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Naturally occurring phytopathogens enhance biological control of water hyacinth (*Eichhornia crassipes*) by *Megamelus scutellaris* (Hemiptera: Delphacidae), even in eutrophic water

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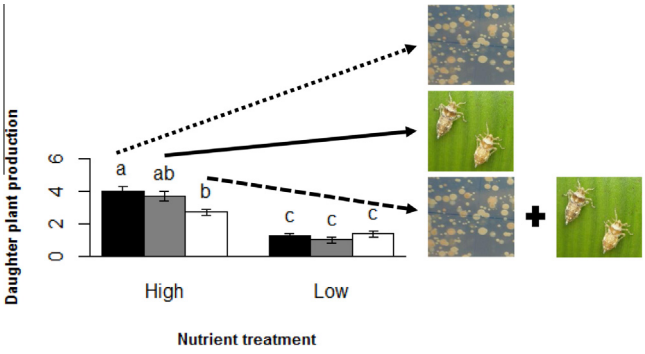
HIGHLIGHTS

- *Megamelus scutellaris* facilitated infection of water hyacinth by fungal pathogens.
- Synergy between phytopathogens and *M. scutellaris* reduced water hyacinth vigour.
- Synergy was observed in eutrophic waters, where the weed is most problematic.
- *Megamelus scutellaris* may complement mycoherbicides for improved weed management.

GRAPHICAL ABSTRACT



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ABSTRACT

Insect biological control agents directly damage target weeds by removal of plant biomass, but herbivorous insects have both direct and indirect impacts on their host plants and can also facilitate pathogen infection. *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) was recently released into South Africa to help control invasive water hyacinth (*Eichhornia crassipes,* Pontederiaceae). We compared the impact of fungicide surface-sterilised and unsterilised *M. scutellaris* individuals and water hyacinth leaves on growth of the weed at two nutrient levels. The survival and reproduction of adult *M. scutellaris* was not reduced by sterilisation. Under high nutrient conditions, unsterilised *M. scutellaris* with unsterilised leaves reduced water hyacinth daughter plant production by 32%, length of the second petiole by 15%, chlorophyll content by 27% and wet weight biomass by 48%, while also increasing leaf chlorosis 17-fold, in relation to control plants under the same nutrient regime. Surface sterilisation of the insect and/or plant surfaces led to a general reduction in these impacts on water hyacinth growth and health. This contrast was less evident under low nutrient conditions. *Megamelus scutellaris* facilitated infection by fungal and other pathogens, thus its biology is compatible with pathogens that could be developed into mycoherbicides. This integrated approach may be ideal for management of infestations of water hyacinth in eutrophic water systems where control has been problematic, both in South Africa and elsewhere.

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1. Introduction

Fungal pathogens are almost ubiquitous in both natural and agricultural environments (Peay et al., 2008). They can have devastating impacts on plant health (Dean et al., 2012), but more often have less obvious sub-lethal effects (Krokene et al., 2010). Some fungal infections are facilitated by insect feeding and the behaviour of phloem-feeding insects in particular aids transmission of plant diseases in general. Planthoppers (Auchenorrhyncha), such as the Delphacidae, are a prominent group of plant-feeders that are known to transmit a wide range of pathogens (viruses, mycoplasma-like organisms (MLOs), bacteria) as well as fungi (Harris and Maramorosch, 1980; Denno and Roderick, 1990). Not all plant-pathogen-vector relationships are economically harmful and the relationship between plant pathogens and their vectors can potentially be used to help control invasive plant species (Conway, 1976; Charudattan et al., 1978; Lambers et al., 2008).

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laub.) (Pontederiaceae) is a free-floating aquatic macrophyte originating from the Amazon basin in South America (Bechara, 1996). It has colonised natural water courses worldwide (Gopal, 1987) and was introduced into South Africa in the early 20th century as an ornamental plant (Cilliers, 1991). Water hyacinth quickly gained the status of the country's most problematic aquatic weed (Hill and Olckers, 2001) with well documented negative socioeconomic impacts, health-related consequences and reductions in native biodiversity (Mailu, 2001; Midgley et al., 2006; Malik, 2007; Coetzee et al., 2014).

Until recently, six arthropods and one pathogen had been released as biological control agents against water hyacinth in South Africa (Coetzee et al., 2011), with notable successes attributed to two weevils, *Neochetina eichhorniae* Warner and *N. bruchi* Hustache (Coleoptera: Curculionidae) (Hill and Olckers, 2001). However, biological control programmes in South Africa and elsewhere have not achieved complete control, especially where the plant is growing in eutrophic, pollution-enriched water (Holm et al., 1977; Coetzee and Hill, 2012). Additional biological control agents have therefore been sought in an effort to achieve more widespread control over water hyacinth (Cordo, 1996; Hill and Olckers, 2001), one of which is *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) (Sosa et al., 2004). This phloem-feeding bug is native to those parts of South America where water hyacinth is present, including Argentina, Brazil, Peru and Uruguay (Sosa et al., 2007). It can reduce water hyacinth growth rates, induce significant tissue damage, and increase plant mortality rates (Tipping et al., 2011). *Megamelus scutellaris* was released first in the USA, in 2010 (Tipping and Center, 2010), and subsequently in South Africa in 2013 (J.A. Coetzee, pers. obs.).

The success of biological control agents against water hyacinth can largely be attributed to the reductions in vigour that are effected by tissue loss (Wilson et al., 2007). Herbivory by the control agents *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae) (Coetzee et al., 2005), *N. eichhorniae* and *N. bruchi* (Center et al., 2005) and *Cornops aquaticum* Bruner (Orthoptera: Acrididae) (Bownes et al., 2010) has been shown to reduce the competitive ability of water hyacinth plants. However, the effects of insect feeding cannot be attributed to herbivory alone (Ripley et al., 2008; Marlin et al., 2013). Venter et al. (2013) demonstrated that pathogens associated with *N. eichhorniae* contributed more than herbivory to a reduction of photosynthesis in water hyacinth. Pathogens are able to significantly decrease productivity and plant growth parameters, including overall fresh weight, photosynthetic rates and daughter progeny numbers (Conway, 1976; Lambers et al., 2008), and can lead to a gradual decline in water hyacinth populations (Charudattan, 1984).

The use of pathogens to control water hyacinth has received relatively little attention, both in South Africa and elsewhere (Charudattan, 2001; Ray and Hill, 2012a, 2012b), although the efficacy of fungal pathogens in controlling water hyacinth has been shown under both laboratory and field conditions (Shabana et al., 1995; Martínez Jiménez and Charudattan, 1998; Ray et al., 2008). Exposure to isolates of two species (*Alternaria eichhorniae* Nagraj and Ponappa and *Fusarium oxysporum* Schltdl) resulted in disease indices (pathogenicity) of 65% and 47% respectively, when applied as mycoherbicidal applications on water hyacinth under laboratory conditions (Ray and Hill, 2012a). Furthermore, the disease indices of these isolates were significantly increased when augmented with feeding by the weevil *N. eichhorniae*, whereby pathogenicity increased by 21.8% for *A. eichhorniae* and 45.2% for *F. oxysporum* treatments. Feeding by *Neochetina* weevils also achieves a significantly greater level of control over water hyacinth when augmented with *Cercospora piaropi* Tharp (Moran, 2005), as does the mite *Orthogalumna terebrantis* Wallwork (Acarina: Galumnidae) when present in combination with *Acremonium zonatum* (Sawada) Gams. (Sanders et al., 1982). These examples support the hypothesis that combined herbivore and fungal pathogen applications can provide greater control of water hyacinth than agents acting alone (Moran, 2005; Martínez Jiménez and Gomez Balandra, 2007).

The phloem-feeding behaviour of *M. scutellaris* suggests it may facilitate fungal disease initiation on water hyacinth (Harris and Maramorosch, 1980). The aims of this study were to determine whether *M. scutellaris* facilitates infection of water hyacinth by fungal pathogens, what the consequences of infection are for water hyacinth vigour, and whether the effects vary according to the water nutrient regime in which water hyacinth is growing.

2. Methods and materials

Cultures of water hyacinth and *M. scutellaris* were maintained at Rhodes University, Grahamstown, South Africa. A consignment of *E. crassipes* plants was collected from the Kubusi River (32.5926° S; 27.4218° E) near Sutterheim, South Africa and used to cultivate additional plants in 3000 L plastic pools housed in greenhouse tunnels made of clear plastic sheeting. Pools were supplied with a constant release nutrient supply (see Section 2.2) from two perforated plastic bottles suspended in the water column, which are replenished approximately every six months. *Megamelus scutellaris* (ex. Argentina via USDA, Fort Lauderdale) was obtained from a colony which was initiated in 2008 and maintained on *E. crassipes* plants.

2.1. Sterilisation of insects and plants

Eichhornia crassipes plants and *M. scutellaris* adults were surfaced sterilised to remove spores of fungal pathogens. Sterilisation of *M. scutellaris* adults was performed by applying a brief spray application of 1.5% Sporekill© (Hygrotech (Pty) Ltd, Pretoria, South Africa), a commercially available anti-fungal solution, to a 10 cm × 15 cm nylon mesh pouch containing 10 insects. *Eichhornia crassipes* leaves and stems were initially treated by rinsing the leaves and stems in tap water and then with sterile distilled water to remove unwanted particulate matter. They were then sequentially immersed for 30 s each in 70% ethyl alcohol (to improve the penetration of sodium hypochlorite), sodium hypochlorite (3.5% w/v), and finally three times in distilled water (Ray and Hill, 2012b). Control (unsterilised) plants and insects were obtained directly from the cultures.

To test the effectiveness of the sterilisation procedures, single *M. scutellaris* adults were vortexed for one minute in 1 ml of deionised water and single leaves of *E. crassipes* were vortexed in 2.5 ml of deionised water. 100 µl aliquots of each solution were then pla-