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Susceptibility of cockroaches (*Gromphadorhina portentosa*, *Nauphoeta cinerea* and *Blaptica dubia*) exposed to entomopathogenic nematodes

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Running head: Cockroach susceptibility to entomopathogenic nematodes

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Abstract

Cockroaches are major pests, vectors of pathogenic bacteria and induce allergies. Current control methods use chemical pesticides, but they can be ineffective and costly and there are reports of resistance in the field, hence new control methods are needed. There are conflicting reports about the susceptibility of cockroaches to entomopathogenic nematodes (EPNs) so we investigated if EPNs could kill several diverse cockroach species including the Madagascan hissing roach (*Gromphadorhina portentosa*), the Lobster roach (*Nauphoeta cinerea*) and *Blaptica dubia*. Female adult cockroaches were exposed to either commercial products containing *Steinernema kraussei* or a combination of *Heterorhabditis* spp. and *Steinernema* spp. at 50 and 150 nematodes per cm^2 for 21 days. We also monitored feeding and the numbers of infective juveniles that were produced from each cockroach corpse. We found that *S. kraussei* were harmless to all cockroach species (at both doses) but when exposed to a mixture of *Heterorhabditis* spp. and *Steinernema* spp. *B. dubia* died after 6 days and its feeding was strongly inhibited. We also found that the mixture of *Heterorhabditis* spp. and *Steinernema* spp. could proliferate in the cadavers of *B. dubia* whilst *S. kraussei* could only reproduce in *G. portentosa* and *B. dubia* but not *N. cinerea*. In conclusion, *S. kraussei* was harmless to all three cockroach species but *B. dubia* were killed when exposed to *Heterorhabditis* spp. and *Steinernema* spp., highlighting the differences in the host range of EPNs.

Keywords: Cockroaches, entomopathogenic nematodes, host range, parasites.

1. Introduction

There are more than 4000 cockroach species throughout the world, which live in forests, grasslands, sand dunes and caves (Bell, 1981). Some pestiferous species such as the American cockroach (*Periplaneta americana*), the German cockroach (*Blattella germanica*) and the Oriental cockroach (*Blatta orientalis*) live in urban environments and feed on decaying food and waste (Eggleston and Arruda, 2001). As well as being a significant pest species these cockroaches can produce asthma-triggering allergens and can vector pathogenic organisms (Rosenstrich et al., 1997; Ahmed et al., 2011). They are usually controlled by using bait formulations containing toxic ingredients such as sulfluramid, fipronil and imidacloprid (Rust et al., 1995). However, multi-chemical resistance to these insecticides has been reported (Ko et al., 2015) and there are health risks associated with using these chemicals, therefore new methods of control are needed.

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae, have been formulated into biological control agents and have been used successfully in targeting multiple agricultural and horticultural pest species (Campos-Herrera, 2015). When applied to soil EPNs seek out their insect hosts and enter through natural body openings such as the mouth, anus or spiracles (Grewal et al., 2001), where they release a lethal symbiotic bacterium: *Xenorhabdus* spp. for *Steinernema* spp. and *Photorhabdus* spp. for *Heterorhabditis* spp. (Forst et al., 1997). The bacteria multiply in the host and cause septicaemia and death within 48 hours (Smart, 1995). The nematodes reproduce within the host, releasing thousands of infective juveniles to find and parasitise new hosts. EPNs are able to kill many different insect species (Gaugler, 2002) however; there are conflicting reports about the host range of EPNs when exposed to cockroaches. For example, Zervos and Webster (1989) state that *Heterorhabditis heliothidis* can kill all instars of *P. americana* but Maketon et al. (2010) reported poor levels of control by *Heterorhabditis* species. Also

Koehler et al. (1992) showed that *Steinernema carpocapsae* could kill cockroaches but their efficacy is dependant on the gender of the cockroach.

Here we decided to investigate the host range of EPNs exposed to three species of cockroach including the Madagascan hissing roach (*Gromphadorhina portentosa*), the Lobster Roach (*Nauphoeta cinerea*) and *Blaptica dubia*. *B. dubia* is found in Central and South America, where it lives in areas of high temperatures and moisture; it is not a serious pest and it commonly used for pet food (Hussein and Hoffmann, 2013). *N. cinerea* has been used in many behavioural and sexual selection studies (Kou et al., 2009) but there is little know about its natural history (Kambhampati et al., 2013). The Madagascar hissing cockroach (*G. portentosa*) is a large, immobile species that lives in dry litter on the tropical rain forest floor in Madagascar and there is little known about its natural ecology and behaviour (Yoder and Grojean, 1997). As only several species of cockroach have been tested for their susceptibility to EPNs and there are conflicting reports of the efficacy of EPNs killing cockroaches (Koehler et al., 1992; Zervos and Webster., 1989; Maketon et al., 2010) we decided to investigate if these three species were susceptible to single species or mixtures of EPNs. Instead of isolating natural species of EPNs we used commercial products containing *S. kraussei* or a mixture of *Steinernema* spp. and *Heterorhabditis* spp. that can be purchased easily (Campos-Herrera, 2015). We used a mixture of EPN species to examine whether a combination of *Steinernema* spp. and *Heterorhabditis* spp. may be more effective than single species. Ultimately, this research could increase our understanding of the host range of commercially important EPNs and discover if these previously untested cockroach species are able to cope with EPN infection.

2. Materials and methods

2.1 Nematodes and insects

Nematodes ("Vine weevil killer" and "Grow your own") were purchased from BASF, U.K. Vine Weevil Killer (VW) Nemasys® contains *S. kraussei* and Grown Your Own (GYO) Nemasys® contains a mixture of *Steinernema* spp. and *Heterorhabditis* spp., the exact species of each was not disclosed by the manufacturer. Nematodes were stored at 15°C until use. Adult female cockroaches (*G. portentosa, N. cinerea* and *B. dubia*) (3-4 months old) were purchased from Zoocentre and stored in non-airtight plastic boxes with egg cartons and were fed lettuce *ad libitum*. They were kept for 14 days prior to experiments to make sure there was no natural infection or death by nematodes or other parasites.

2.2 Assessing the susceptibility of cockroaches to EPNs

Boxes (12.5 cm x 5.6 cm x 6 cm) were filled with 40 g of soil. Cockroaches were exposed to either 3,500 or 10,500 nematodes per box, which corresponds to the field application rates of 50 or 150 nematodes per cm^2, respectively (Campos-Herrera, 2015). It must be noted that we could not distinguish between the *Steinernema* spp. and *Heterorhabditis* spp. in Grown Your Own (GYO). There were also 3 plastic boxes, which received no nematodes and acted as the control. Five female adult cockroaches were added to each box and three discs of lettuce (3.5 cm diameter) were added as food. After every 3-4 days the food was removed and the amount that had been eaten was quantified and new food was added (also 3.5 diameter lettuce discs) for 18 days. The amount eaten was quantified by tracing around the lettuce discs on 1 x 1 mm graph paper and counting the total squares eaten. In each treatment the survival and feeding of 15 cockroaches was monitored and the whole experiment was repeated three times (45 cockroaches tested in total for each treatment). Any dead cockroaches were placed in a White trap (White, 1927) to assess if the nematodes could

reproduce in the cadaver. They were left for 28 days after which the numbers of infective juveniles present in the surrounding water were quantified.

2.3 Statistical analysis

Survival of cockroaches was assessed using a Two way ANOVA and Tukey's post hoc test. The numbers of nematodes produced in dead cockroaches were compared using a Student's t test. All statistics were performed using www.vassarstats.net.

3. Results

3.1 Assessment of *G. portentosa, N. cinerea* and *B. dubia* exposed to EPNs

S. kraussei was harmless to all three cockroach species (*G. portentosa*, *N. cinerea* and *B. dubia*). There were no significant differences between the survival of each cockroach species exposed to 50 and 150 nematodes per cm^2 and the controls ($P>0.05$) (Fig 1). Similarly, as well as having little effect on the survival of the three cockroach species *S. kraussei* did not affect the amount each cockroach had eaten when exposed to 50 and 150 nematodes per cm^2 after 18 days ($P>0.05$) (Fig 2).

To investigate the susceptibility of cockroaches further we concentrated on using *B. dubia* and exposed this species to a combination of *Steinernema* spp. and *Heterorhabditis* spp. at 50 and 150 nematodes per cm^2 for 21 days. In contrast to exposing the cockroaches to *S. kraussei*, survival of *B. dubia* was significantly reduced by both doses compared to the control after day 8 ($P<0.05$) (Fig 3A). There was no significant difference between the survival of *B. dubia* exposed to 50 and 150 nematodes per cm^2 ($P>0.05$). The amount that *B. dubia* ate was also significantly less that the control cockroaches after day 4 ($P<0.05$) (Fig 3B). Therefore, a combination of EPNs can kill *B. dubia* compared to only *S. kraussei*.

3.2 Assessment of *G. portentosa*, *N. cinerea* and *B. dubia* as suitable hosts for EPN reproduction

S. kraussei was able to reproduce in *G. portentosa* and *B. dubia* but not *N. cinerea* (Table 1). Seven *N. cinerea* died during infection by *S. kraussei* but after 28 days after being placed in a White trap there were no nematodes produced. In contrast, the combination of nematodes used (*Steinernema* spp. and *Heterorhabditis* spp.) killed cockroaches after 6 days infection and reduced cockroach feeding but also the nematodes were able to reproduce in the cadavers. Specifically, the combination of *Steinernema* spp. and *Heterorhabditis* spp. nematodes were found in great abundance (Table 1) in cockroaches exposed to 50 and 150 nematodes per cm^2, although there was no significant difference between the nematodes produced from cockroaches exposed to each dose ($P>0.05$).

4 Discussion

The host range of EPNs when exposed to cockroaches is poorly known and from 4000 cockroach species (Bell, 1981) only several have been tested for their susceptibility to EPNs including *P. brunnea*, *B. germanica*, *P. americana* and *B. orientalis* (Appel et al., 1993; Baker et al., 2012; Corpus and Sikorowski, 1992; Maketon et al., 2010; Morton and Garcia del Pino., 2013; Puza and Mracek, 2010). All of these cockroach species were shown to be susceptible to *S. carpocapsae*, which is different to our results which show that *S. kraussei* was unable to kill *G. portentosa*, *N. cinerea* and *B. dubia*. The reasons for resistance to *S. kraussei* could be due to gender of cockroaches, application techniques or differences in foraging behaviour of EPNs.

We used female *B. dubia* cockroaches in our experiments, which could have affected our results, as there are differences in the susceptibility of male and female cockroaches to

EPNs. For example, males of *P. americana*, are killed faster by *S. carpocapsae* than females (Morton and Garcia del Pino, 2013). The reason for this difference in gender susceptibility is unknown but it could be because the male genitalia is easier for EPNs to enter than females, which has been observed in *P. americana* exposed to *S. carpocapsae* (Morton and Garcia-del-Pino, 2013). It has also been suggested that the immune response of female cockroaches is much stronger than males (Sheridan et al., 2000).

As well as differences in gender susceptibility different application methods of EPNs can affect the survival of cockroaches. For example, *B. germanica* was found to be highly susceptible to *S. kraussei*, *S. carpocapsae* and *S. affine* when confined in a 1.5 ml Eppendorf tubes or applied directly onto the body of the cockroach (El-Kady et al., 2015; Puza and Mracek, 2010; Skierska et al, 1976) but when placed in Petri dishes the cockroaches were found to be highly resistant, as they were able to detect the nematodes and avoid areas where they were present (Puza and Mracek, 2010). Similarly, Ahmad et al. (2010) showed that when added to Petri dishes *P. americana* was resistant to *S. masoodi*. As well as avoidance behaviour cockroaches, such as *P. americana*, have the ability to groom their legs and displace EPNs (Koehler et al., 1992; Morton and Garcia-del-Pino, 2013). These studies demonstrate the importance of direct contact of nematodes to the cockroach body and that cockroaches have the ability to detect and avoid EPNs and to groom away any invading nematodes.

Foraging behaviour and the ability to detect and avoid host-associated cues may also play a role in the susceptibility of insects. For example, it has been shown that from several EPNs tested, *S. scapterisci* responded best to cricket and cricket-derived odorants and was the most pathogenic species to cricket hosts (Dillman et al., 2012). In terms of cockroach-associated cues used by EPNs to find hosts these will include: host volatiles, CO2 and soluble cues including faeces (Dillman et al., 2012). Cockroach faeces contains ammonia, which is

repellent to *S. glaseri*, *H. bacteriophora* and *S. carpocapsae* (Grewal et al, 1993a). *Heterorhabditis bacteriophora* and *H. zealandica* were found to be the most sensitive to ammonia from both *P. americana* and *B. germanica*, which hindered the infection of these cockroaches by the nematodes (Zervos and Webster, 1989; Grewal et al 1993a). Some EPN species, such as *S. scapterisci*, are able to successfully parasitise adult cockroaches (*B. germanica*) due to being less sensitive to the ammonia of cockroach faeces (Grewal et al 1993b). These differences in behaviour exposed to host cues would ultimately affect the infection of cockroaches by nematodes if they were innately averted from ammonia containing faeces. However, aversion behaviour could be reduced by adding a polymer to nematodes during application to increase nematode contact with cockroaches. For example, Schroer et al. (2005) showed that by adding a surfactant-polymer to *S. carpocapsae* it significantly increased their efficacy in controlling *Plutella xylostella*.

We found that a combination of *Steinernema* and *Heterorhabditis* spp. could kill *B. dubia* (although 35-40% of cockroaches were still alive after treatment). Other studies have successfully shown that a mixture of EPN species can be more efficient than application of single species. Neumann and Shields (2008) showed that a combination of *S. carpocapsae* and *H. bacteriophora* provided significant protection against the alfalfa snout beetle (*Otiorhynchus ligustici*) and reduced survival of the larvae more than *S. carpocapsae* alone. Presumably, the combination of the bacterial symbionts of each nematode (*Photorhabdus* spp. and *Xenorhabdus* spp.) (Forst et al., 1997) were too toxic for *B. dubia* compared to the introduction of *Xenorhabdus* species by *S. kraussei* alone.

We have shown that *G. portentosa*, *N. cinerea* and *B. dubia* are highly resistant to *S. kraussei*, which could be due to differences in gender, application techniques or nematode foraging behaviour. However, when *B. dubia* was exposed to a combination of *Steinernema* spp. and *Heterorhabditis* spp. they were killed and their feeding was strongly inhibited. These

results expand the host range of EPNs when exposed to several cockroach species and highlight the ability of these nematodes to cause mortality when used as a combination of genera.

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Figure legends

Fig 1: Survival of *G. portentosa* (A), *N. cinerea* (B) and *B. dubia* (C) exposed to 0 (black), 50 *S. kraussei* per cm^2 (grey) and 150 *S. kraussei* per cm^2 (black dashed) for 21 days. Bars represent ± one standard error.

Fig 2: Mean percentage feeding of *G. portentosa* (A), *N. cinerea* (B) and *B. dubia* (C) exposed to 0 (black), 50 *S. kraussei* per cm^2 (grey) and 150 *S. kraussei* per cm^2 (black dashed) for 18 days. Bars represent ± one standard error.

Fig 3A: Survival of *B. dubia* exposed to 0 (black), 50 *Steinernema* spp. and *Heterorhabditis* spp. per cm^2 (grey) and 150 *Steinernema* spp. and *Heterorhabditis* spp. per cm^2 (black dashed) for 21 days. Bars represent ± one standard error.

Fig 3B: Mean percentage feeding of *B. dubia* exposed to 0 (black), 50 *Steinernema* spp. and *Heterorhabditis* spp. per cm^2 (grey) and 150 *Steinernema* spp. and *Heterorhabditis* spp. per cm^2 (black dashed) for 18 days. Bars represent ± one standard error.

Table 1: Numbers of nematodes produced from dead *G. portentosa*, *N. cinerea* and *B. dubia* that were exposed to *S. kraussei* or *Steinernema* spp. and *Heterorhabditis* spp. for 21 days.