Heterorhabditis Bacteriophora: An Ecofriendly Biological Control Agent

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Abstract

The entomopathogenic nematode, *Heterorhabditis bacteriophora*, is an environmentally safe alternative to chemical pesticides. It is half of a symbiotic relationship with the bacteria, *Photorhabdus luminescens* which lives in the nematode gut. *Heterorhabditis bacteriophora* has a wide range of susceptible insects making it a very effective alternative to current biological control practices. The nematode has been proven to be safe to humans, non-target insects, wildlife, fauna, and water. For this reason, as well as consumers' increasing consciousness of health issues, *Heterorhabditis bacteriophora* should be considered as a viable alternative and researched more thoroughly.

Keywords: *Heterorhabditis bacteriophora, Photorhabdus luminescens,* Biological Control, entomopathogenic nematode

Introduction

Heterorhabditis bacteriophora (Hb) is a microscopic entomopathogenic nematode (EPN) that is commonly referred to as a roundworm [Inman et al., 2012]. It is a non-segmented worm that is tapered at both ends (shown in Figure 1). The nematode is one half of a mutualistic relationship with the bacteria *Photorhabdus luminescens* and dwells naturally in soils around the world [Upadhyay et al., 2015]. Due to *Heterorhabditis bacteriophora* naturally occurring in soil, it has been used as a natural biological control agent by growers for many years [Lord, 2005]. The use of this nematode as a biocontrol agent is environmentally safe to humans, animals, plants, other insects [Lacey et al., 2001] and the environment. Hb is economically beneficial because of its ease of use and application, its relative ease of production and its low cost when compared to chemical biological control agents in use today [Lacey et al., 2001]. The presence of the *Heterorhabditis* nematode in itself is not enough to cause virulence in the insect host. Only when *Photorhabdus luminescens* bacteria are present in the gut of the nematode is virulence shown in insect hosts [Holmes et al., 2016]. With this being said, the relationship is not obligate but mutualistic because the nematodes provide a route inside the host for the bacteria and in return, the bacteria kill the insect host and provide food for the reproduction of both partners involved [Ciche and Ensign, 2002].

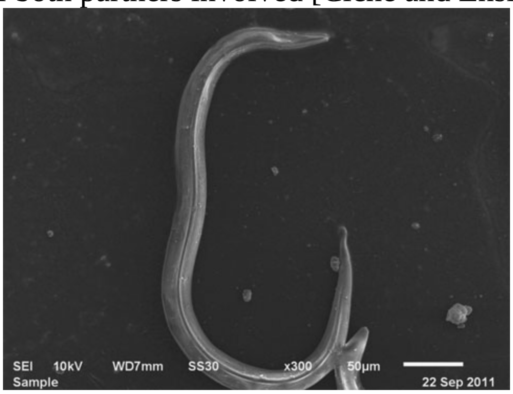

Figure 1. *Heterorhabditis bacteriophora* nematode shown under a scanning electron microscope [Inman et al., 2012].

Relationship with *Photorhabdus luminescens*

Heterorhabditis bacteriophora, is part of a symbiotic relationship with the bioluminescent bacteria *Photorhabdus luminescens* (Figure 2) which lives in the nematode gut [Ehlers, 2001]. Once the nematode and its symbiont bacteria are inside the insect host's hemocoel, *P. luminescens* allows the nematode to survive inside the insect host's body by secreting toxins and antimicrobials directly into the haemocoel [Gulley et al., 2015]. This is done to prevent an immune response against *H. bacteriophora* as well as preventing invasion of the host by other bacteria. *Photorhabdus luminescens* also produces enzymes that break down the carcass of the host in order to provide nutrients for both partners' consumption during reproduction [Gerdes et al., 2015; Patterson et al., 2015]. Each partner of this symbiotic relationship can live without the other in a laboratory setting proving that the relationship is not an obligatory relationship. However, *Heterorhabditis bacteriophora* can only form this type of symbiotic relationship with the bacterium *Photorhabdus luminescens* [Kooliyottil et al., 2013].

Figure 2. Cadavers of the wax moth expressing bioluminescence after infection by H. bacteriophora 2 days previous [Poinar and Grewal, 2012].

Heterorhabditis bacteriophora as a Biological Control Agent

The use of EPNs as biological control agents is not a new idea. In fact, *Heterorhabditis bacteriophora* strains have been known as biological control agents since the 17th century [Smart, 1995]. However, their use was not taken seriously until the 1930s. EPNs have been used to protect a variety of different plants [Upadhyay et al., 2013] such as, citrus [Abd-Elgawad, 2013], turf [Johnigk et al., 2004], Cotton [Shapiro-Ilan et al., 2002], mushrooms [Georgis et al., 2006], Apples, Pears, and Nuts [Lacey and Shapiro-Ilan, 2003] because they are pathogenic towards more than 200 insect host species [Shapiro-Ilan et al., 2002] from different orders such as, Diptera, Coleoptera, and Lepidoptera [Pilz et al., 2014]. Animal grazing lands and field crops such as coffee, sugarcane, wheat, as well as orchard crops, generally receive chemical, organic pesticides. The percentage of crops currently treated with biocontrol agents is at about 55% [Thakore, 2006]. This is significant because the total amount of organic crops is estimated to be 4.2% of the world's farm land. In 2004, the United States had the fourth largest amount of organic farm land (Table 1) with 950,000 hectares. This represents 0.23% of the world-wide total farm lands. Organic farming has been shown to allow greater crop growth, better soil health, as well as more natural nutrients and minerals for plant growth [Thakore, 2006]. This means that farmers will be able to grow healthy crops for many more years than farmers using conventional chemical pesticides [Smart, 1995]. The public's increasing awareness of the health risks and the harmful effects of chemical synthetic pesticides is a factor encouraging farmers to switch to biocontrol agents. Examples of the harmful effects of chemical pesticides are the development of resistance and the possibility of meat and milk contamination [Georgis, 2006]. The Food Quality and Protection Act of 1996 has pressed for the regulation of chemical pesticides and has encouraged growers to start to look towards organic biological control agents [Shapiro-Ilan et al., 2002]. The use of biopesticides has risen since the early 2000s in which they were at 1.7% in 2003, 2.5% in 2006 and 4.2% in 2010 [Thakore, 2006]. If growers were to cease the use of synthetic pesticides, then crop yields would increase [Thakore, 2006].

Region	Percent of Global Organic Farmland	Number of Hectares (millions)
Oceania	40%	17.5
Europe	27%	11.5
Latin America	15%	6.6
Asia	8%	3.4
North America	7%	3.1
Africa	3%	1.2

Table 1. The total organic farming land on each continent in 2013 [White, 2015].

Safety of *Heterorhabditis bacteriophora* as a Biological Control Agent

Heterorhabditis bacteriophora has been shown to have no effects towards humans, non-target insects, the environment, or the fauna [Smart, 1995]. It has also been shown to be sustainable and environmentally safe [Susurluk and Ehlers, 2008]. However, it has been suggested that the use of *Heterorhabditis bacteriophora* as biocontrol agents could allow for the nematodes to become too aggressive of a pesticide causing them to wipe out beneficial insects [Lord, 2005]. An argument countering this reasoning is the *H. bacteriophora* nematodes are effective against many different insects in laboratory trials [Hazir et al., 2003], however, in field trials, they possess a much more narrow range of insects which would prevent them from moving on to kill beneficial insects. Once the host insects are no longer present, the nematodes will die off [Susurluk and Ehlers, 2008]. Ideally, nematodes will recycle in the soil after the initial application for years afterwards. However, factors such as low efficacy under bad conditions, timing of initial application, temperatures, and insect availability affect the number of nematodes present [Smart, 1995].

Modified *Heterorhabditis bacteriophora* Nematodes

The use of transgenic nematodes with heat tolerance genes as biological control agents could potentially allow nematodes to infect and survive in mammals due to their ability to tolerate the warmer temperature. It has been shown that transgenic nematodes would not pose any extra threats to the environment when applied to fields in wide-scale trials [Wilson et al., 1999]. In a study comparing heat tolerance and desiccation tolerance, strains with heat tolerance genes caused slightly higher levels of virulence and expressed a better ability for host penetration and reproduction compared to strains with desiccation tolerance [Mukuka et al., 2010]. The use of inbred lines has also been shown to possess beneficial traits that are superior to those of their parent cells [Bai et al., 2005].

Commercial Production

There are a few things that must occur before the wide spread acceptance and use of EPNs as biological control agents will happen. First, it is important to increase virulence and performance under conditions that are not ideal. The species must be able to tolerate variation in the environment. Second, increased profit for production companies and lower retail costs for grower. Third, ease of application as well as greater persistence and shelf life [Lacey et al., 2001]. A method to allow infected juvenile nematodes to survive longer in temperatures greater than 35 degrees Celsius will allow nematodes to be transported easier as well as increase their shelf life. Currently, this is a major obstacle currently facing the wide spread acceptance of EPNs [Mukuka et al., 2010]. Short term exposure to temperatures higher than 35 degrees Celsius can lower infected juvenile reproduction ability, activity, and viability [Mukuka et al., 2010]. This could be extremely costly to producers because the product is no longer effective against insect pests. Other than temperature sensitivity, other major disadvantages of nematodes are specificity (the range of insects susceptible), time needed to kill the host, and the cost compared to chemical pesticides [Lacey et al., 2001]. Application methods of the nematodes must be considered. These must either be overcome or alleviated by the advantages in order to be an economically viable solution to the pesticide problem.

EPNs are mass produced in liquid media [Johnigk et al., 2004] using bioreactors [Inman et al, 2012]. These bioreactors are extremely costly and for smaller companies, may not be feasible. For that reason, many companies pull their nematode products off the shelves and quit production shortly after they begin. However, the developments in the equipment and technologies may allow for faster production which would allow for profits to be higher and more attractive in the eyes of producers [Hazir et al., 2003].

Issues with *Heterorhabditis bacteriophora* Use

Ploughing fields is a normal part of farming field crops, however, it causes an immediate decrease in the number of nematodes present in fields (Figure 3). It has been shown that the use of a harrow to till land had a much less dramatic effect of the nematodes [Susurluk and Ehlers, 2008].

Heterorhabditis bacteriophora strains are known for having low tolerances to both heat and desiccation. Because of that, it has been suggested that these strains be genetically modified to tolerate a higher temperature, desiccation, or a combination of both [Segal and Glazer, 2000]. One *Heterorhabditis bacteriophora* strain, KKMH1, has been found to be heat tolerant [Seenivasan and Sivakumar, 2014]. KKMH1 was isolated from dry regions and is more tolerant towards desiccation than other strains. However, other reports indicated that *H. bacteriophora* strain KKMH1 possessed no tolerance towards rapid desiccation [Seenivasan and Sivakumar, 2014]. *Heterorhabditis bacteriophora* was shown to be very tolerant to heat when compared to *H. indica,* and *H. megidis* [Mukuka et al., 2010].

Storage is also a potential issue with *Heterorhabditis bacteriophora* due to their inability to survive in temperatures above 35 degrees Celsius. This makes transportation difficult as well as significantly limits the shelf life of the product. In turn, this can limit the use of EPNs as biological control agents [Smart, 1995].

The cost of treating crops with nematodes can be expensive. The effectiveness of an application of EPNs relies heavily on conditions such as temperature, rainfall, and soil conditions [Pilz et al., 2014]. Due to this, repeated applications might be required to maintain the level of control over the pests. This is expensive and work intensive. As strains of nematodes become more hardy, more growers will invest in them [Smart, 1995].

Nematodes can be applied to soil by many different methods, including foliar applications and surface applications [Lacey and Geogis, 2012]. Foliar application of EPNs has been shown to be the least effective due to intolerance to desiccation. It is possible that the engineering of a desiccation tolerance gene could be added to make foliar applications more feasible, but as of this writing, it has not been accomplished. Surface applications are when nematodes are applied directly onto the soil. It is normally paired with irrigation to sustain the nematodes until they can migrate into the soil. Surface applications have been recorded as being the most successful method [Lacey and Geogis, 2012]. Since nematodes in the infected juvenile stage (IJs) are capable of tolerating higher pressures, they can be applied using conventional methods. EPN water suspensions are sprayed directly onto the soil allowing them to infect the insect pest while they are moving over or through the soil [Lacey and Geogis, 2012]. One study suggested that the use of a one-patch application (applying the nematodes in only one portion of the field) was ineffective and did not reduce the grub population. However they discovered that the use of a uniform application method as well as a nine patch method (applying nematodes in 9 evenly spaced areas) was effective in reducing the grub population after a year [Wilson et al., 2003]. The application patterns are shown in Figure 4.

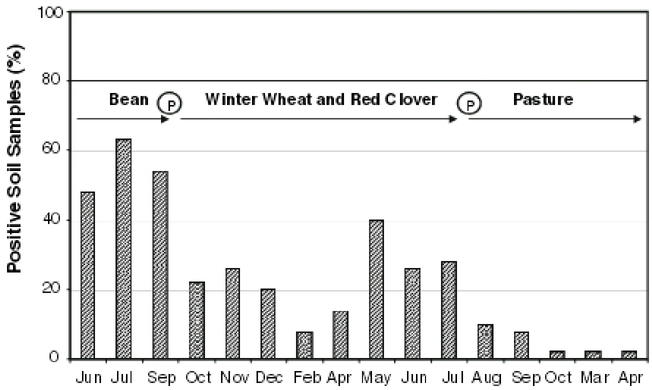

Figure 3. The percentage of soil samples from a field of beans followed by wheat and red clover and then a pasture containing *Heterorhabditis bacteriophora*. Samples were taken over a period of two years beginning in July 2002 and ending in August 2004. After April 2003 no nematodes were recovered. ‘‘P’’ indicates when ploughing occurred [Susurluk and Ehlers, 2008].

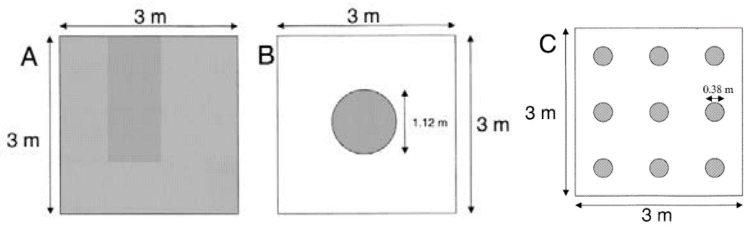

Figure 4. Application pattern of three nematode application strategies to 3 by 3 meter plots: (A) uniform application, (B) one patch application, (C) nine patch application [Wilson et al., 2003].

Effectiveness of *Heterorhabditis bacteriophora*

Entomopathogenic nematodes are cruisers which makes them more effective against insect hosts which are less mobile below the soil surface [Shapiro-Ilan et al., 2002]. Due to this, nematodes are more effective in fine textured soils which retain moisture in the upper layers of the soil where most insects reside [Shapiro-Ilan et al., 2002]. Soils with low moisture levels reduce nematodes mobility which greatly hinders their host-finding success [Ebssa et al., 2001]. EPNs can generally only survive in dry soils for a maximum of 2-3 weeks [Pilz et al., 2014] making it difficult to use them as biological control agents in arid regions. *Heterorhabditis bacteriophora* and other EPNs have been tested for suppression against the Black vine weevil, Japanese beetle, White grubs, and Chafers. The results are shown in Table 2. It was shown that *H. bacteriophora* is an excellent agent against the Japanese beetle. It works well against the Black vine weevil and White grubs, and a fair match against Chafers [Shapiro-Ilan et al., 2002]. Another study showed that White grubs could sustain a *Heterorhabditis bacteriophora* population for years after the initial application in undisturbed areas such as golf courses and turf [Pilz et al., 2014]. This shows that, in theory, the use of EPNs as biological control agents is highly effective. However, in reality, there are other forces acting against nematodes in the soil. Such forces include natural predators such as mites and other nematodes [Rosenhein et al., 1995].

Pest	Nematode Species	Host Suitability (% Suppression)	Number of References in Analysis
Black vine weevil (*Otiorhynchus sulcatus*)	*Heterorhabditis bacteriophora* *Steinernema feltiae* *S. carpocapsae*	Good (71) Good (75) Fair (58)	7 3 5
Colorado potato beetle (*Leptinotarsa decemlineata*)	*S. carpocapsae*	Fair (57)	3
Corn rootworms (*Diabrotica* spp.)	*S. carpocapsae*	Good (61)	7
Japanese beetle (*Popillia japonica*)	*H. bacteriophora* *S. carpocapsae* *S. glaseri*	Excellent (80) Fair (47) Good (63)	7 6 3
White grubs (*Phyllophaga* spp.)	*H. bacteriophora*	Good (72)	3
Chafers (*Cyclocephala* spp.)	*H. bacteriophora*	Fair (59)	3
Sciaridae (*Lycoriella* spp. and *Bradysia* spp.)	*S. feltiae*	Excellent (89)	5
Leafminer (*Liriomyza trifolii*)	*S. carpocapsae*	Good (66)	3
Black cutworm (*Agrotis ipsilon*)	*S. carpocapsae*	Excellent (86)	5
Diamondback moth (*Plutella xylostella*)	*S. carpocapsae*	Fair (56)	3
Corn earworm (*Helicoverpa zea*)	*S. riobrave*	Excellent (90)	4
Borers (*Synanthedon* spp.)	*S. feltiae*	Excellent (86)	4
Spodoptera spp.	*S. carpocapsae*	Poor (27)	3
Imported fire ant (*Solenopsis invicta*)	*S. carpocapsae*	Poor (25)	3

Table 2. An analysis of various insect hosts for suitability for EPNs [Shapiro-Ilan et al., 2002].

Heterorhabditis bacteriophora and Chemical Pesticides

There is concern about whether or not EPNs can safely be used with conventional chemical pesticides or best used on a rotational basis. Agricultural researchers have suggested that high exposure to inorganic fertilizers can inhibit the ability of nematodes to infect insect hosts. However, short exposures actually increased the nematodes ability to infect insect hosts [Stuart et al., 2006]. This suggests that chemical fertilizers may cause the nematode populations to die off, but the use of chemical fertilizers in rotation might also increases nematodes effectiveness [Denoth, 2002].

Conclusion

The notion of using the entomopathogenic nematode *Heterorhabditis bacteriophora* as a biological control agent against insect pests has been around for years but it is only recently starting to gain popularity with growers. As the movement for healthier foods and environmentally friendly pesticides are being called to replace chemical pesticides, the popularity of EPNs has grown. The benefits of using biological control agents are safety to humans, plants, wildlife, and the environment. Also better nematodes mass production technologies are being developed. These aspects will allow the use of *Heterorhabditis bacteriophora* to become a widely accepted, economical environmentally friendly alternative to conventional chemical pesticides currently being employed by growers.

Acknowledgments

Financial support was provided in-part, by: Farm Bureau of Robeson County, North Carolina; University of North Carolina at Pembroke (UNCP) Office of the Provost and Academic Affairs; UNCP Department of Chemistry and Physics and UNCP Thomas Family Center.

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