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Host plant colonisation by arbuscular mycorrhizal fungi stimulates immune function whereas high root silicon concentrations diminish growth in a soil-dwelling herbivore

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ABSTRACT

Plant nutritional quality is dependent on soil nutrients and co-evolved soil microbial symbionts. Most plants associate with arbuscular mycorrhizal (AM) fungi, which alter their nutritional quality and silicon (Si) uptake from the soil. High Si concentrations reduce plant nutritional quality and can act as an effective defence both aboveground and belowground. The growth and immune function of insect herbivores is dependent on the quality of their host plants, hence the AM symbiosis and Si concentrations can impact insect growth and immunity via changes in host plant quality. The effects of AM fungi or Si on root herbivores are poorly quantified, while impacts on insect immunity are unknown. We investigated the effects of host plant colonisation by AM fungi and high root Si concentrations on plant quality alongside the growth of a root feeding insect and the immune response to entomopathogenic nematode infection.

Two sugarcane varieties (*Saccharum* species hybrids L.) were grown under fully factorial treatment combinations of ± Si and AM/non-AM. Root feeding insects (*Dermolepida albohirtum* Waterhouse) fed on the plants and their immune function was assessed in a bioassay, while insect growth and root consumption were assessed in a feeding trial. We found high Si concentrations decreased insect growth and root consumption, the latter by 71%. Insect growth was reduced on plants associated with AM fungi, which was dependent on Si treatment and plant variety. Insect immunity increased by 62% on AM colonised plants, which negatively correlated with insect growth. These results demonstrate that the impacts of the AM symbiosis on root feeding insects can depend on Si availability and plant variety. Our study suggests that AM fungi can prime insect immunity, independent of host plant quality or Si concentrations, and the negative effects of AM fungi on soil dwelling insects involves immune function stimulation which, due to a growth-immunity trade-off, results in growth reduction.

1. Introduction

The nutritional quality of plants is dependent on soil fertility but also interactions with co-evolved microbial symbioses. For example, arbuscular mycorrhizal (AM) fungi associate with the majority of land plants, a symbiosis that is based on the transfer of soil nutrients such as phosphorus (P) and nitrogen (N) in exchange for plant photoassimilates in the form of hexose sugars (Garcia et al., 2016; Smith and Read, 2008). AM fungi colonise plant roots and can benefit the host plant by improving water and nutrient uptake which then improves plant growth, vigour and nutrient concentrations (Treseder, 2013), which are important indicators of plant quality (Mattson, 1980). The growth and performance of insects which feed on living plant material is largely determined by the quality of their host plants. As such, it is not surprising that the plant AM status can impact the performance of herbivorous insects, as plant concentrations of N as well as P are known to affect

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herbivore performance (Elser et al., 2000). Surprisingly, only a handful of studies have investigated the impacts of the AM symbiosis on soil dwelling insects (Johnson and Rasmann, 2015), despite the fact that these organisms share the soil environment and interact directly with the same organ of the host plant, the roots. Of these studies, all, except one (Currie et al., 2011), have found that AM colonisation of the host plant negatively impacts root feeding insects, yet the mechanisms remain unclear. Indeed, there is arguably a large evolutionary selection pressure for AM fungi to negatively impact root feeding insects (Johnson et al., 2016) as root herbivory can decrease photosynthesis, thereby reducing the photoassimilates available for the fungus, while also reducing root mass available for colonisation (Zvereva and Kozlov, 2011).

Interestingly, in addition to increasing P and N uptake, AM fungi can increase plant silicon (Si) uptake from the soil (Clark and Zeto, 1996; Garg and Bhandari, 2016; Kothari et al., 1990) and have recently been observed to increase plant Si in soils with low Si concentrations, with negative impacts on root herbivore performance (A. Frew, Unpublished results). The positive effects of Si application on plant resistance to both abiotic and biotic stresses are well known (Ma, 2004), and has been shown to significantly increase crop growth and resistance to pests and pathogens in the field (Guntzer et al., 2012). As such, the positive effects of Si application on plant growth and resistance is a promising avenue of research for improving crop production and sustainable pest management (Frew et al., 2016a). Indeed the efficacy of high plant Si concentrations in reducing the performance of herbivorous insects is well documented (Massey and Hartley, 2009; Reynolds et al., 2009) and has very recently been shown to be effective against root feeding insects (Frew et al., 2016a, 2016b). Si is taken up by plants from the soil as monosilicic acid where, it accumulates and naturally polymerises to form solid silica (SiO_2) phytoliths (Ma and Yamaji, 2015). The deposition of these silica phytoliths increases plant toughness, while reducing palatability and digestibility of the plant tissue (Massey and Hartley, 2009). Therefore, high Si concentrations reduce plant quality for insect herbivores.

The nutritional quality of plants not only impacts the growth of herbivorous insects, but also their immune defences against natural enemies in the soil environment (Lee et al., 2008; Schmid-Hempel, 2005; Triggs and Knell, 2012), where reductions in plant quality can reduce insect immune function (Gherlenda et al., 2016; Lee et al., 2008). In the soil environment, insects come into contact with various natural enemies including entomopathogenic nematodes, fungi, viruses, bacteria and predators. Several of these have been used as bio-control agents, for example, the entomopathogenic fungi *Beauvaria bassiana* and *Metarhizium anisopliae* (Meyling and Eilenberg, 2007), as well as several species of entomopathogenic nemadotes (Georgis et al., 2006). An understanding of how plant Si concentrations and the AM symbiosis can impact insect immunity, growth and plant consumption, could better inform pest management for increased efficacy of biocontrol strategies in the field. Yet no studies, to our knowledge, have investigated the effects of high Si concentrations or host plant colonisation by AM fungi on the immune function of an insect herbivore, aboveground or belowground. The primary defence mechanisms of the insect immune system occur through encapsulation and melanisation (Smilanich et al., 2009). Phenoloxidase (PO) is a key enzyme to the production of melanin (Cerenius and Söderhäll, 2004), which is used in the melanisation and encapsulation processes to eliminate an invading body. As such, PO activity can indicate the immune function of an insect (Cotter et al., 2004; Cotter and Wilson, 2002; Triggs and Knell, 2012).

The effects AM fungi and Si, as soil factors, on plant quality, highlight their potential to impact insect growth and immune function via changes in host plant quality. Therefore, we investigated the impacts of plant AM colonisation and high root Si concentrations on plant root quality using sugarcane (*Saccharum* species hybrids L.), a known Si accumulator that forms associations with AM fungi. We also examined the related impact of these factors on the growth and immunity of a root feeding insect using the larvae of the greyback cane beetle (*Dermolepida albohirtum* Waterhouse), colloquially known as the canegrubs, significant pests to the Australian sugar industry. We assessed insect immune function in a bioassay using natural enemies of soil dwelling insects, entomopathogenic nematodes. Silicon uptake efficiency varies substantially between plant varieties (Soininen et al., 2013), as well as their responsiveness to AM colonisation (Sawers et al., 2008). Therefore, we examined the effects of high root Si concentrations and the AM symbiosis on two varieties of sugarcane with distinct breeding lineages, one perceived to be resilient to biotic stresses (Q240) including pathogens (e.g. brown rust (*Puccinia melanocephala*)) and canegrub herbivory, and one more susceptible to pathogens and insect herbivory (Q200) (Sugar Research Australia Ltd, 2016).

Insect immune function, and overall performance, is largely determined by the nutritional quality of their host plants, and host plant quality is affected by the plant AM status and by Si concentrations. Therefore, we investigated the impacts of high root Si concentrations and plant colonisation by AM fungi on the performance and immunity of the canegrub. We assessed plant responses by measuring plant total biomass and root mass alongside root C:N ratio and P concentrations to examine plant growth and nutritional quality, as well as root Si concentrations to assess Si based root defences. We evaluated the impact on the canegrubs by allowing them to feed on intact plants for three weeks and measuring growth and also immune function (through assessment of phenoloxidase (PO) activity). We also carried out a feeding trial to measure the impacts on canegrub relative growth rates and their relative root consumption.

2. Materials and methods

2.1. Experimental set-up

A factorial experiment with three factors including 'AM fungi', 'Si' and 'root herbivore' in a fully crossed design was carried out using 80 sugarcane plants of variety Q200 and 80 of variety Q240 grown from single-eye cuttings. Plants were germinated in trays of gamma irradiated potting mix (Richgro© All Purpose Potting Mix), receiving tap water *ad libitum* for two weeks in a shade house. All plants were then transferred to 3.7 L pots with gamma irradiated soil sourced from a sugarcane field in Queensland, Australia, fully described in Frew and Johnson (2016) as 'soil A' and exhibiting low Si concentrations of 1392 mg/kg (plant available Si: 23 mg/kg) (see Supplementary data Appendix A, Table A1 for soil nutrient breakdown).

To apply the AM treatments, half of the plants were inoculated with approx. 400 AM fungal spores from a commercial inoculum, Start-up Super© (Microbe Smart Pty. Ltd. Melrose Park DC, South Australia) comprising spores from four species identified as *Glomus etunicatum*, *G. coronatum*, *G. intraradices* and *G. mosseae*. These are referred to as AM plants, while the uninoculated plants are referred to as non-AM plants. Spores were extracted from the inoculum using wet sieving and a sucrose centrifugation extraction method (Daniels and Skipper, 1982). All pots also received microbial filtrate (300 mL) to standardise the microbial community within each pot at the initiation of the treatment. This filtrate was created by using the extraneous extraction solution (without spores) from the AM fungal inoculant.

To apply the Si treatments, half of the plants received 200 mL of