YERSINIA SP. (MH96) – A POTENTIAL BIOPESTICIDE OF MIGRATORY LOCUST *LOCUSTA MIGRATORIA* L.

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ABSTRACT

Locusta migratoria L. is a recognised global pest that feeds on a wide range of graminaceous crops and pasture. The activity of the novel entomopathogenic bacterium *Yersinia* sp. (MH96) against *L. migratoria* was investigated in the laboratory. *Locusta migratoria* were allowed to feed on ryegrass (*Lolium multiflorum*) carrying *Y. sp.* residues, and mortality was observed daily for 5 days. At constant temperatures of either 23.5 or 37°C, *Y. sp.* caused 100% mortality of 1st instar *L. migratoria* after 72 h. At constant temperatures of 23.5°C, mortality amongst 2nd-3rd instar *L. migratoria* after 48 h was ca 34%, rising to 100% mortality after 5 days. When 3rd instar *L. migratoria* were exposed to *Y. sp.* cultured at either 25°C or 37°C, mortality was higher in individuals maintained at 23.5°C compared to 37°C. This result was attributed to the phenology of the bacterium at elevated temperatures. These initial studies indicate that *Y. sp.* has promise as a biopesticide against *L. migratoria*, especially against early instars.

Keywords: entomopathogen, biopesticide, locusts, locust control, instar stage.

INTRODUCTION

Locusta migratoria L. (Orthoptera: Acrididae) (migratory locust) is a significant pest in Africa, the Middle East and Australia with hoppers or adults causing substantial and often devastating losses to a wide range of crops (Anon. 1982). The species has the widest distribution of any acridoid, comprising temperate and tropical parts of the eastern hemisphere (Anon. 1982). *Locusta migratoria* is found in New Zealand, but because of cooler temperatures, swarming behaviour is absent and the insect does not achieve pest status.

Overseas, various control techniques and management strategies are employed to control not only *L. migratoria*, but the many other plague locust species that form swarms and devastate large areas of vegetation (Lecoq 2001; Lomer et al. 2001). Control of locusts has traditionally relied on synthetic insecticides, and while this will remain the primary control for plagues, the detrimental impacts to the environment (Lecoq 2001) have driven the search for a biological control option as part of an integrated pest management approach to locust management (Lomer et al. 2001). An IPM approach can include development of alternative control agents, such as microsporidia, entomophthoralean fungi and parasitoids (Lomer et al. 2001, Anon. 2006).

Pathogens, unlike predators and parasitoids, can be relatively cheaply mass-produced and applied to provide direct control during a locust plague (Scanlan et al. 2001). Of the entomopathogens known to be active against locusts, the fungi *Metarhizium anisopliae* var. *acridum* Driver & Milner and *Beauveria bassiana* (Bals.-Criv.) Vuill. have been employed with some success against locusts (Lomer et al. 2001; Milner & Hunter 2001). Large scale field trials in Australia with *M. anisopliae* var. *acridum* formulated as Green Guard® (FI-985), gave significant control of migratory locust, *L. migratoria*, and Australian plague locust, *Chortoicetes terminifera* (Walker) (Hunter et al. 1999;

Long & Hunter 2005), but not against the spur-throated locust (*Austracris guttulosa*) (Walker) (Milner & Hunter 2001). In addition, there is often an 8-14 day lag between pathogen attachment, germination and penetration of the spore, and mortality (Hunter et al. 1999), allowing continued foliage consumption.

The entomopathogenic bacterium *Yersinia* sp. (MH96), isolated from a diseased grass grub larva (*Costelytra zealandica*) (White) from New Zealand soils, has shown high oral activity against members of Coleopteran and Lepidopteran species, as well as the Orthopteran *L. migratoria* (Hurst & Glare 2006). The bacterium is highly virulent causing death through secretion of toxin followed by rapid invasion of the host haemolymph overwhelming the insect within 72 h of infection (Hurst et al. 2007). Many members of *Yersinia* spp. behave differently at 37°C compared to lower temperatures of 25°C (Chester & Stotzky 1976; Logue et al. 2000), a scenario that could influence product efficacy during field application. The potential of *Y. sp.* (MH96) as a potential biopesticide for locust control was assessed using the migratory locust *L. migratoria* L. The ability of *L. migratoria* to tolerate elevated temperatures made it the ideal candidate to assess the relative efficacy of *Y. sp.* (MH96) at 37°C.

METHODS

Insects

Locusts (*Locusta migratoria*) were supplied from a laboratory colony (Biosuppliers Limited) that had been through ca 200 generations. Insects were held in 8 litre plastic vented containers with tissue on the floor, at 25°C for 1-2 days without food until required. The paper was moistened with distilled water twice per day until the experiment was set up.

Bacterium culture

Yersinia sp. (MH96) was grown in Luria-Bertani (LB) broth at either 25°C or 37°C where indicated. Cultures were incubated with shaking at 250 rpm in a Raytek orbital incubator, in 250 ml conical flasks containing 50 ml LB broth grown overnight resulting in $\sim 1.30 \times 10^{10}$ colony forming units/ml.

Operating parameters

The temperatures selected for the experiments were 24°C or 37°C, which is within the range of temperatures (14-43°C) experienced by early instar *L. migratoria* in Queensland, Australia. Of the five *L. migratoria* instars, 1st to 3rd instar stages were used for assessment of *Y. sp.* (MH96) efficacy (Table 1). The actual temperatures experienced by the insects during the experiment, both in the environment chamber and during bioassay/food replacement assessments, were measured using temperature loggers (TinyTag Ultra 2). The actual mean (min., max.) temperatures recorded in the environment chambers and during the bioassay/feed replacement assessments were 23.5°C (22.2-25.1°C) and 37.0°C (23.8-38.2°C). Typically 3-4 replicates were undertaken per experiment using 7-9 insects per treatment (Table 1). All experiments were carried out in the laboratory in environmental chambers at the indicated temperature with a light:dark of 14:10 h. Fresh untreated ryegrass was replaced as required in each treatment with each container misted with distilled water once the food was replaced. Each foliage replacement took between 15-20 min.

TABLE 1: Bioassay parameters of *Yersinia* sp. (MH96) against *L. migratoria* used in the three experiments.

Expt	No. replicates for each treatment	Insects/container (Instar)	Bacteria growth temperature	Locust exposure temperature(s)
1	Control (4); <i>Yersinia</i> (4)	8-9 (1 st)	25°C	23.5 or 37°C
2	Control (3); <i>Yersinia</i> (4)	8 (2 nd -3 rd)	25°C	23.5°C
3	Control (4); <i>Yersinia</i> (4)	7 (3 rd)	25 or 37°C	23.5 or 37°C

Insect bioassay

Yersinia sp. (MH96), grown overnight at 25 or 37°C, was mixed with distilled water containing 0.01% Duwett (siloxane polyalkyleneoxide copolymer) in a 1:1 ratio. LB broth mixed with an equal amount of distilled water containing 0.01% Duwett served as the control. The solutions were applied to the point of run-off to the foliage of glasshouse-grown ryegrass *Lolium multiflorum* (cv. Tama) using a Badger Air-Brush (Model 350 TM). The plant roots had been sealed in plastic bags (100 mm x 80 mm) using a nylon zip tie. Once the foliage had been treated, it was placed in the laboratory at 25°C and the residue allowed to dry for 1-1.5 h. A single bouquet was then placed horizontally in a 1.75 litre vented polycarbonate plastic container. *Locusta migratoria* were anaesthetised with CO₂ and then 7, 8 or 9 individuals were placed in each 1.75 litre container. Once they had recovered from exposure to CO₂ (approximately 3-6 min) the containers were placed in the environment chambers at 23.5 or 37°C. Mortality was observed every 1-2 days over a 5 day period.

Data analysis

Mean mortality on days 2 and 5 was analysed using the Generalised Linear Model (GLM) procedure for modelling binomial proportions employing a logit link function (McCullagh & Nelder 1983). Chi-square analysis was performed to test for significant differences in mortality with analyses carried out using GenStat (Version 10, VSN International Ltd, Hemel Hempstead, UK).

RESULTS

Toxicity of *Y. sp.* (MH96) towards 1st instar *L. migratoria* at 23.5 or 37°C

At both 23.5 and 37.0°C there was rapid mortality after 48 h with 94.0±2.41% (±SEM) and 97.0±3.77% of 1st instar *L. migratoria* dead, respectively, and all dead after 72 h. None of the control *L. migratoria* died over the 5-day experimental period (Fig. 1). Although not quantified, visual observations showed that at 23.5°C, little bacteria-treated ryegrass was consumed compared with the control, yet despite reduced consumption, mortality occurred within 24 h of exposure to the bacteria.

FIGURE 1: Cumulative mortality of 1st instar *L. migratoria* maintained at 23.5°C or 37°C when consuming ryegrass treated with *Yersinia* sp. (MH96) that was cultured at 25°C.

Toxicity of *Y. sp.* (MH96) to 2nd – 3rd instar *L. migratoria* at 23.5°C

Mortality of the 2nd – 3rd instar stage was less than that observed for 1st instar *L. migratoria* with only 34±12.87% dead after 48 h. However, this was significantly higher than the mortality observed in the controls ($\chi^2 = 7.30$, $P=0.035$) (Fig. 2). By day 5, all *L. migratoria* exposed to *Y. sp.* had died compared to ca 10±5.0% of the controls ($\chi^2 = 75.56$, $P<0.001$). Similar to observations in experiment 1, consumption of bacteria-treated foliage was less than that observed in the controls.

FIGURE 2: Cumulative mortality of 2nd–3rd instar *L. migratoria* maintained at 23.5°C when consuming ryegrass treated with *Yersinia sp.* (MH96) that was cultured at 25°C

Toxicity of *Y. sp.* (MH96) grown at 25 or 37°C towards 3rd instar *L. migratoria* held at 23.5 or 37°C

The temperature at which the bacteria had been cultured had a significant effect on subsequent *L. migratoria* mortality. For 3rd instar *L. migratoria* exposed to bacteria grown at 25°C and then maintained at 23.5°C, mortality after 48 h was 33±9.9%. This compared to mortality of 18±8.4% for *L. migratoria* exposed to bacteria that was grown at 37°C and then maintained at 23.5°C ($\chi^2 = 5.61$, $P=0.026$). By day 5, 100% of the *L. migratoria* exposed to bacteria grown at 25°C and then maintained at 23.5°C had died compared to 71±3.4% of *L. migratoria* exposed to bacteria that was grown at 37°C and then maintained at 23.5°C ($\chi^2 = 259.05$, $P<0.001$) (Fig. 3a).

The temperature at which the locusts were held had a more marked effect on mortality than the temperature at which the bacteria was cultured. Mortality of 3rd instar locusts held at 37°C and exposed to bacteria cultured at 25 or 37°C was only 14±6.6% and 7±4.8% after 5 days, respectively (Fig. 3b). There were no significant differences in mortality between bacteria culture treatments at either the 2 or 5 day assessment periods. Irrespective of the temperature at which the bacteria was cultured, the overall mortality of *L. migratoria* maintained at 23.5°C (58±11.1%) and 37°C (8±6.3%) at the end of the bioassay was significantly different ($\chi^2 = 11.9$, $P= 0.002$).

Locusta migratoria exposed to 37°C rapidly consumed the ryegrass irrespective of the temperature at which the bacteria was grown. It was necessary to replace the ryegrass in the controls in the first 24 h and every 24 h thereafter. For *L. migratoria* exposed to bacteria-treated ryegrass, the rate of consumption was less but ryegrass still had to be replaced with untreated foliage in the first 24 h for half the replicates and every 24–48 h thereafter. At 23.5°C, consumption of bacteria-treated foliage was less than that observed in the controls, as in experiments 1 and 2.

FIGURE 3: Cumulative mortality of 3rd instar *L. migratoria* maintained at (a) 23.5°C or (b) 37°C when consuming ryegrass treated with *Yersinia* sp. (MH96) that was cultured at 25 or 37°C

DISCUSSION

This work has shown that *Yersinia* sp. is highly pathogenic to 1st-3rd instar *L. migratoria*, with highest activity towards the 1st instar stage. Optimal lethality occurred when the bacteria was cultured at 25°C and applied at 23.5°C. The decreased bacterial entomopathogen efficacy for later instars has been reported elsewhere (Bowling et al. 2007; Tounou 2007). Tounou (2007) found that mortality of *Oedaleus senegalensis* (Krauss) and *Schistocerca gregaria* (Forskål) to the microsporidian *Paranosema locustae* Canning was concentration dependent, with younger instars being more susceptible than older instars and longer median survival time recorded in later instars compared to the younger instars. For *L. migratoria* this was most pronounced when *Yersinia* sp. was cultured at 37°C and fed to 3rd instar locusts that were maintained at 37°C (Fig. 3b) compared to similarly cultured bacteria where the locusts were maintained at 23.5°C (Fig. 3a). This could indicate a lag in the establishment of *Y. sp.* (MH96) where the rapid rate of food consumption at elevated temperatures results in a heightened throughput of the inoculum resulting in a reduction in efficacy. Alternately many *Yersinia* spp. exhibit altered growth dynamics at 37°C, producing cells with changes in cell wall components (Chester & Stotzky 1976; Logue et al. 2000) that may in turn alter the survival dynamics of *Y. sp.* (MH96) in the insect system. Both of these scenarios need to be tested.

Studies have shown that locusts have an active behavioural thermoregulation seeking a preferred body temperature of 38–41°C for significant periods of the day (Blanford & Thomas 2000). In addition many locust species are able to thermoregulate to overcome fungal infections (Gardner & Thomas 2002), and for this reason field applications of entomopathogenic fungi have lead to variable results (Blanford & Thomas 1999, 2000; Ouedraogo et al. 2004). Studies on isolates of *M. anisopliae* (MaPL40, MaPL32) against wingless grasshopper (*Phaulacridium vittatum*) (Sjöstedt) found that at temperatures over 30°C there was a significant reduction in sporulation and a decline in locust mortality (Barrientos-Lozano et al. 2002). This study has showed significant efficacy of *Y. sp.* towards 1st instar *L. migratoria* at 37°C but efficacy was low for 3rd instars. Behavioural thermoregulation has been observed in *L. migratoria* following infection with *Metarhizium anisopliae* var. *acridum* (Ouedraogo et al. 2004), and future research will need to investigate any behavioural response by *L. migratoria* to infection by *Y. sp.* (MH96) in the temperature range of 38–41°C. However, Figure 1 suggests that if *Y. sp.* (MH96) is cultured and potentially formulated at a lower temperature (e.g. 25°C), efficacy at elevated temperatures at the time of application could be maximised. Concurrent observations identified a significant reduction in food consumed in treated versus the control *L. migratoria* indicating that foliage sprayed with *Y. sp.* (MH96) will not be quickly consumed. However, laboratory trials have indicated that very little foliage needs to be consumed to cause a lethal dose (M.R. McNeill, unpubl. data). An indirect result of this is that inoculated but uneaten vegetation would be available to infect the next locust, a situation that could be ideal for the control of moving bands of locust hoppers where extensive patches of vegetation are often consumed (Steadman 1990). *Yersinia sp.* shows a high level of pathogenicity against *L. migratoria*, and holds promise as an entomopathogen providing rapid kill of early instars. The potential against other key locust pests, such as Australian plague locust, *Chortoicetes terminifera* (Walker), spur-throated locust (*Austracris guttulosa*) (Walker) and desert locust *Schistocerca gregaria* (Forskål), still needs investigation. Not all Orthoptera will necessarily be susceptible to *Y. sp.*, as laboratory experiments against black field cricket (*Teleogryllus commodus*) (Walker) found little toxicity against either neonate or adult stages (M.R. McNeill, unpubl. data). The effect of residue age and temperature on efficacy and the formulation of *Y. sp.* as a biocontrol agent are areas for further investigation if this entomopathogen is to be viable control agent under field conditions. However, the results reported here demonstrate *Y. sp.* (MH96) has promise as a future entomopathogen, at least against *L. migratoria*.

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