularly in an integrated pest management approach, also taking into account their possible interference with other biocontrol agents such as nematodes: the survival of entomopathogenic nematodes, such as *Heterorhabditis* and *Steinernema*, is indeed progressively and negatively affected by an increase of the viral suspension (Salama and Abd-Elgawad, 2002).

The potential of bacteria to control *Rhynchophorus* species is known only for RPW, but with contradictory results probably depending on the bacterial species and the virulence levels. An important role in avoiding bacterial infections could be played by the polar surface fraction of extracts from adults and large larvae of RPW that inhibits Gram-positive bacteria such as the commercial product of *B. thuringiensis* (Mazza et al., 2011a). So, this aspect deserves future investigations and the potential use of pathogenic bacteria would consider the strong antimicrobial activity shown by several stages of RPW (Mazza et al., 2011a). Data on these agents being scanty, more virulent natural pathogen strains should be identified and their efficacy in pathogenicity improved.

The use of fungi, in particular indigenous strains such as *B. bassiana* and *M. anisopliae*, obtained from a naturally infected RPW should be seriously taken into account for biological control, also because of their sublethal effects. Fungi are particularly suitable for the control of concealed and aggregated insects, such as *Rhynchophorus* species, for several features. In Italy and in other European countries, RPW is spreading in urban contexts characterized by low biodiversity levels, and the use of fungi could be thus implemented in these areas due to the restricted multi-targeting spectrum of these agents and to the lack of harmful effects on human health. Moreover, their use could also improve other control methods, such as the Sterile Insect Technique (SIT), by spreading the pathogen within an already established population, as suggested by Llácer et al. (2013). Specific research is also needed to isolate more virulent strains (see Cito et al., 2014) and to determine the optimum conditions for the fungal epizootic in the weevil populations.

Entomopathogenic nematodes can provide effective biological control for some important pests of commercial crops (Burnell and Stock, 2000). They have many attributes to be a good biological control agent: they are environmentally safe and acceptable, can be produced in large quantities in the laboratory and be easily applied in the field (Liu et al., 2000). The management of palm pests with entomopathogenic nematodes is potentially fruitful, but unfortunately several factors negatively affect their use in integrated pest management programs applied to RPW control. It seems crucial to deep aspects concerning the isolation of local nematodes, to maintain their vitality, infectiveness and virulence, and to discover the defense mechanism inhibiting the reproduction of some EPN species into the RPW bodies. Moreover, the frequent systematic application of treatments and their costs should be considered for weevil management.

The possible ecological role of most mites is still poorly known and field and laboratory studies could assess their feeding habits and potential use as biocontrol agents. Some authors hypothesized that some mites associated with RPW are not simply phoretic and that their presence could affect the red palm weevil's fitness by reducing lifespan (e.g. Mazza et al. 2011b) or by hampering flight behavior (Atakan et al., 2009b). It is noteworthy to consider, mainly in the areas of new introduction of the RPW, the high density of Uropodine mites carrying fungal spores, associated to the weevils and inhabiting the palm trees (Mazza G., pers. obs.): mites may exert a double/composite effect by overweighting and limiting the diffusion of the insect and by increasing the spreading of fungal agents.

Among parasitoid and predatory insects, only Diptera deserve favorable consideration, as demonstrated for *B. menezesi* against *R. palmarum*. The level of parasitism was high and this natural enemy was observed throughout the year, which is encouraging for its use as a component of integrated pest management. The efficiency of the mass rearing technique of Diptera parasitoids will be improved though their correct identification and the study of their biological cycles.

According to the data we presented, fungal biocontrol agents seem to be the best biocontrol agents: they fit the biocontrol guidelines both for attributes and perspective/context use. For *R. ferrugineus*, in particular, in addition to the reasons for healthy needs, the urban and peri-urban contexts where the presence of this pest can spread, restrict the spectrum of possible target hosts, lowering the multitargeting and overlapping actions of these agents. This may be a way to evade association/competition with complex web connections in communities and to simplify the application protocols of these strategies and to limit at maximum the unbalance of the environmental equilibrium.

The biological control of *Rhynchophorus* palm weevils could not be a utopian or a cost-consuming goal, at least in association with other techniques addressed toward long term sustainable and environmentally friendly control strategies. However, host-range tests and risk assessments of the most promising agents should be given high priority to avoid any potential-post-release threats to non-target species in introduced areas. Long-term monitoring is also necessary to evaluate the impact of biological control agents in newly invaded plant and animal communities.

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