MICROBIAL CONTROL OF THE PECAN WEEVIL, *CURCULIO CARYAE*[1]

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ABSTRACT

The pecan weevil, *Curculio caryae* (Horn), is a key pest of pecans throughout the Southeastern US, Oklahoma, Kansas, and parts of Texas. The objective of this paper is to provide an updated review and analysis of the potential for using microbial control to suppress *C. caryae*. No protozoan and only one virus disease has been reported in *C. caryae*, and thus the microbial control potential of these groups appears to be limited. Pathogenic bacteria found in *C. caryae* include the genera *Enterobacter, Pseudomonas,* and *Serratia,* and rickettsia-like organisms. Bacteria appear to have only low to moderate potential as microbial control agents of *C. caryae* due to low infectivity, poor virulence, or perhaps safety issues. Several fungi in the sub-division Deuteromycotina have been found to occur naturally in *C. caryae,* the most common being *Beauveria bassiana* and *Metarhizium anisopliae*, which have considerable potential as control agents. Several nematodes have been reported in *C. caryae,* but only the genera *Steinernema* and *Heterorhabditis* appear to have any promise as microbial control agents. Although these nematodes possess only low to moderate virulence to *C. caryae* larvae, some species, particularly *Steinernema carpocapsae*, are highly virulent to adults. Overall, microbial control research on *C. caryae* is in its early stages and would benefit from identifying more pathogen strains and species as candidate control agents as well as elucidating factors affecting efficacy and methods of improvement.

INTRODUCTION

The pecan weevil, *Curculio caryae* (Horn), is a key pest of pecans throughout the Southeast (Mizell 1985). The insects have a two or three-year life-cycle (Harris 1985). Adults emerge from soil in late July-August, feed on developing nuts, and oviposit into the nuts after dough stage begins (Harris 1985). Larvae develop within the nut and fourth instars drop to the soil where they burrow to a depth of 8-25 cm, form a puparium, and overwinter. The following year approximately 90% of the larvae pupate and spend the next 9 months in the soil as adults (Harris 1985). The remaining 10% of the population spend 2 years in the soil as larvae, emerging as adults in the third year (Harris 1985).

Control recommendations for the pecan weevil currently consist solely of above-ground applications of chemical insecticides (e.g., carbaryl) to suppress adults (Ellis et al. 2000, Harris 1999). Late season applications of carbaryl, however, can result in resurgence of damaging aphid populations because carbaryl suppresses certain aphid predators (e.g., coccinellids) but does not suppress the pecan aphid complex (Dutcher and Payne 1985). Due to the problems associated with aphid resurgence, as well as other environmental and

[1]Coleoptera: Curculionidae

regulatory concerns, research on developing alternative control strategies is warranted. Microbial control agents represent one of the potential alternatives to chemical insecticides.

Microbial control is the manipulation of pathogens (or their by-products) to suppress pest populations (Tanada and Kaya 1993). Insect pathogens that may be used in microbial control include virus, bacteria, protozoa, fungi, and nematodes (Tanada and Kaya 1993). In lieu of describing the biology of these groups (which is beyond the scope of this manuscript), I refer interested readers elsewhere [e.g., Tanada and Kaya (1993), Fuxa and Tanada (1987), and Lacey (1997)].

Fuxa (1987) describes four approaches to microbial control. The introduction approach encompasses establishment of the pathogen in a pest population where the microbial agent did not previously exist. In this approach, permanent suppression of the pest is generally expected. Inundative augmentation is an "insecticidal" approach to microbial control for which short-term suppression with little or no recycling is expected. Inoculative augmentation is intermediate between the two previously mentioned approaches; it entails periodic introduction with some recycling expected. The fourth approach is environmental manipulation or conservation (i.e., increasing the naturally occurring microbial agent's population indirectly without actual addition of pathogen units).

The objective of this paper is to provide an updated review and analysis of the potential for using microbial control to suppress *C. caryae*. Microbial control can be considered during certain periods of *C. caryae*'s life cycle. It is unlikely that any control strategy, chemical or biological, could be successful against *C. caryae* when larvae are protected in the nut; thus, the insects must be targeted during periods when they are "exposed". Furthermore, unlike with chemical insecticides, foliar sprays with microbial agents are unlikely to be effective due to environmental constraints (e.g., sensitivity to ultraviolet light and desiccation) (Fuxa 1987) and cost. Microbial control of *C. caryae* currently emphasizes targeting larvae, after they drop from the nut, or emerging adults. The literature pertaining to pathogens of *C. caryae* has been reviewed previously by Sikorowski (1985) and briefly by Fuxa et al. (1998).

PROTOZOA AND VIRUSES

Although protozoan diseases have been reported in other curculionids [e.g., the pales weevil *Hylobius pales* (Herbst), the pitch eating weevil *Pachylobius picivorus* (Gemar), and the white pine weevil *Pissodes strobi* (Peck)] (Fuxa et al. 1998), this pathogen group has not been reported to occur in *C. caryae*. Protozoan diseases of *C. caryae* are yet to be discovered. Even if such a pathogen were found, however, the microbial control potential would likely be low because most entomopathogenic protozoa have low virulence causing chronic infections which do not kill the host (Fuxa 1987). On the other hand, chronic diseases may have utility if using an introduction/establishment approach to microbial control.

Only one virus has been found in *C. caryae*. In larvae, Adams et al. (1997) found a spherical non-occluded virus in heart tissue and virus-like particles (VLP) in some other tissues (e.g., muscle sheath tissues near the hindgut). Some degeneration of infected tissues was observed, but it was not extensive (Adams et al. 1997). Although normal physiological processes can be inhibited by viruses and VLPs similar to the one described by Adams et al. (1997), the extent of deterioration due to this *C. caryae* disease, and its distribution among natural populations, will have to be investigated before potential as a control agent can be determined.

BACTERIA

Several soil-inhabiting bacteria have been reported to occur naturally in *C. caryae* larvae. Sri-Arunotai et al. (1975) found *Pseudomonas aeruginosa* (Schroeter) and *Serratia marcescens* Bizio to be natural pathogens of *C. caryae*. Sikorowski (1985) reported finding the above mentioned bacteria as well as other *Enterobacter* spp. and *Pseudomonas* spp. The potential of the genera *Enterobacter, Pseudomonas,* and *Serratia* as microbial control agents is limited by their status as opportunistic pathogens (i.e., the bacteria have difficulty penetrating the host's gut and generally only enter insects already under stress although once inside the hemocoel they can be quite virulent) (Sikorowski 1985, Tanada and Kaya 1993). Additionally, *Pseudomonas* and *Serratia* are opportunistic pathogens of humans (Tanada and Kaya 1993), thus raising safety issues when considering these organisms for microbial control.

Rickettsia-like organisms (RLOs) were found in *C. caryae* larvae infecting the midgut heart, trachea, and Malpighian tubules (Adams et al. 1997). These infections caused complete degeneration of fat body cytoplasm (Adams et al. 1997). The RLOs found in *C. caryae* resemble those included in the genus *Rickettsiella* (Adams et al. 1997), which are pathogenic to insects (some RLOs such as most *Wolbachia* spp. that occur in insects are non-pathogenic) (Tanada and Kaya 1993). *Rickettsiella* spp. can infect humans (Tanada and Kaya 1993), hence the safety of the RLO found in *C. caryae* will have to be considered if the microbial control potential of this pathogen is pursued further.

Only one test has been conducted with a bacterial entomopathogen to determine potential for microbial control of *C. caryae*. A laboratory assay tested the effect of *Bacillus thuringiensis tenebrio* on *C. caryae* adults (Ring and Snow 1988). The insects were exposed to pecans that were dipped in a bacterial suspension (Ring and Snow 1988). No significant effect on *C. caryae* survivorship was detected (Ring and Snow 1988). To date, the potential for microbial control of *C. caryae* with bacteria appears to be quite low.

NEMATODES

Entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) have been reported to occur naturally in pecan weevils. Harp and Van Cleave (1976) observed *Steinernema* sp. (=*Neoaplectana*) in *C. caryae* larvae and pupae. Nyczepir et. al. (1992) discovered a strain of *Heterorhabditis bacteriophora* Poinar in *C. caryae* larvae. Sikorowski (1985) reported several other parasitic nematodes in the pecan weevil: *Pristionchus* sp. (Diplogasterida: Diplogasteridae), *Panagrolaimus* sp. (Rhabditida: Panagrolaimidae), and a mermithid (Stichosomida: Mermithidae) (genus not confirmed). *Panagrolaimus* spp. are not parasitic and the reported association was likely phoretic (Kaya and Stock 1997) and offers no potential for microbial control. Although most diplogastrids have little or no biocontrol potential (Gaugler 1987), the *Pristionchus* sp. found in pecan weevil may be useful because a related species, *P. uniformis,* has been reported to have some potential versus the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Fedorko 1971). Mermithids can also have potential as biocontrol agents (Kaya 1993). However, there are no reports of follow-up work on the *Pristionchus* sp. and mermithid isolated in *C. caryae*. On the other hand, the potential of *Steinernema* and *Heterorhabditis* spp. as *C. caryae* control agents has received considerably more attention.

Various studies have examined *C. caryae* larval susceptibility to entomopathogenic nematodes. In a micro-plot field experiment, Tedders et al. (1973) observed 67% mortality in *C. caryae* larvae treated with *Steinernema carpocapsae* (Weiser) (=*Neoaplectana dutkyi*). However, the relative success of Tedders et al. (1973) was likely due only to the excessive rate of application used (i.e., more than 8,000 infective juveniles per cm^2, which is approximately

100 to 300 times recommended rates) (Georgis and Hague 1991, Georgis et al. 1995). Using the standard rate of 25 infective juveniles per cm^2 in a greenhouse study, Nyczepir et al. (1992) reported less than 35% *H. bacteriophora* infection in *C. caryae* larvae. Similarly, Smith et al. (1993) observed low levels of nematode infection (less than 30%) in a simulated field study with rates of 31-78 infective juveniles per cm^2, using the nematodes *H. bacteriophora* (Georgia strain), *H. bacteriophora* (HP88 strain), *S. carpocapsae* (All strain), and *Steinernema feltiaė* (Filipjev) (SN strain).

Certain entomopathogenic nematode species may be highly effective against a particular pest; whereas, others may be only moderately effective or ineffective (Georgis and Gaugler 1991, Shapiro-Ilan et al. 2002a). Currently more than 30 species of entomopathogenic nematodes are recognized (Hominick et al. 1997) and there are numerous strains of each (Poinar 1990). Therefore, Shapiro-Ilan (2001a) conducted a laboratory study aimed at expanding the range of nematode species tested for virulence to *C. caryae* larvae. Yet the level of *C. caryae* mortality observed was low to moderate (not more than 60%) for all of the nine species and 15 strains tested, and no significant differences in virulence were detected among the species (Table 1, Figs. 1 and 2). Shapiro-Ilan (2001a) demonstrated that nematode virulence to *C. caryae* larvae is substantially less compared with virulence to the Diaprepes root weevil, *Diaprepes abbreviatus* (L.), a weevil that is currently controlled commercially by entomopathogenic nematodes in citrus orchards (Shapiro-Ilan et al. 2002a).

TABLE 1. Entomopathogenic Nematodes Used in this Study[a].

Species	Strain	Abbreviation
Heterorhabditis bacteriophora Poinar	Baine	HbBai
H. bacteriophora	Hb	Hbhb
H. bacteriophora	NJ1	HbNj1
H. bacteriophora	HP88	HbHp8
H. bacteriophora	Oswego	HbOsw
H. bacteriophora	TF	HbTf
H. indica Poinar, Karunakar & David	Original isolate	HiOri
H. indica	Hom1	HiHom
H. marelatus Liu & Berry	IN	HmIn
H. marelatus	Point Reyes	HmPoi
H. megidis Poinar, Jackson & Klein	UK211	HmUk2
H. zealandica Poinar	NZH3	HzNzh
Steinernema riobrave Cabanillas, Poinar & Raulston	355	Sr355
S. carpocapsae (Weiser)	All	ScAll
S. feltiae (Filipjev)	SN	SfSn
S. glaseri (Steiner)	NJ43	SgNj4

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Additionally, susceptibility of *C. caryae* larvae to nematodes was shown to decrease further with larval age (Shapiro-Ilan 2001a). Thus, Shapiro-Ilan (2001a) concluded that suppression of *C. caryae* larvae with entomopathogenic nematodes is unlikely to be cost effective unless improvements are made on the nematodes or the manner in which they are applied.

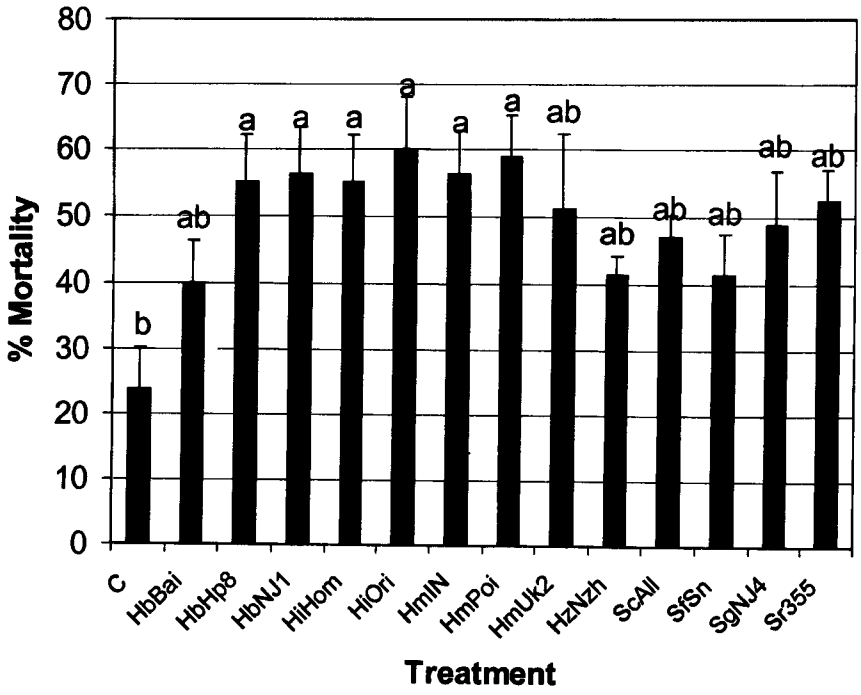

FIG. 1. Nematode-induced mortality of *Curculio caryae* larvae. Different letters above bars indicate statistical significance (Student-Newman-Keul's test, $P < 0.05$). See Table 1 for description of nematode treatment abbreviations; C, control (water). Reprinted with permission of the Entomological Society of America, J. Econ. Entomol. 94: 7-13.

Adult pecan weevils may be more amenable to microbial control with entomopathogenic nematodes than the larval stage (Shapiro-Ilan 2001b). A laboratory study conducted under parallel conditions used for the larvae (Shapiro-Ilan 2001a) indicated high virulence of several nematodes to pecan weevil adults (Fig. 3) (Shapiro-Ilan 2001b). *Steinernema carpocapsae* was particularly virulent killing close to 100% of the weevils; *Steinernema riobrave* Cabanillas, Poinar, and Raulston and *H. bacteriophora* also showed some potential (Fig. 3).

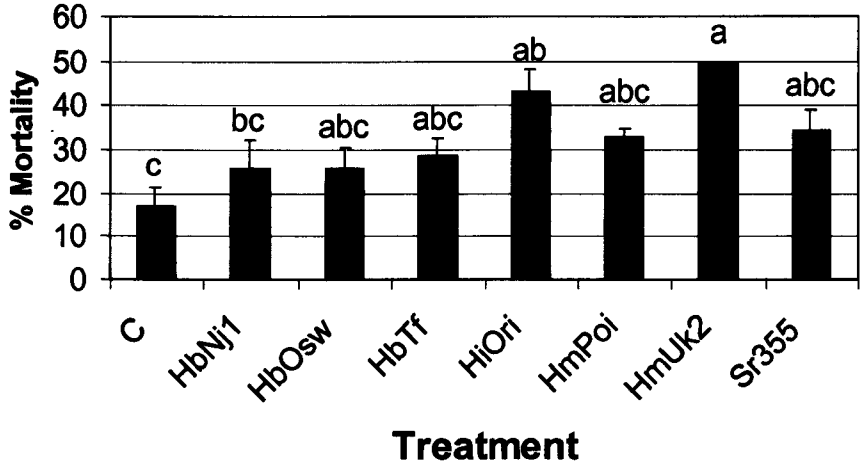

FIG. 2. Nematode-induced mortality of *Curculio caryae* larvae ($P < 0.05$). See Table 1 for description of nematode treatment abbreviations; C, control (water). Reprinted with permission of the Entomological Society of America, J. Econ. Entomol. 94: 7-13.

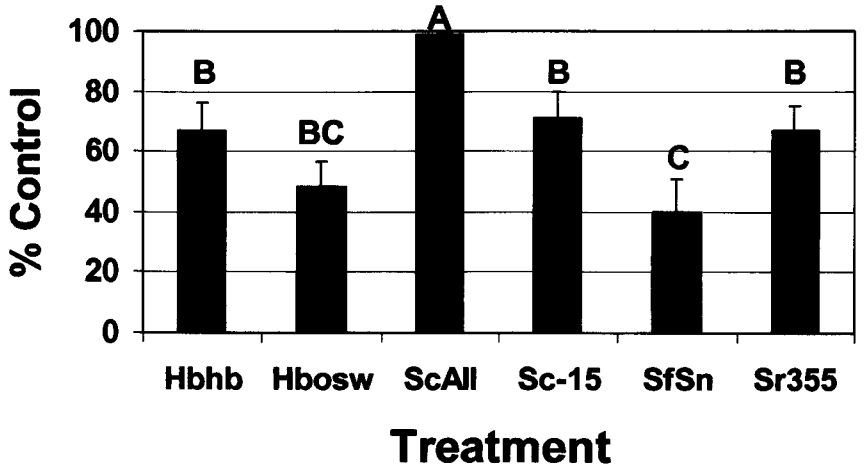

FIG. 3. Percentage control of adult *Curculio caryae* (corrected with Abbott's formula) with entomopathogenic nematodes. See Table 1 for description of nematode treatment abbreviations. Weevils were exposed to nematodes continually for four days except for Sc-15, for which exposure time was 15 min. Letters above bars indicate statistical differences among treatments ($\propto = 0.05$). Reprinted with permission of the Georgia Entomological Society, J. Entomol. Sci. 36: 325-328.

For a number of weevil species, it is the larval stage that is more susceptible to entomopathogenic nematode infection than the adult stage [e.g., the fuller rose beetle, *Asynonychus godmani* Crotch (Morse and Lindegren 1996), the sweetpotato weevil, *Cylas formicarius* (F.) (Mannion and Jansson 1992), *Cosmopolites sordidus* (Gemar) (Pena et al. 1991), and the West Indian sugarcane weevil, *Metamasius hemipterus* (Oliver) (Giblin-Davis et al. 1996]. This trend has also been observed in various other Coleoptera (Geden et al. 1985, Georgis et al. 1991, Theunis 1998). It appears the pecan weevil is an exception, at least for susceptibility to *S. carpocapsae* (Shapiro-Ilan 2001b).

Expanding on the study by Shapiro-Ilan (2001b), I tested several additional nematode species for virulence using methods based on those described by Shapiro-Ilan (2001a, b). Briefly, 250 infective juveniles (less than 16 days old) were pipetted onto soil in plastic cups (3-4 cm i.d., 3.5 cm deep) filled with moist soil (14% final moisture level) and containing one adult weevil each. The nematodes tested were *H. indica* Poinar, Karunakar, and David (Hom1 strain), *H. megidis* Poinar, Jackson, and Klein (UK211 strain), *H. marelatus* Liu and Berry (Point Reyes strain), *S. carpocapsae* (All strain), and *S. glaseri* (Steiner) (NJ43 strain) (a control received only water). Weevil mortality was recorded after four days of incubation at 25°C. Treatment differences were detected through analysis of variance and the Student-Newman-Keul's multiple range test; data were arcsin transformed prior to analysis (SAS Institute 1985). Similar to the results reported by Shapiro-Ilan (2001b), *S. carpocapsae* caused 100% mortality and showed greater virulence to *C. caryae* adults compared with all other nematodes tested (Fig. 4). *Heterorhabditis indica* caused significantly greater weevil mortality relative to the control whereas *H. megidis, H. marelatus,* and *S. glaseri* did not (Fig. 4).

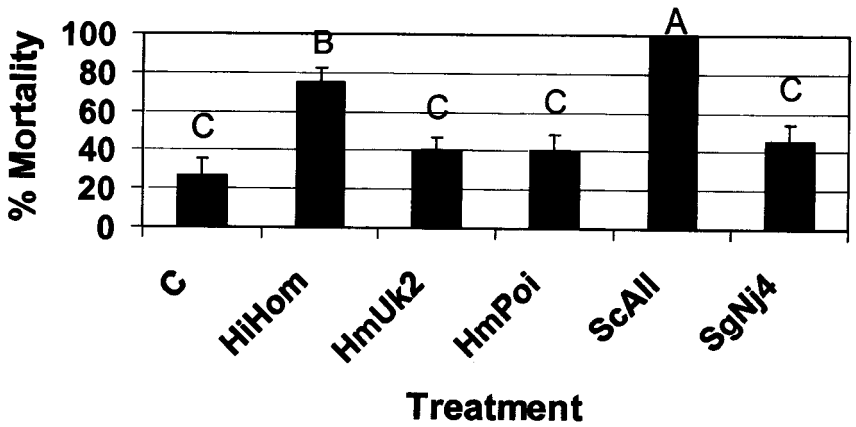

FIG. 4. Nematode-induced mortality of *Curculio caryae* adults. Different letters above bars indicate statistical significance ($P < 0.05$). See Table 1 for description of nematode treatment abbreviations; C, control (water).

Steinernema carpocapsae appears to be a good candidate for control of *C. caryae* adults. One approach may be to apply entomopathogenic nematodes in a narrow (perhaps 1 to 2 m) band around each pecan tree. The adult weevils that crawl to the tree trunk would then be

infected as they pass the area of application. If nematodes were applied as such, the cost of application would be reduced relative to treating a broader area (e.g., the entire orchard). However, if this approach were used, weevils would have to be infected with nematodes after only a relatively short exposure time. The data indicate that *S. carpocapsae* has the potential to infect *C. caryae* and cause > 70% mortality after 15 minutes of exposure (Fig. 3). *Steinernema carpocapsae* is a good candidate for this "banding" application approach because the nematode has an ambushing foraging strategy (sits and waits for a potential host to pass and then attaches to it) and remains near the soil surface when applied there (Lewis et al. 1992, Moyle and Kaya 1981). If the banding method is not successful, an alternative approach might be to broadcast nematodes to control *C. caryae* adults under the soil or as they are emerging. This broadcast approach could also be appropriate for nematodes with more of a "cruiser" search strategy (to actively seek out the host) (Lewis et al. 1992) such as *S. riobrave* or *Heterorhabditis* spp (Grewal et al. 1994). Compared with the banding method, broadcasting would require application of nematodes to a wider area within the orchard (thus increasing cost). Future research will be required to test both banding and broadcast application of entomopathogenic nematodes. Parameters that must be investigated include rate and area of application, irrigation requirements, and persistence of control.

FUNGI

Of all the pathogen groups that infect *C. caryae*, the fungi have received the most attention. Most studies have focused on two fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin, which have been reported to occur naturally in *C. caryae* larvae (Sri Arunotai et al. 1975, Swingle and Seal 1931). Other entomopathogenic fungi reported to occur in *C. caryae* larvae include *Synnematium* sp. (= *Hirsutella* sp.) (Harp and Van Cleave 1976), *Sorosporella* sp., *Paecilomyces* sp., and *Aspergillus flavus* Link (Sikorowski 1985). All these fungi are in the subdivision Deuteromycotina (see Tanada and Kaya 1993). With one exception in *Aspergillus* sp. (Tedders et al. 1973), only *B. bassiana* and *M. anisopliae* have been investigated for their potential as microbial control agents of *C. caryae*.

Laboratory studies have indicated relatively high virulence of some fungal isolates to *C. caryae*. Tedders et al. (1973) found up to 84 and 76% mortality of *C. caryae* larvae using *B. bassiana* and *M. anisopliae*; whereas, mortality from *Aspergillus* sp. was not significantly different from the control. Harrison et al. (1993) tested 7 isolates of *B. bassiana* and observed varying degrees of virulence with up to 73% larval mortality using 10^7 conidia/ g soil. Varying degrees of virulence to *C. caryae* adults were also observed among *B. bassiana* isolates (Harrison et al. 1993).

Field studies have indicated varying levels of efficacy against *C. caryae* larvae and adults. Tedders et al. (1973) reported up to 67% *C. caryae* larval mortality from *B. bassiana* (no rate of application provided), but this level of efficacy has not been equaled or surpassed since. Harrison et al. (1993) reported < 35% larval mortality from *B. bassiana*, but this was likely due to a low rate of application (i.e., 1.2×10^8 conidia/ha; a recommended rate for most *B. bassiana* applications is in the realm of 10^{13}/ha or more) (Booth et al. 2000, James and Elzen 2001). However, Gottwald and Tedders (1983) observed < 30 and ≤ 6% larval mortality from *B. bassiana* and *M. anisopliae* applications, respectively, using relatively high rates (up to 10^7 conidia/g soil). In the same study, higher efficacy was observed toward adults (i.e., 72 and 50% suppression from *B. bassiana* and *M. anisopliae* applications, respectively) (Gottwald and Tedders 1983).

The promising nature of *B. bassiana* and *M. anisopliae* as control agents of *C. caryae*, has led to several investigations concerning the ecology of the system and factors affecting

efficacy. The fungus appears to be widely distributed in pecan orchards where it has been surveyed (Harrison and Gardner 1991, Shapiro-Ilan et al. 2003). Harrison and Gardner (1991) studied the distribution of *B. bassiana* in Georgia pecan orchards and found the fungi present in 19 out of 19 orchards surveyed with an average range among orchards of 33-1342 colony-forming units/g soil. Transmission of *B. bassiana* from infected *C. caryae* adult to healthy adult has been demonstrated (Gottwald and Tedders 1983), and similar horizontal transmission among larvae can be facilitated by the expansive hyphal growth from infected cadavers (e.g., 8.55 cm in diameter) (Gottwald and Tedders 1984). Release of conidia from *B. bassiana* and *M. anisopliae* increases with vibration and at relative humidity below 50% (Gottwald and Tedders 1982). Additionally, red-infrared radiation can increase conidia release in *B. bassiana*; whereas, the effect is variable in *M. anisopliae* (Gottwald and Tedders 1982). *Beauveria bassiana* releases 1–200 times more conidia than *M. anisopliae*.

Pesticides may have a suppressive effect on the potential of *B. bassiana* and other entomopathogenic fungi to control *C. caryae*. Tedders (1981) reported that six fungicides commonly used in pecan orchards inhibited growth of *B. bassiana*. Effects of fungicides on the entomopathogenic fungi varied (e.g., Triphenyltin hydroxide was the most suppressive fungicide to both *B. bassiana* and *M. anisopliae* whereas the effect of Benomyl was greater on *B. bassiana* than *M. anisopliae*, and Dodine, Sulphur, and Dinocap were relatively less suppressive to both fungi) (Tedders 1981). Several herbicides used in pecan management were also found to be suppressive to *B. bassiana* growth, but the effect appeared to dissipate when the fungus was applied seven days after herbicide application (Harrison and Gardner 1992). Shapiro-Ilan et al. (2002b) demonstrated that harmful pesticide effects on *B. bassiana* can be overcome through artificial selection or isolation of naturally resistant strains.

Research indicates entomopathogenic fungi, particularly *B. bassiana* and *M. anisopliae*, have potential to control *C. caryae*. *Beauveria bassiana* may be a superior candidate because isolates tested thus far appear to be more virulent (Tedders et al. 1973, Gottwald and Tedders 1983) and produce more conidia, which would favor continuous control through recycling. *Beauveria bassiana* could potentially be used as a barrier treatment to infect larvae near the soil surface as they drop from nuts and begin to burrow (Harrison et al. 1993). A drawback to this approach is that the larvae emerge from nuts over several months (e.g., October to December) (Boethel and Eikenbary 1979, Harris and Ring 1979), and thus lack of fungal persistence (Storey et al. 1987) could be a hindrance, requiring multiple applications.

Adult control might be achieved using the banding method described in the nematode section above. Gottwald and Tedders (1983) demonstrated promise in this approach, and Shapiro, Gardner, and Cottrell (unpublished data) observed up to 90% suppression using this approach within the first week after application. However, because it can take ten days for *B. bassiana* to cause death (Sikorowski 1985), the fungus may not be capable of sufficiently preventing *C. caryae* ovipostion, which occurs an average of 6.5 days after adults emerge (Criswell et al. 1975). *Metarhizium anisopliae* kills *C. caryae* larvae faster than *B. bassiana* (i.e., within one to three days) (Sikorowski 1985). If this trend holds true for adults then *M. anisopliae* might be a more suitable candidate for the banding method. On the other hand, fungus-infected insects can reduce feeding and other behaviors prior to death (Tanada and Kaya 1993). Future research is required to determine if such pre-death behavior in *B. bassiana* infected *C. caryae* adults will adequately reduce feeding and oviposition damage. Further research is also required to determine other aspects of the feasibility for entomopathogenic fungi to control *C. caryae*, e.g., fungal persistence, rates of application, irrigation requirements, and causes of variation in efficacy.

CONCLUSIONS

Although studies on microbial control for suppression of *C. caryae* began more than 70 years ago (Swingle and Seal 1931), research in this area is in its early stages. Microbial control research can be divided into four successive stages: (1) identification of candidate pathogens that show potential for control of the target pest, (2) initial field testing (3) determination of factors that affect field efficacy for that particular pathogen-pest system, and (4) improvement of efficacy based upon findings in the previous stages. With the exception of some of the studies on fungi, microbial control research has been limited to phase 1 and 2.

Additional research is required to identify candidate microbial agents. Only a few limited surveys for pecan weevil pathogens have been reported (e.g., Sri-Arunotai et al. 1975, Harrison and Gardner 1991, Shapiro-Ilan et al. 2003). More extensive survey work could lead to discovery of additional strains or species of *C. caryae* diseases from all pathogen groups. Once the pathogenicity of isolated organisms is established through Koch's postulates (Tanada and Kaya 1993) and identification confirmed by established experts, the disease causing agent can be assayed for microbial control potential. In addition to finding new strains and species of *C. caryae* diseases through surveys, many entomopathogens already in culture remain to be tested for pathogenicity and virulence to the weevil, even in the groups most studied already (i.e., the nematodes and fungi). In selecting pathogens to screen versus *C. caryae*, consideration should be given to the host specificity and history of virulence to related insects. For example, some pathogens that are quite host specific (e.g., many of the viruses and some protozoans) (Fuxa 1987), and are associated with insect species not closely related to *C. caryae* can be ruled out; whereas, other groups with wider host ranges and recorded success versus Curculionidae or other Coleoptera (e.g., many entomopathogenic nematodes and fungi) (Tanada and Kaya 1993) have greater chance of success.

Pathogens that show some promise for microbial control of *C. caryae* in field trials should be investigated further to determine factors affecting efficacy. Some parameters that should be defined include requirements for irrigation, formulation, and application rates and methodology, as well as overall compatibility with the agroecosystem and other management strategies. Although many of the environmental parameters affecting successful microbial control have been defined (e.g., soil type, moisture, ultraviolet radiation) (Georgis and Gaugler 1991, Fuxa 1987), it can be beneficial to characterize these factors within the specific system one is working in.

Once factors affecting efficacy are defined (and weaknesses are identified) several approaches can be used to enhance microbial control potential of *C. caryae*. Increased efficacy might be obtained through improved formulations or novel application methods. For example, a novel method of application being investigated currently is distribution of nematodes in their infected hosts (Shapiro-Ilan et al. 2001). Control can be improved or persistence of suppression enhanced through environmental manipulation/conservation approaches such as using cover cropping or moving livestock through the orchard (Fuxa 1987). Studies on pathogen persistence, reproductive rate, and transmission are prerequisite to implementation of environmental manipulation/conservation. Combinations of pathogens with other pathogens or chemical agents may have a synergistic effect on insect suppression (Koppenhöfer and Kaya 1997, Koppenhöfer et al. 2000). Finally, genetic improvement can be used to enhance traits important to successful microbial control (Gaugler 1987). One study in the realm of genetic improvement of *C. caryae* pathogens has been reported in which the investigators determined variable levels of virulence among mutant populations of *B. bassiana* (Champlain et al. 1981).

In addition to having a pathogen that can efficaciously suppress the target insect, a successful microbial control program must possess favorable economics (Shapiro-Ilan et al.

2002a). Currently it appears that fungi and nematodes, particularly *B. bassiana, M. anisopliae*, and *S. carpocapsae*, have the greatest potential as microbial control agents of *C. caryae*. The cost of treating an entire hectare with *B. bassiana* (e.g., Mycotrol® ES) at 5 x 10^{13} conidia/ha is approximately $67 USD (Ben Rogers, Emerald BioAgriculture Corp., personal communication), and the cost of treating one hectare with *S. carpocapsae* (e.g., BioVector® 25) at 2.5 x 10^{9} infective juveniles/ha is approximately $188 USD (Mike Dimock, Certis USA, personal communication). The actual cost per hectare if using these products for *C. caryae* control at the recommended rates would be substantially less because the entire soil surface would not have to be treated. *Curculio caryae* larvae only occur within the drip line of pecan trees (Harris 1975), and their abundance has been found to increase closer to the trunk (Raney et al. 1970). Thus, the maximum area that would require microbial treatments is within the drip line of each tree. Significant further reductions in treated area requirements could be obtained by using the banding method for adult control; conceivably, 10% or less of the orchard's soil surface would need to be treated with a 1 m band around each trunk. Field tests are underway to determine the feasibility of this approach.

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