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Entomopathogenic and plant pathogenic nematodes as opposing forces in agriculture

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Abstract

Plant-parasitic nematodes (PPN) are responsible for substantial damages within the agriculture industry every year, which is a challenge that has thus far gone largely unimpeded. Chemical nematicides have been employed with varying degrees of success, but their implementation can be cumbersome, and furthermore they could potentially be neutralizing an otherwise positive effect from the entomopathogenic nematodes (EPN) that coexist with PPNs in soil environments and provide protection for plants against insect pests. Recent research has explored the potential of employing EPNs to protect plants from PPNs, while providing their standard degree of protection against insects. The interactions involved are highly complex, due to both the three-organism system and the assortment of variables present in a soil environment, but a strong collection of evidence has accumulated regarding the suppressive capacity of certain EPNs and their mutualistic bacteria, in the context of limiting the infectivity of PPNs. Specific factors produced by certain EPN complexes during the process of insect infection appear to have a selectively nematicidal, or at least repellant, effect on PPNs. Using this information, an opportunity has formed to adapt this relationship to large-scale, field conditions and potentially relieve the agricultural industry of one of its most substantial burdens.

Graphical Abstract

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Keywords

Entomopathogenic nematodes; Plant parasitic nematodes; Infection; Immunity; Parasitism

1. Introduction

Nematodes are a fairly vast phylum, and many of the species in the group also happen to be parasitic, opportunistically inhabiting a range of hosts that include plants, insects and animals (Dillman and Sternberg, 2012; L'Ollivier and Piarroux, 2013; Quist et al., 2015). Naturally, depending on the type of organism a nematode has infected and the context in which the infection is taking place, a nematode's success in terms of survival and parasitism can either be in line with, or sharply opposed to, human health or economic interests. Research into the interactions between the host immune system and nematode virulence mechanisms have therefore garnered considerable interest and support in the hope that these interactions can be mediated beneficially (Castillo et al., 2011; Babu and Nutman, 2014; Govere and Smant, 2014).

In particular, nematodes can have a large impact on agriculture through their effect on populations of insects and plants alike. Those nematodes that are entomopathogenic, or insect parasitic, can generally be thought of as advantageous, and the *Heterorhabditis* and *Steinernema* genera of entomopathogenic nematodes (EPNs) have been employed specifically and intentionally as biocontrol agents for insect pests (Ehlers, 2001; Ffrench-Constant et al., 2007). Plant-infectious nematodes, on the other hand, account for approximately 5% of crop yield loss by limiting root growth, plant size and photosynthetic rate (Gheysen and Mitchum, 2011; Kyndt et al., 2013). One of the confounding issues with this situation then is that because nematodes present in a plant's environment may be having opposite effects, an unspecific nematicidal treatment is eliminated as a viable strategy for crop protection, especially if EPNs are maintaining a population of insect pests below a harmful threshold, which may not be immediately apparent. These two kinds of nematodes may also have little competitive effect on each other, as efforts to suppress plant-parasitic *Meloidogyne parityla* nematodes with *Steinernema feltiae* produced inconsistent and marginal results (Shapiro-Ilan et al., 2006), although this may be a species-dependent effect, as other studies using different pairings of EPNs and plant-parasitic nematodes (PPNs) have shown more affirmative findings (Molina et al., 2007).

With this dynamic in place, guiding nematode interactions to a desired result may require a significant amount of tact and subtlety that will rely on a thorough knowledge of plant and insect immunity as it relates to nematode infection. It is important to consider as well that these two forms of immune response will have strong and fundamental differences. In general, insect innate immunity consists of mechanisms that lead to the production of antimicrobial peptides (AMPs) and reactive oxygen species (ROS), and cellular functions involving phagocytosis, encapsulation and nodulation (Welchman et al., 2009; Viljakainen, 2015), but plants, lacking a cellular response, will be incapable of responding to nematodes with functions such as encapsulation, which is the most common insect immune response to a metazoan invader (Jones and Dang, 2006; Muthamilarasan and Prasad, 2013; Honti et al., 2014; Vlisidou and Wood, 2015). A further examination of these two immune systems and

their respective responses to nematode parasites will outline the primary mechanisms of insect and plant resistance.

2. The insect immune response to EPNs

A nematode infection of an insect host begins when a nematode of the infectious juvenile (IJ) stage attaches to the cuticle of the insect, penetrates through the various natural openings, such as the spiracles or mouth, and establishes itself in the hemolymph after advancing into the body cavity (Griffin, 2012). Once established, the nematode will release its mutualistic bacteria into the host hemolymph, *Photorhabdus* bacteria in *Heterorhabditis* nematodes and *Xenorhabdus* bacteria in *Steinernema* nematodes, either through regurgitation or esophageal pumping of the bacteria down through the intestine and out of the anus (Goodrich-Blair, 2007; Waterfield et al., 2009). These bacteria then go on to release toxic and immunosuppressive compounds, eventually leading to the death of the host by septicemia (Ffrench-Constant et al., 2007; Herbert and Goodrich-Blair, 2007). The release of nematode mutualistic bacteria does not occur immediately, however, and is instead delayed, by 30 min in the case of *Heterorhabditis*, and 4 – 6 h for *Steinernema* (Li et al., 2007). This means that the host insect has a window, granted of a variable timeframe depending on the species, in which it may neutralize the parasite before being forced to compensate for the additional challenge of the bacterial infection. In a general way, nematode avirulence is primarily achieved by initiating hemolymph clotting, activating a melanization reaction, and encapsulating the nematode in layers of hemocytes. Clot formation is based on the activity of soluble factors in the hemolymph, including transglutaminase, which will bind foreign bodies, including *Photorhabdus* (in the case of transglutaminase), and form microclots that can be incorporated into networks of fibers produced by hemolymph and triggerin that will further isolate the pathogen (Hyrsl et al., 2011; Toubarro et al., 2013). The melanization reaction, which is technically part of the humoral response, although it functions in close association with the cellular response, is comprised of the conversion of the inactive precursor prophenoloxidase to active phenoloxidase (Eleftherianos and Revenis, 2011), which generates the indole groups used to form melanin that then binds the nematode and supports the destruction of the parasite with ROS (Castillo et al., 2011). The cellular response, although thus far underrepresented, then plays perhaps the most crucial role by rapidly encapsulating the pathogen in compacted layers of hemocytes, which is a process that can be initiated within minutes of exposure, and potentially prevents the release of bacteria into the hemolymph (Satyavathi et al., 2014).

The release of bacteria by nematodes is clearly a challenge to the host that, once initiated, is difficult to overcome, as each nematode can release 50 – 200 bacteria directly into the hemolymph (Goodrich-Blair, 2007; Wu et al., 2014). The products of these bacteria can undermine much of the immune system, both cellular and humoral, by releasing toxic components that are capable of damaging hemocytes, and enzymes such as the RTX-like metalloprotease of *Photorhabdus* that can cleave hemolymph proteins involved in regulating host immune effector genes (Bowen et al., 2003; Rodou et al., 2010; Vlisidou et al., 2012). The success of the insect immune system in overcoming an infection by nematode parasites is therefore based largely on its ability to prevent the release of these bacteria and their immunosuppressive products, and indeed, a correlation between survival and the degree of

encapsulation has been demonstrated (Li et al., 2007; Eleftherianos et al., 2010). A number of variables in the immune response, as viewed between different species of insects, reflect this point through their ability to influence host survival (Castillo et al., 2011). One such variable is the starting point of encapsulation. As mentioned previously, nematodes eject their mutualistic bacteria from the mouth and anus (Snyder et al., 2007), and therefore it would follow that the most effective method of encapsulation would be to cover these openings first in order to prevent the release of bacteria. Concordantly, encapsulation of *Heterorhabditis* by *Manduca sexta* hemocytes is initiated at the head and tail of the nematode (Li et al., 2007). In the Colorado potato beetle, *Leptinotarsa decemlineata*, however, encapsulation is initiated in the middle of the nematode, near the esophageal region, possibly in response to secretory-excretory pore exudates (Ebrahimi et al., 2011). This mechanism could represent a disadvantage for the insect, as the nematode could potentially eject bacteria during the encapsulation process and well before the process is complete. The time frame of the encapsulation process measured against the timing of bacterial release is also important, as both are represented by a spectrum of a fairly wide range. Encapsulation may begin within minutes of exposure, but completion of the task involves multiple stages, each with their own timing (Stanley et al., 2009; Jiang et al., 2010). Depending on the species involved in the interaction, the formation of multiple layers of hemocytes can take 45 min – 2 h, the complete encapsulation of the nematode 2 – 4 h, and partial melanization 16 – 24 h, as was observed with combinations of *Heterorhabditis bacteriophora*, *L. decemlineata*, and *Galleria mellonella* (Ebrahimi et al., 2011). These differences could be crucial in determining the survival of the insect based on the delay of bacterial release by the nematode, which as mentioned previously can be as brief as 30 min or as long as 4 – 6 h.

The insect immune response has been described in some detail, but one factor that has not yet been discussed is that nematode parasites are also capable of evading detection by the immune system, in which case the mechanics of a cellular response would be largely irrelevant (Brivio et al., 2005; Castillo et al., 2011). Lytic surface coat proteins, hydrophobic exudates, and lipopolysaccharide-like binding proteins produced by nematodes can all facilitate the parasite's evasion of encapsulation (Li et al., 2009; Brivio et al., 2010; Mastore et al., 2014). Overall, the interaction can then be characterized as a highly complex interplay between the genotypes of the insect, the nematode and its mutualistic bacteria, which although perhaps difficult to predict, does provide a number of potential targets for control that could be beneficial to agriculture if applied appropriately. In the context of eliminating insect pests, strains could be developed that produce the proteins necessary for evasion, or nematodes could be generated that have significantly decreased delays in the ejection of their bacterial endosymbionts. Future research may do well to investigate the factors that generate differences in ejection timing, as nematodes that can overwhelm the insect immune system before being encapsulated would likely serve as much more efficient biocontrol agents.

3. The plant response to root knot nematodes (RKNs)

PPNs, and in particular, Root Knot Nematodes (RKNs) of the genus *Meloidogyne* carry out a slightly different parasitic protocol than their entomopathogenic counterparts (Curtis,

2007). RKNs initiate an infection by penetrating host root tips as second stage juveniles (J2) after hatching from eggs in the soil (Caillaud et al., 2008). Pharyngeal secretions from the nematode then induce proximal cells to undergo mitosis repeatedly without cytokinesis, such that a cluster of hypertrophic, multinucleated “giant” cells, referred to as a “gall”, is formed and serves as a food source for the nematode, which is then sedentary (Melillo et al., 2014; Davies et al., 2015). In addressing this invasion, the plant is limited to a program more similar to the insect humoral system than cellular system, as plants lack the specialized cell types required for a cellular response. The specific programs that moderate resistance to RKNs are still poorly understood, but progress has been made toward clarifying how some plants are able to gain relative advantages when employing their immune mechanisms against a nematode parasite.

A number of fundamental mechanisms appear to play a role in RKN resistance, including R genes such as *Mi 1.2* in tomato plants, which encodes a member of the nucleotide binding leucine rich repeat gene family (Villevet et al., 2015), and the hypersensitive response, which appears to be a commonly employed mechanism for limiting RKN feeding capacities. In particular, a resistance gene referred to as *RMcl(blb)* in potatoes is known to limit nematode virulence by influencing the concentration of Ca^{2+} in root cells near the nematode (Davies et al., 2015). In this system, *RMcl(blb)* apparently induces the upregulation of the calcium-dependent protein kinase CDPK4, which may play a role in the release of ROS in response to a Ca^{2+} influx, possibly then inducing cell death characteristic of the hypersensitive response and preventing the nematode from forming giant cells as a resource reservoir. It is important to note, however, that the hypersensitive response is not always required for resistance to RKNs. Alfalfa for instance, does not display a hypersensitive phenotype in response to infection, but instead is apparently capable of resistance by denying nematodes access to the developing vascular cylinder of the root (Postnikova et al., 2015).

The salicylic acid (SA) and jasmonic acid (JA) pathways have also been identified as potential proponents of immunity. JA biosynthesis and signaling component genes have been found to be upregulated after RKN infection, and exogenous application of JA has been found to reduce root egg masses in tomato plants (Zhou et al., 2015). Exogenous application of the SA analog benzothiadiazole (BTH) to tomato plants also led to a decrease in gall formation, which is believed to be the result of cell-wall stiffening due to increased H_2O_2 production, lignin accumulation, and peroxidase activity in response to the BTH exposure (Melillo et al., 2014). Furthermore, when exposed to BTH, parenchymal cells surrounding the feeding site underwent a change in Tap1 anionic peroxidase expression and subsequently appeared to entrap the nascent giant cells, potentially preventing them from expanding further, perhaps similar to a rudimentary, extemporaneous cellular response.

Enhancing resistance in crop plants to *Meloidogyne* would be remarkably beneficial to agriculture as an industry, but there are still significant voids in the available information about the plant immune mechanisms that are capable of conferring this resistance. Much of the information available is limited to a level of detail that refers only to functional groups such as oxidative stress or ubiquitination as means of resistance (Kyndt et al., 2012; Villevet et al., 2015), and in cotton, overexpression of *Meloidogyne*-induced cotton 3 (MIC3) was able to dramatically reduce egg production by *Meloidogyne incognita*, but the function of

MIC3 remains entirely unknown, and it does not appear to have any identifiable functional domains (Wubben et al., 2015). The interaction between plant parasitic nematodes and their hosts therefore represents an exciting field, potentially rich with novel interactions that in the future could be used to develop more stable and resistant crop plants.

4. The suppression of PPNs with EPNs

There is a collection of research describing interactions between EPNs and PPNs with regard to the ability or tendency of EPNs to interfere with the normal activity of PPNs. Fortunately, the available research allows a number of stepwise conclusions to be made about this system, based on specific experimental characteristics, such as the species of EPN being used and the presence or absence of an insect host, which indicate strongly that the symbiotic bacteria of EPNs may be responsible for the observed adverse effects on PPNs due to the production of deterrent factors from these symbiotic bacteria together with the factors that are produced to weaken or kill an insect host. The fundamental concepts of the interactions are generally straightforward, but the array of details that can affect the system will prove to be remarkably complex.

Using EPNs to deter PPNs for the benefit of a plant is a task that becomes rapidly more and more complex once the environment is transitioned from a controlled laboratory setting to the endlessly variable conditions present in an agricultural soil environment. Control of the system requires a method that accounts for, or simplifies, interactions between both types of nematodes and the plants, the two groups of nematodes, and certain insect species that act as pests of the plants and hosts of the EPNs. Moreover, each of the four parties encompasses a myriad of species that are not all likely to interact in the same way. Certain EPNs may be more effective against certain species of PPNs, and some plant species may not be as attuned to the benefits of specific EPNs. A number of studies have, however, thoroughly examined specific instances of the four-part interaction, providing individual pieces of information that have begun to form a pattern. Superficially the results can appear to lack a robust quality, or otherwise look inconsistent, but the specific successes that have appeared demonstrate a clear potential for the biological control of PPNs with EPNs.

One notable characteristic that separates the studies done to date is the application method used to introduce the EPNs, which can involve direct application or the use of an insect host cadaver as a delivery vessel. The more common method examined so far has been the direct introduction of IJ EPNs in aqueous solution (Kepenekci et al., 2015), although this specific technique has produced fairly mixed results. In another study, EPNs were applied in greenhouse and laboratory assays in order to monitor their effect on the *Meloidogyne javanica* infection process in tomatoes and soybeans (Fallon et al., 2002). The specific EPNs used were *Heterorhabditis indica*, as well as *S. feltiae* and *Steinernema riobrave*, but the only effect observed was a reduction in *M. javanica* root penetration after the introduction of *S. feltiae*. All other treatments were similar to water control treatments with regard to root penetration, *M. javanica* egg production and plant biomass, which generally can be interpreted as an insufficient degree of effect to be considered suppression. Pérez and Lewis (2002) however, observed strong suppressive effects, albeit with a significantly altered approach in terms of experimental setup. In this study, the ability of the same *Steinernema*

spp., *S. feltiae* and *S. riobrave*, together with *H. bacteriophora*, was monitored for protective capacity when co-incubated under laboratory conditions with tomato seedlings and *M. incognita* (PPN) eggs. The results showed that the seedlings treated with the two *Steinernema* spp. had fewer *M. incognita* juveniles infecting their roots, and that these nematodes also went on to produce fewer eggs. Furthermore, all EPN introduction time points from 2 weeks prior to *M. incognita* infection to 2 weeks after were effective, and increases in the concentration of *Steinernema* were correlated with an enhanced suppressive effect, although low and high concentrations both resulted in suppression. Grewal et al. (1999) also examined tomato root penetration by *M. incognita* in sterilized sand, and the ability of *Steinernema* spp. to suppress it, but found that the application of live *Steinernema* did not have a protective effect. The scattered positive results that have arisen from the application of IJs seem to indicate a potential capacity of EPNs for direct interference with PPNs, but arguments could be made that this is merely an effect of highly variable experimental designs. In the case of the Pérez and Lewis (2002) study for instance, a control was not in place to identify any differences between control and treatment trials in reference to the ability of *M. incognita* eggs to hatch properly. These assays may have simply appeared to provide evidence of protection due to the partial destruction or manipulation of the *M. incognita* eggs by the *Steinernema* IJs.

Another significant factor that undoubtedly guides the results of these studies is that they have been performed under greenhouse or laboratory conditions, which highlights the importance of another study that identified a suppressive capacity in EPNs. The authors performed a field study of golf course turf grass that included *M. incognita* and aqueous application of *S. riobrave*, and found that *S. riobrave* was as effective as chemical nematicide, if not more so (Grewal et al., 1997). One consideration that comes with this result is that the natural life cycle of EPNs, and namely their interactions with and the ability to infect insect hosts, may play a role in the suppression of PPNs, even if the nematodes alone do not possess the ability to interfere with the infection process. EPNs that have infected a host have been found to display greater infectivity, increased dispersal and increased survival compared with nematodes in aqueous suspension (Shapiro and Glazer, 1996; Shapiro and Lewis, 1999; Pérez et al., 2003). Consistent with this notion, a number of studies have performed similar tests to those completed with IJs in aqueous suspension, but instead co-incubated plants and PPNs with insect cadavers that had been infected with EPNs prior to the assay. Some of these studies also performed duplications of their assays with IJs in aqueous suspension, allowing for a direct comparison between the two methods within the same experimental design. One such study found that insect hosts infected with *Steinernema carpocapsae*, *S. feltiae*, and *S. riobrave* were all capable of repelling *M. incognita* from tomato roots during a greenhouse trial, while the application of IJs failed to promote a decrease in root penetration (Grewal et al., 1999). Other similar studies, however, remain mottled with inconsistency or limited results, although with a trend towards suppression. For example, a previous study found an 18% reduction in egg masses from *Meloidogyne parityla* during greenhouse assays in pecans after the application of *S. riobrave*-infected hosts as well as increased dry-root weight when plants were treated with *S. feltiae*-infected hosts (Shapiro-Ilan et al., 2006). Generally, however, the results were not strong enough to conclude that *M. parityla* could be suppressed with the entomopathogenic

species tested. Interestingly, another study found evidence of suppression by an *H. bacteriophora* isolate (Rama Caida) when used as a treatment for *M. incognita* infection in pepper and summer squash (Del Valle et al., 2013). The number of *M. incognita* eggs was reduced in both plants 60 days p.i. Oddly, the *Steinernema* spp. tested did not produce suppressive results, although the species was *Steinernema diaprepesi* rather than the more commonly used *S. feltiae* or *S. riobrave*.

The next challenge then is to explain why a suppressive effect might generally be more likely to occur if the EPN being used as a treatment is first allowed to infect an insect host. Perhaps the most striking change between these two treatments is the fact that if the EPN has infected a host, its mutualistic bacteria will have been released into the host where they will have begun to secrete components that aggressively enhance the infection process. As mentioned, PPNs do not infect cooperatively with mutualistic bacteria, so a reasonable assumption is that they would be less tolerant of the products of an EPN's mutualistic bacteria. Some evidence has been generated to support this notion and even demonstrate that the cell-free culture supernatants of these bacteria may be sufficient to repel PPNs. As early as 1997, investigators demonstrated this effect with cell-free culture filtrates of the mutualistic bacteria of *S. carpocapsae*, *S. feltiae* and *S. riobrave*, namely *Xenorhabdus nematophilus*, *Xenorhabdus bovienii* and *Xenorhabdus R*, respectively (Grewal et al., 1997). All three of these filtrates were nematocidal and demonstrated 98 – 100% mortality when tested against *M. incognita* IJs, thus building on previous research indicating that *Xenorhabdus* spp. produce ammonia, indole and stilbene derivatives, and that the stilbene derivatives and ammonia have selective nematocidal capacities (Paul et al., 1981; Hu et al., 1988; Richardson et al., 1988). In their 2002 study, Pérez and Lewis also suggested that their results may have been partially due to the activity of *Xenorhabdus* spp. (Pérez and Lewis 2002), and in a 2015 study, the authors were able to demonstrate that dipping tomato plants in *X. bovienii* spent medium supernatants is capable of suppressing *M. incognita* egg mass numbers and increasing plant height compared with infected, but untreated, controls (Kepenecki et al., 2015). This result was also compared with a less effective *Photorhabdus luminescens* treatment, which is consistent with the more stable suppressive results observed when *S. feltiae* is used for biocontrol rather than *H. bacteriophora* (Lewis and Grewal, 2005).

The suppressive effects of *Xenorhabdus* filtrates have therefore been somewhat well demonstrated in the context of PPNs, but moreover, the effects of *Xenorhabdus* have also been well examined in the context of repelling scavengers in general, likely as a method of preventing the nematode host of the bacteria from being destroyed while reproducing within an infected insect (Zhou et al., 2002). The authors characterized a component of *X. nematophila* supernatants that is capable of repelling the Argentine ant *Linepithema humile*. The repellent component was found to be filterable, heat stable, acid sensitive and small enough to pass through a 10 kDa pore-size membrane, as well as present in some form in *P. luminescens*, although optimally present in supernatants after 132 h compared with the 108 h of *Xenorhabdus* cultures, which may provide at least a partial explanation of the more consistent efficacy of *S. feltiae*. These observations were later expanded in a well-controlled study in which the feeding behaviors of ants, crickets and wasps on nematode, freeze and

axenic nematode-killed insects were monitored (Gulcu et al., 2012). The results showed that in general, the tested species would not feed on an insect killed at least 2 days previously by a nematode with its mutualistic bacteria. Crickets were, however, found to feed on insects infected by axenic nematodes, insects infected with *Serratia marcescens*, and freeze-killed insects, strongly indicating that the mutualistic bacteria were responsible for the deterrent effect.

EPNs therefore have demonstrated a strong potential, through their mutualistic bacteria, as a possible biocontrol method for PPNs (Fig. 1). Applying this potential would represent a tremendous advantage to the agriculture industry, but some of the subtleties involved in effectively introducing and sustaining the suppressive effects will require some additional work and research. The current information available seems to indicate that *S. feltiae*, and specifically *X. bovienii*, is the strongest candidate. As mentioned previously, *S. feltiae* has had the most consistent suppressive results, and its mutualistic *X. bovienii* has been suggested to produce a higher or otherwise more effective concentration of the deterrent factor (Gulcu et al., 2012). The aforementioned studies seem to indicate that *S. feltiae* contributes to suppression primarily as a vessel, which means a number of methods could be used to deploy *X. bovienii* in the field, although each with relative strengths and weaknesses. For instance it was suggested that plants could be dipped in *Xenorhabdus* culture filtrates, but this may only be effective transiently, as the stability of the nematicidal substances and deterrent factor in soil remain unknown (Kepenecki et al., 2015). Employing this method commercially would also require that nematodes still be used to harbor the *Xenorhabdus* at least occasionally, as *Xenorhabdus* can revert to a secondary form spontaneously in long-standing laboratory cultures, and fail to produce the substances that are associated with its cooperative action in *Steinernema* spp. (Zhou et al., 2002). The application of IJs carries its own challenges as well, however. The common application rate of IJs that has been shown to be effective is 2.5 billion IJs per hectare (Lewis et al., 2001), but this concentration is naturally reduced rapidly after application (Smits, 1996) due to a lack of hosts, temperature fluctuations, UV radiation, dessication (Kaya, 1990), and antagonists present in the soil (Kaya, 2002). A treatment that effectively suppresses PPNs will therefore require some enhancement in the stability or maintenance of *Steinernema*, or their associated *Xenorhabdus* spp., in a soil environment. This is a broad statement, but the possible solutions are just as broad. Additional research may develop a stable *Xenorhabdus* strain that innocuously associates with a variety of plant species, or a method for efficiently co-seeding a soil environment with EPNs and an insect host could be designed. Regardless of the specific method eventually used to address the biocontrol of PPNs, the presently available information leaves the field poised for a solution to a long-standing and high priority obstacle to agriculture.

Additional research would also need to be done in order to determine whether the deterrent factor(s) being produced by *Xenorhabdus* is specific to certain nematode species or if the nematodes are responding to a broad-spectrum factor. EPNs may benefit from specifically deterring PPNs in that PPNs damage plants, and that these plants are also a food source for the insect pests that the EPNs consume, but a concomitantly plausible theory would be that certain insect pests could also benefit from the activity of PPNs, which may exhaust the

immune resources of the plant and reduce its capacity for defense against insect herbivory. The exact balance and thresholds of benefit and detriment are likely to be highly variable however, as the species involved could dramatically affect long term outcomes based on specific immune responses as well as relative consumption and reproduction rates, among other factors. Despite this difficulty, an attempt to isolate a factor specific to the repulsion or killing of PPNs would likely be a worthwhile pursuit because the field use of one specific factor could greatly increase efficiency and reduce the possibility of detrimental, non-specific effects.

5. Concluding remarks

Nematodes are ubiquitously involved in many human interests, in terms of health, as over 100 species of nematodes are human parasites, and with regard to the global economy as nematodes can function as plant, livestock or insect parasites. In particular, because nematodes play a dual role in agriculture as both biocontrol agents of harmful insect pests and pathogens of plants themselves, a more detailed and complete understanding of how nematodes interact with the immune systems of their respective hosts could provide a strong advantage in developing strategies to mitigate the damage caused by plant pathogens and enhance the efficacy of EPNs as a biocontrol mechanism. An immunological approach may be especially beneficial as it can target specific nematode groups rather than employing a broad nematicidal agent, which could equally promote deleterious effects. Some fields of research that may be of particular interest include the pathways that dictate the timing of mutualistic bacteria ejection in EPNs, the mechanisms by which plants are able prevent RKN species from entering their root systems, and the precise nature of the relationship between EPNs and PPNs, especially with regard to the effect of EPN bacterial factors on PPNs, as this field may provide methods for control that are both efficiently applicable and effective.

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Highlights

- Entomopathogenic nematodes coexist with plant pathogenic nematodes
- Nematodes interact with the immune systems of their respective hosts
- Plant pathogenic nematodes suppress entomopathogenic nematodes
- Factors from entomopathogenic nematodes can repel plant pathogenic nematodes
- Novel strategies are required to control plant pathogenic nematodes in the field

Fig. 1.

A representative illustration of the context in which an entomopathogenic nematode (EPN) (*Steinernema* nematodes) can interfere with the infection process of a plant pathogenic nematode (PPN) (*Meloidogyne* nematodes). The EPNs are seen entering the insect host cadaver (1) where they then release their mutualistic *Xenorhabdus* bacteria into the hemolymph (2). A number of bacterial factors are then produced (3), some of which are selectively nematicidal, resulting in the repulsion of, or interference with, the plant pathogenic species (4). The plant root to the right that is not protected by nematicidal factors is shown to be burdened with nematode-induced root gall formation.