RECRUITMENT OF ENTOMOPATHOGENIC NEMATODES TOWARD *PANICUM BISULCATUM* ROOTS DAMAGED BY SCARAB LARVAE

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Introduction

Belowground, plants are facing various threats such as pathogens and insect herbivores (Johnson et al., 2016). In grasslands, the collective biomass of scarab root pests can reach or even exceed that of sheep grazing above ground in certain Australian pastures (Britton, 1978). Grassland plants are yet well armed to defend their roots (Moore and Johnson, 2016). As one strategy among their arsenal, insect damaged roots emit volatile organic compounds in the ground (Rasmann et al., 2005). These volatiles diffuse in the soil matrix (Hiltpold and Turlings, 2008) and attract entomopathogenic nematodes (EPNs) (Rasmann et al., 2005) toward the injured tissues (Hiltpold et al., 2011). EPNs are obligatory parasites (Dillman et al., 2012a). After infection, the insect host dies of septicaemia caused by a symbiotic bacterium (Burnell and Stock, 2000). The EPNs reproduce inside the host cadaver and, once space is lacking, break through and forage for other sustainable hosts (Gaugler et al., 1997). Such indirect induced defence has been described in various plant species (Boff et al., 2001;Van Tol et al., 2001;Rasmann et al., 2005;Ali et al., 2010;Hiltpold et al., 2010;Rasmann et al., 2011;Turlings et al., 2012;Santos et al., 2014) as well as in grass relatives (Rasmann et al., 2005;Köllner et al., 2008).

We hypothesised that, when damaged by root insect herbivores, wild grasses would emit volatile cues in the soil matrix and consequently attract EPNs.

Material and Methods

Plants

Panicum bisulcatum Thunb. (Poales: Poaceae) were grown from seed in potting mix (Osmocote®, Scotts LLC, USA) in a glasshouse (30°C, 80% RH, natural light regime) over one month. They were watered as needed to ensure an optimal growth.

Insects

African Black Beetle (ABB, *Heteronychus arator* Fabricius (Coleoptera: Scarabaeidae)) larvae were collected in a pasture on the Hawkesbury Campus (Western Sydney University, NSW, Australia) in January 2015 two to three weeks before the experiments and maintained in potting mix (Osmocote®, Scotts LLC, USA) at 25°C.

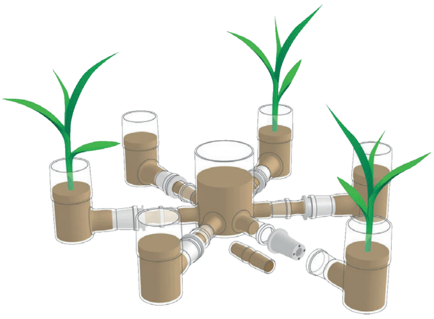
Entomopathogenic nematodes

The EPN species *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) was obtained from Ecogrow Environment Pty Ltd (NSW, Australia) and stored at 6°C. One day prior the behavioural experiments, EPNs were extracted from the storage material in a Baermann funnel (Baermann, 1917) and transferred in water in a cell culture flask (Sigma Aldrich).

EPN behavioural assays with insects

So-called 6-arm belowground olfactometers (Figure 1) were used to assess the behaviour of the EPNs in response to insect root damage cues. On day 1, plant roots were washed from all potting mix particles and plants were individually transplanted in sand (10% moisture w:w) in glass pots (Figure 1). One ABB larva was added to half of the pots with plants and one ABB larva was added to one pot without a plant. The remaining three glass pots were filled with moist sand only and all pots covered with aluminium foil to prevent evaporation. To allow the establishment of the volatile gradients, on day 2, all pots were connected to the central arena, which was then filled with 10% moist sand and covered with aluminium foil. On day 3, ca. 2,000 EPN viable individuals were injected in 1 ml of water in the centre of the central arena. On day 4, 24 h later, the olfactometer was disassembled and EPNs were extracted from each arm using Baermann traps (Baermann, 1917). Finally, the number of EPNs in each arm was recorded. This set-up was replicated five times with new plants in the same glasshouse where plants were grown (30 °C, 80% RH, natural light regime)..

Figure 1.
Schematic representation of the 6-arm belowground olfactometer. (See Rasmann et al. (2005) for a detailed description.)



EPN behavioural assays with methyl jasmonate induction of the plants

To assess if EPNs would be attracted toward plants induced with methyl jasmonate (MeJA, Sigma Aldrich, a plant hormone released to trigger plant defence pathways in response to insect feeding), we followed the same procedure as above, but instead of insect larvae, plants were induced with 150 µg of MeJA (Röder et al., 2011) and MeJA only was applied to one third of the pots. This set-up was replicated five times.

Statistical analysis

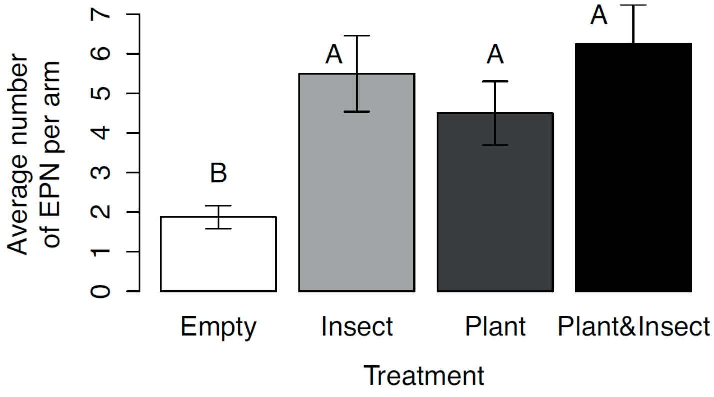
Quasi-likelihood functions were used to compensate for the over-dispersion of nematodes within the olfactometer (Turlings et al., 2004). The response of the EPN to the different arm treatments was fitted by maximum quasi-likelihood estimation in R version 3.0.2, and its adequacy was assessed through likelihood ratio statistics and examination of residuals (Turlings et al., 2004).

Results and Discussion

Despite the severe damage to the roots, EPNs were not attracted toward pots containing both plants and insects compared with those containing plants and grubs alone, yet empty pots attracted significantly less

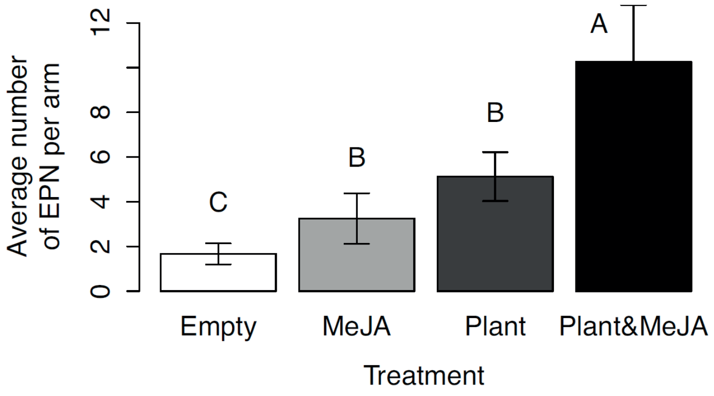
EPNs (Figure 2, $F_{3,44} = 13.002$, $P < 0.001$). This might be due to the fact that ABB larvae were clipping portions of the roots away from the main root system before feeding on the detached roots (I. Hiltpold, personal observation). This could be a behavioural strategy by the insect to avoid plant inducible defences and only have to cope with constitutive defences. Yet insignificant, EPNs tended to be more numerous in the arms with grubs only than with plants only (Figure 2). This is in accordance with the fact that several insects species have been shown to emit volatiles triggering EPN foraging behaviour (e.g. Dillman et al., 2012b).

Figure 2.
Attraction of entomopathogenic nematodes (EPN) to the different treatments applied to the six arms of the belowground olfactometer when the plants were induced with insect larvae. Bars indicate SEM and letters statistical differences.



The distribution between the different arms of the 6-arm belowground olfactometers was significantly different from random when MeJA was applied (Figure 3, $F_{3,44} = 10.69$, $P < 0.001$). Significantly more EPNs were recorded in the arm where plants were induced with MeJA than in any other arm (Figure 3). This demonstrates that *Panicum bisulcatum* has the potential to emit volatile blends attracting EPNs to protect its roots from insect herbivory after induction of its defences. However, as roots fed on were detached from the plant, such inducible defences could not have been triggered, resulting in the lower EPN attraction observed in Figure 2.

Figure 3.
Attraction of entomopathogenic nematodes (EPN) to the different treatments applied to the six arms of the belowground olfactometer when the plants were induced with methyl jasmonate (MeJA). Bars indicate SEM and letters statistical differences.



The volatile profile of the induced or non-induced roots were not analysed because the quantity of sampled root material was too small. Current data, however, shows that *P. bisulcatum* has the potential to produce volatiles attracting insect natural enemies, although it appears that scarab larvae can evolve behavioural adaptions to avoid this defence mechanism.

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