Entomopathogenic nematodes against foliage feeding crucifer pests in the tropics

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Abstract
Use of entomopathogenic nematodes against foliage pests is commonly perceived to be limited by their tolerance to temperature, desiccation and UV radiation. The effect of these abiotic factors on the infective juveniles (ijs) of two isolates of Steinernema spp. (SSL85), two isolates of Steinernema spp. (M87), Heterorhabditis n.sp. and H. indicus recovered from selected sites within Peninsular Malaysia was examined. Infectivity at different temperatures was found to differ both within and between species, with optimal infection generally at 25 °C. Desiccation studies revealed more marked differences between the isolates. For example, at 80% relative humidity, survival of approximately 51% of ijs of Steinernema spp. (SSL85/25) was observed, compared with 13% for Steinernema spp. (M87/45). Prior exposure of ijs suspended in water droplets to simulated solar radiation resulted in a general decline in % mortality of Plutella xylostella larvae in subsequent bioassays but no marked reduction in mean infection. These preliminary studies are encouraging as they suggest that entomopathogenic nematodes can tolerate, within defined limits, the major abiotic factors faced in the foliar environment. The results are discussed in terms of application under foliar conditions.

Key words: Entomopathogenic nematodes; temperature; desiccation; solar radiation; Plutella xylostella.

Introduction
With both the increase of pesticide resistance and greater awareness of the environmental impact of pesticides on the environment, alternative control agents for employment in the IPM of the major lepidopteran pests of crucifers are continually being sought. One such alternative is entomopathogenic nematodes.

Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) have been used with varying degrees of success in the biocontrol of a wide range of insect pests. Success has been greatest against soil-dwelling insects (e.g. Klein, 1990) and insects of cryptic habitats (e.g. Begley, 1990). Use of entomopathogenic nematodes against foliar insects has been limited, and has by and large been unsuccessful, with few exceptions (e.g. Begley, 1990). The reasons generally quoted for lack of success in this more hostile environment are desiccation, UV radiation and temperature stress caused to the nematodes.

In any biocontrol programme, one of the most important things is the selection of the most suitable isolate/species of nematode. This selection process must encompass the entire process ranging from ease of mass culture through to efficacy under field conditions. The entomopathogenic nematodes used in current study were isolated in Malaysia (Mason et al., 1996) and selected following mass screening of the infective juveniles (ijs) against third instar larvae of Plutella xylostella, one of the major lepidopteran pests of crucifers in Malaysia. The results of a series of preliminary experiments conducted to assess the effect of the major abiotic factors on the ijs of these isolates are presented below.

Materials and Methods
Nematode cultures
Six isolates were assessed: two of Steinernema spp. (SSL85: 25 & 43); two of Steinernema spp. (M87: 3 & 45); Heterorhabditis indicus and Heterorhabditis sp. All cultures were maintained at 20 °C on late instar larvae of Galleria mellonella L. (The Mealworm Co., Sheffield, U.K.), with ijs being used within six days of emergence.

Culturing of P. xylostella
A UK isolate of P. xylostella (Rothamstead insecticide susceptible strain) was obtained from Dr. M. Furlong (IACR-Rothamstead, Harpenden, Herts., UK). Cultures were maintained at 20 °C on 4–6 week-old Chinese cabbage, Brassica oleracea var. capitata (’Tip Top’).

Effect of temperature on infection
The sand tube assay of Fan & Hominick (1991) was used to assess infectivity of ijs at a range of temperatures: 10, 15, 20, 25, 30, 35 and 40 °C. To 25ml of moist sand in 30ml universal tubes, 200 ijs suspended in 1ml of tap water were added. One late instar G. mellonella larva was added per tube. A screw top lid was fitted to each tube and the tubes inverted to ensure contact between the larva and sand. For each
nematode isolate, there were 20 tubes per test temperature. Tubes were incubated at the test temperature for 72 hours. The larvae were then removed, washed in tap water and further incubated at 20 °C (as required) prior to dissection. Both prevalence (percent insect mortality) and intensity of infection were assessed.

**Desiccation tolerance**

Desiccation tolerance was examined at 25 °C at two relative humidities (r.h.): 40% and 80%. Infective juveniles were desiccated according to the method of Perry (1977). Approximately 50–100 ijs suspended in a small volume of distilled water were pipetted onto a glass microscope slide. Excess water was removed using filter paper. The slide was then immediately placed in the r.h. chamber. Relative humidities were maintained using glycerol/water solutions (Grover & Nicol, 1940). There were five replicates per treatment. After the required period of desiccation, slides were removed and flooded with distilled water. The re-suspended ijs were assessed after 24 hours and the number of living nematodes recorded. Those ijs not responding to mechanical stimulation were considered to be dead.

**Exposure to simulated tropical sunlight**

The effect of exposure to simulated tropical sunlight was assessed by irradiating the ijs using a 1000W solar simulator (Oriel Corp., Stratford, CT, USA). The test arena consisted of a 5cm diameter Petri dish in which a Chinese cabbage leaf disk (5cm diameter) was embedded on top of 1% agar (1–2mm depth). Infective juveniles were applied to the leaf disks in 1µl droplets. Thirty droplets were pipetted evenly onto each leaf disk, giving approximately 200 ijs per leaf disk. Exposure to simulated tropical sunlight was for 0, 5, 10, 15, 20 and 25 minutes with five replicates per exposure time. Following exposure, four, third instar *P*. *xylostella* larvae were added to each arena to assess infectivity of the irradiated ijs. The dishes were sealed and incubated at 25 °C for 48 hours. Insect mortality (prevalence) and intensity of infection were then assessed. Controls were as above with the exception that no nematodes were added to the droplets.

**Statistical analysis**

All analyses were performed using the statistical package GLIM (Royal Statistical Society, London, 1985), allowing generalised linear modelling to be conducted. Analyses of variance and analyses of covariance were conducted using the binomial error distribution with logit link function, allowing significance to be tested using $c^2$ values (Crawley, 1993). Overdispersion was corrected for as required by either adjusting the scale parameter (equal sample sizes) or using Williams’ procedure (unequal sample sizes), allowing F-tests to be used to test for significance (Crawley, 1993). Significance is reported at the 5% level.

**Results**

**Effect of temperature on infection**

Infection occurred within the temperature range of 15–35 °C, with no infection at either 10 °C or 40 °C. Within this range, percent mortality of *G*. *mellonella* larvae displayed little variation with temperature (results not shown). In contrast, the mean number of ijs infecting *G*. *mellonella* larvae varied significantly across the temperature range (Figure 1), with most isolates displaying optimal infection at 25 °C. These results are encouraging as they show that ijs of each of the isolates assessed can infect (and cause mortality) at temperatures likely to be experienced in both highland and lowland crucifer production areas in Malaysia.

**Desiccation tolerance**

Survival of ijs following desiccation at 40% r.h. was not significantly different between the nematode isolates (p>0.05) (Figure 2). All isolates could survive desiccation at 40% r.h. for at least 30 minutes, with the exception of *Steinernema* sp. (M87/45). Although survival was not significantly (p>0.05) different between the isolates, some interesting trends emerge. For example, both heterorhabditid species showed markedly high survival at 10 minutes (over 90%) followed by a massive decline to approximately 30% survival at 20 minutes, with survival then tailing off. In contrast, the steinernematids generally showed a large decline in survival after only five minutes, with further decline in survival being more gradual. *Steinernema* sp. (M87/3) displayed a gradual decline in survival: nearly 70% of the ijs surviving at 20 minutes, although by 30 minutes less than 3% were alive.

In marked contrast to the large drops in survival noted at 40% r.h., the decline in desiccation survival at 80% r.h. was more gradual (Figure 3). For example, survival after 30 minutes was still over 50% for each of the isolates. Desiccation survival was not significantly (p>0.05) different between the isolates.

**Exposure to simulated tropical sunlight**

Exposures of 15 minutes and longer resulted in the evaporation of all visible water from the droplets containing the ijs. The results for percent mortality and mean infection (Figures 4 and 5, respectively) show that the ijs were capable of infecting and causing appreciable mortality of third instar *P*. *xylostella* following exposure to simulated sunlight for at least 10 minutes after all visible water had evaporated. Even allowing for the variability displayed in the data for percent mortality, the overall trend with increasing time was for a reduction in percent mortality (Figure 4). Mean infection (Figure 5), in contrast to % mortality, remained relatively constant over time [except *Steinernema* sp. (M87/3)].

**Discussion**

The results from the present laboratory study suggest that ijs of selected isolates/species of entomo-
Figure 1. The effect of temperature on mean infection (%) of Galleria mellonella by Malaysian entomopathogenic nematodes (n=20)

Figure 2. Survival (%) of Malaysian entomopathogenic nematodes following desiccation at 40% RH on glass slides

Figure 3. Survival (%) of Malaysian entomopathogenic nematodes following desiccation at 80% RH on glass slides

Figure 4. Mortality (%) of third instar Plutella xylostella larvae by entomopathogenic nematodes (in 1 µl droplets) exposed to simulated tropical sunlight for different times (n=20)

Figure 5. Mean infection (%) of third instar Plutella xylostella by entomopathogenic nematodes (in 1 µl droplets) exposed to simulated tropical sunlight for different times (n=20)
Pathogenic nematodes have the potential for application in the foliar environment. For example, the temperature range for infection of *P. xylostella* larvae encompasses the range likely to be encountered in Malaysia – in either highland or lowland crucifer growing regions. Although surface temperatures on foliage, are likely to be higher (40 °C or more) due to exposure to direct sunlight, this, in terms of field efficacy, can be overcome by carefully timing the application to be early in the morning or in the late afternoon/early evening.

Similarly, although the method used to assess desiccation survival does not faithfully mirror field conditions, the results obtained, especially at 80% r.h., are very encouraging as they show that survival of the ijs is approximately 13–51% after 40 minutes. The glass slide method used induces a very rapid dehydration of the ijs and while rapid dehydration will be norm in the foliar environment it may not be as extreme due to the influence of the micro-climate surrounding the leaves. Indeed, both Glazer (1992) and Bauer et al. (1995) have observed elevated survival (in terms of infectivity) of entomopathogenic nematodes on leaf surfaces with dense pubescence. The present results, using this rapid dehydration and rehydration method, further suggest that, following evaporation of droplets under field conditions, it may be feasible to re-spray using water to rehydrate the desiccated ijs and perhaps increase the efficacy of the ijs. Such a strategy will only be feasible if low volume application methods are used (Lello et al., 1996). Further studies will be conducted into desiccation survival of the ijs using methods which reflect foliar conditions following field application.

The present results also suggest that ijs can survive, infect and cause lethal infections following exposure to simulated tropical sunlight, at least under defined conditions. These results differ from earlier publications (e.g. Gaugler et al., 1992) but this can be accounted for by the different methods used. The present study applied ijs in discrete water droplets, mirroring to a certain extent the situation following field application. It was encouraging that the ijs caused infection and mortality even after the water droplets had evaporated. This may be due to maintenance of a high r.h. following irradiation, so that although there was no visible water, the conditions at the leaf surface-air surface interface (i.e. ‘micro-climate’) remained favourable for infection.

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References