

Interactions between Entomopathogenic Fungi and Nematodes

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Entomopathogenic nematodes (EPNs) belonging to the genera *Steinernema* Travassos (Rhabditida: Steinernematidae) and *Heterorhabditis* Poinar (Rhabditida: Heterorhabditidae) are obligate and lethal parasites of insects. Their infective juveniles (IJs), non-feeding and usually soil dwelling, hold in their foregut symbiotic bacteria that play an important and essential role in killing susceptible insects. Entomopathogenic fungi, mainly Ascomycetes, are regularly found infecting insects in the environment, especially in the soil. The species of the genera *Metarhizium* Sorokin, and *Beauveria* Vuill. are the best known entomopathogenic fungi. These organisms usually attach to the external body of insects by conidia adhering to the host's cuticle. Under the right temperature and humidity conditions, these spores germinate, grow as hyphae, and colonize the insect's body. After a few days (4–7), the insect is usually killed, especially by fungal toxins, and new spores are formed in or on the insect (sporulation), ready to be spread in the environment.

Keywords: entomopathogenic fungi ; entomopathogenic nematodes ; synergy

1. Introduction

Both entomopathogenic fungi and nematodes are pathogens/parasites with a broad host range ^{[1][2][3][4][5][6]} and they largely share an ecological niche, and often are isolated from the same soil samples. For instance, Tarasco et al. ^[7] reported co-occurrence of *Steinernema ichnusae* Tarasco, Mracek, Nguyen, and Triggiani (Rhabditida: Steinernematidae) and *Beauveria bassiana* s.s. (*sensu stricto*) (Bals-Criv.) Vuill. (Hypocreales: Cordycipitaceae) in the samples from an oak forest in Sardinia, Italy.

In response to competition, parasites and pathogens exhibit a diverse array of strategies that improve their chances of growth or reproduction over competitors ^[8].

2. Nematode Adaptations for Interactions with Entomopathogenic Fungi

One of the common modes of adaptation by nematodes to entomopathogenic fungi involves the avoidance of competition, and this has been demonstrated by Barbercheck and Kaya ^[9]. In their experiments, a major part of the infective juveniles (IJs) of *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) were repelled from the insects infected with *B. bassiana* s.l. (*sensu lato*). Similarly, in dual infection with the fungus *Cordyceps fumosorosea* (Wize) (Hypocreales: Cordycipitaceae), the invasion rate of *Steinernema feltiae* Bovien (Rhabditida: Steinernematidae) was lower in comparison with the nematode-only application ^[10]. Nevertheless, in both studies, the avoidance was only partial, as some infective juveniles migrated towards and penetrated the fungus-infected larvae.

Once the nematodes enter the fungus-infested insects, strong competition for resources occurs. Barbercheck and Kaya ^[11] have shown that the growth of *B. bassiana* s.l. in *Galleria mellonella* L. (Lepidoptera: Pyralidae) was inhibited in dual infection with *S. feltiae*, if the nematodes were applied simultaneously or 12 h after the fungus. The authors suggested that the main cause of inhibition was the bacterial symbiont of *S. feltiae*, *Xenorhabdus bovienii*. Since then, *Photorhabdus* and *Xenorhabdus* bacteria have been shown to produce many compounds with an antifungal activity. For instance, hydroxy–stilbenes (isopropylstilbene) produced by *Photorhabdus luminescens* effectively suppressed fungal human pathogens *Aspergillus flavus* Link (Eurotiales: Trichocomaceae), *Aspergillus fumigatus* Fresenius (Eurotiales: Trichocomaceae), *Botrytis cinerea* Pers (Helotiales: Sclerotiniaceae), *Candida tropicalis* Berkhout (Saccharomycetales: Saccharomycetaceae), and *Cryptococcus neoformans* (San Felice) Vuill (Tremellales: Tremellaceae) ^[12]. Gualtieri et al. ^[13] demonstrated that *Xenorhabdus nematophila* produces antifungal PAX peptides that suppress serious plant and human fungal pathogens. Similarly, the secondary metabolites of *Xenorhabdus budapestensis* and *Xenorhabdus szentirmaii* suppress plant pathogenic fungus *Phytophthora nicotianae* Breda de Haan (Peronosporales: Peronosporaceae)

[14]. The metabolites from *X. szentirmai* proved effective against four plant-pathogenic fungi, *Monilinia fructicola*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, and *Fusarium oxysporum* [15]. Cimen et al. [16] identified fabclavines as broad spectrum antifungal bioactive compounds responsible for the antifungal activity of *X. szentirmai*.

The secondary metabolites of *Xenorhabdus* and *Photorhabdus* bacteria were found to be effective during the competition of entomopathogenic nematodes and fungi within insect hosts. For instance, *X. nematophila* inhibited the growth of *B. bassiana* s.l. on agar plates [17]. In another study, *Photorhabdus luminescens* inhibited the growth and conidial production of *Metarhizium anisopliae* (Metch.) Sorokin, *B. bassiana* s.l., *Beauveria brongniartii* (Saccardo) Petch (Hypocreales: Cordycipitaceae), and *C. fumosorosea* [18]. Similarly, Tarasco et al. [7] demonstrated that extracts from *Xenorhabdus bovienii* inhibited the growth of *B. bassiana* s.s.

3. Fungal Adaptations for the Interactions with Entomopathogenic Nematode

During dual infections with entomopathogenic fungi, numerous studies recorded negative effects on the nematodes as well [10][19][20]. Naturally, entomopathogenic fungi produce many toxic metabolites in order to kill their insect hosts [21][22]; however, some compounds were found to have an antibiotic effect that is believed to protect the fungus against antagonistic microorganisms, or to prevent saprophytic microbes in the host cadaver [23]. Ansari et al. [18] demonstrated that the crude extract of *M. anisopliae* s.l. inhibited the growth of *P. luminescens* and *Xenorhabdus poinarii*. Similarly, fungal extracts from *B. bassiana* s.s. impaired the growth of *X. bovienii* [7].

Recently, Hummadi et al. [24] revealed that entomopathogenic fungus *Metarhizium brunneum* (Petch) (Hypocreales: Clavicipitaceae) produces volatile organic compounds that are highly toxic to the infective juveniles (IJs) of the EPN, *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora*, and these compounds can shape the interaction of these pathogens in the rhizosphere. These findings suggest that the interactions between entomopathogenic fungi and nematodes also occur outside the host.

4. Outcomes of the Interaction

Both entomopathogenic nematodes and fungi possess numerous adaptations for competition with each other. Barbercheck and Kaya [11] observed that these pathogens rarely co-produce progeny in infected hosts, and one of them usually prevail. The authors also observed that the nematode progeny production decreased with the time between the exposure of the hosts to *B. bassiana* s.l. and nematodes, and the fungus was detrimental to the development of *S. feltiae* and *Heterorhabditis heliothidis* (Khan, Brooks, and Hirschmann) (Rhabditida: Heterorhabditidae) when applied to the insect more than 48 h before nematodes [11]. Barbercheck and Kaya [25] hypothesized that the two day period corresponds to the time that circulating hyphal bodies appear in the fungus-infected host. When nematodes are applied after this period, they are unable to successfully develop in the host, and *B. bassiana* s.l. develops exclusively. Such an exclusion could be attributed to indirect interactions related to competition for the same host resources [26]. Similarly, less virulent strains of *M. anisopliae* s.l. applied 2 days before *H. bacteriophora* decreased nematode reproduction [20].

In simultaneous applications, nematodes usually outcompete the fungus [11]. Ansari et al. [27] observed that in simultaneous application, the combination with *Heterorhabditis megidis* Poinar, Jackson, and Klein (Rhabditida: Heterorhabditidae) and *Steinernema glaseri* Steiner (Rhabditida: Steinernematidae) was totally detrimental for the reproduction of *M. anisopliae* s.l. Interestingly, Shaurub et al. [28] observed the opposite situation, when maximum IJ yields of *Steinernema riobrave* Cabanillas, Poinar, and Raulston (Rhabditida: Steinernematidae) and *H. bacteriophora* were recorded in *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) previously exposed to *B. bassiana* s.s. Interestingly, Molina et al. [20] observed that a highly virulent fungal isolate, *M. anisopliae* s.l. totally inhibited the reproduction of *H. bacteriophora* even when applied simultaneously with the nematodes, and reduced nematode reproduction when applied after the nematodes. This observation suggests that the fungus directly interacts with the nematodes via the production of metabolites that are toxic to symbiotic bacteria or nematodes. Toxicity to bacteria is more probable, as the crude extract of *M. anisopliae* s.l. was found to be toxic to bacteria, while it had no toxic effects on *H. megidis* and *S. glaseri* even at the highest concentration [18].

As mentioned above, Barbercheck and Kaya [11] observed that these pathogens rarely co-produce progeny, and this was confirmed by several other studies. For instance, Wu et al. [29] reported that after the joint application of *H. bacteriophora* and *H. megidis* with *B. bassiana* s.s. and *M. anisopliae* s.s., no southern masked chafer white grub, *Cyclocephala lurida* Bland (Coleoptera: Scarabaeidae) showed both fungal sporulation and nematode development. On the other hand, Tarasco et al. [7] observed both *S. ichnusae* and *B. bassiana* s.s. developed in *G. mellonella*. The authors described that

both pathogens started the infection process in different parts of the host body and further developed in these defined spaces and competed in the haemocoel to conquer every available space. Therefore, the reproduction of both pathogens within one host is obviously possible, but this phenomenon is likely very rare.

It can be concluded that the interactions between entomopathogenic fungi and nematodes are very competitive and, in general, the nematodes appear to be stronger competitors due to their faster infestation and development inside the host. Nevertheless, in particular pathogen species and strain combinations, the outcome can be different.

5. Effect of the Entomopathogenic Nematode-Entomopathogenic Fungi Interactions on the Host

As was demonstrated above, the relationship between entomopathogenic fungi and the nematodes is mostly antagonistic, where one or both competitors are negatively affected. Nevertheless, the effect of dual pathogen infection can have an additive or synergistic effect on host mortality and can be used to increase the effectiveness of both pathogens in biological control. Ansari et al. [27] suggested that the mechanism of synergy in the insects infected with the nematodes after the fungus could lie in the fact that fungal infection stresses the host by affecting its food intake and body homeostasis while consequently decreases its mechanisms to overcome nematode infection that are very effective in healthy grubs [30][31]. In addition, the insects infected with the fungus respire more and attract entomopathogenic nematodes that follow gradient of carbon dioxide [32][33].

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