
Article:
Sutton, GF, Compton, SGA orcid.org/0000-0002-1247-8058 and Coetzee, JA (2016) Naturally occurring phytopathogens enhance biological control of water hyacinth (Eichhornia crassipes) by Megamelus scutellaris (Hemiptera: Delphacidae), even in eutrophic water. Biological Control, 103. pp. 261-268. ISSN 1049-9644

https://doi.org/10.1016/j.biocontrol.2016.10.003

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

Authors G.F. Sutton [a]†, S.G. Compton [ab] & J.A. Coetzee [c]

Affiliations [a] Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa; [b] School of Biology, University of Leeds, LS2 9JT, United Kingdom; [c] Department of Botany, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa.

Corresponding author † Guy F. Sutton; email: guysutton41@gmail.com

Highlights

- The herbivorous biological control agent Megamelus scutellaris facilitated fungal pathogen infection on Eichhornia crassipes (water hyacinth) by creating feeding scars, which act as fungal entry sites.
- Phytopathogens in conjunction with M. scutellaris herbivory reduced water hyacinth growth more than either factor alone.
- Synergy between phytopathogens and M. scutellaris reduced water hyacinth vigour under eutrophic water conditions, where the weed is most problematic in South Africa.
- Megamelus scutellaris may complement mycoherbicides for management of water hyacinth in eutrophic water systems.

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 fungus-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%, lengths of the second petiole by 15%, chlorophyll content by 27% and wet weight biomass by 48%, while also increasing leaf chlorosis 17-fold. Surface sterilisation of the insect and/or plant surfaces led to a significant reduction in these impacts on water hyacinth growth and health. This contrast was much less evident under low nutrient conditions. Megamelus scutellaris facilitated infection by fungal and other pathogens by creation of pathogen entry sites during feeding. Its biology is therefore compatible with fungal pathogens that could be developed into mycoherbicides, and such an 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.

Key words Biological control; Plant-fungi interaction; Eutrophication; Phytopathogens; Herbivore-fungi interaction; Invasive aquatic macrophyte

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 utilised 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

74 Warner and N. bruchi Hustache (Coleoptera: Curculionidae) (Hill and Olckers, 2001).
75 However, biological control programmes in South Africa and elsewhere have not
76 achieved complete control, especially where the plant is growing in eutrophic,
77 pollution-enriched water (Holm et al., 1977; Coetzee and Hill, 2012). Additional
78 biological control agents have therefore been sought in an effort to achieve more
79 widespread control over water hyacinth (Cordo, 1996; Hill and Olckers, 2001), one of
80 which is Megamelus scutellaris Berg (Hemiptera: Delphacidae) (Sosa et al., 2004).
81 This phloem-feeding bug is native to those parts of South America where water
82 hyacinth is present, including Argentina, Brazil, Peru and Uruguay (Sosa et al.,
83 2007). It can reduce water hyacinth growth rates, induce significant tissue damage,
84 and increase plant mortality rates (Tipping et al., 2011). Megamelus scutellaris was
85 released first in the USA, in 2010 (Tipping and Center, 2010), and subsequently in
86 South Africa in 2013, by the Biological Control Research Group at Rhodes
87 University.
88 The success of biological control agents against water hyacinth can largely be
89 attributed to the reductions in vigour that are effected by tissue loss (Wilson et al.,
90 2007). Herbivory by the control agents Eccritotarsus catarinensis (Carvalho)
91 (Hemiptera: Miridae) (Coetzee et al., 2005), N. eichhorniae and N. bruchi (Center et
92 al., 2005) and Cornops aquaticum Bruner (Orthoptera: Acrididae) (Bownes et al.,
93 2010) has been shown to reduce the competitive ability of water hyacinth plants.
94 However, the effects of insect feeding cannot be attributed to herbivory alone (Ripley
95 et al., 2008; Marlin et al., 2013), and Venter et al. (2013) demonstrated that weevil-
96 borne pathogens contributed more than herbivory to a reduction of photosynthesis in
97 water hyacinth. Pathogens are able to significantly decrease productivity and plant
98 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 vector fungal pathogens or facilitate fungal disease initiation on water hyacinth (Harris & Maramorosch 1980). The aims of this study were to determine whether M. scutellaris vectors fungal pathogens and/or facilitates infection on water hyacinth, 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.

2.2. Methods and Materials

Cultures of water hyacinth and M. scutellaris were maintained at Rhodes University, Grahamstown, South Africa. Water hyacinth was obtained from stock cultures, originally sourced from the Kubusi River, Sutterheim, South Africa (32.5926° S; 27.4218° E), and cultivated in 3000 L plastic pools erected in enclosed tunnels. Pools were supplied with a constant release nutrient supply (see section 2.2) from two perforated plastic bottles that were replenished approximately every six months. Megamelus scutellaris (ex. Argentina via USDA, Fort Lauderdale) have been maintained on plants obtained from the stock culture since 2008. Under our rearing conditions the insect is dimorphic for wing length, although most individuals are brachypterous (possess short or rudimentary wings).

2.1. Sterilisation of insects and plants

Surface sterilisation of E. crassipes and M. scutellaris adults was performed to remove any fungal pathogens present. 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 x 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 seconds each in 70% ethyl alcohol (to improve the

148 penetration of sodium hypochlorite), sodium hypochlorite (3.5% w/v), and finally
149 three times in distilled water (Ray and Hill, 2012b). Control (unsterilised) plants and
150 insects were obtained directly from the cultures.
151 To test the effectiveness of the sterilisation procedures, single M. scutellaris
152 adults were vortexed for one minute in 1 ml of deionised water and single leaves of
153 E. crassipes were vortexed in 2.5 ml of deionised water. 100 µl aliquots of each
154 solution were then plated onto both Potato Dextrose Agar (PDA) and Rose Bengal
155 Chloramphenicol Agar (RBCA) (Biolab©, Merck, Modderfontein, South Africa). The
156 media were then incubated for 72 hours at both 27 ℃ and 32 ℃, and the colony
157 forming units per ml (CFU/ml) counted. Each test of sterilisation effectiveness was
158 replicated five times with each growth medium at each temperature, providing a total
159 of 20 M. scutellaris and 20 E. crassipes leaf replicates. Negative controls were
160 employed by plating an aliquot of 100 µl of deionised water.
161 Effectiveness of sterilisation was determined by performing a two-way
162 analysis of variance (ANOVA) on the CFU/ml between sterilised and control insects
163 and plants, with culture medium and incubation temperature as factors. Statistical
164 analyses and graphing were performed in R Studio© ver. 2.15.3 (The R Foundation
165 for Statistical Computing, 2013).
166
167 2.2. Herbivory and insect/plant sterilisation experiments
168 Herbivory and pathogen infection experiments were performed using M.
169 scutellaris to determine the control agent's ability to facilitate fungal pathogen
170 infection while feeding on water hyacinth. Additionally, bottom-up mediation was
171 investigated by monitoring the effect of both the biological control agent and the
172 presence/impact of any fungal pathogens on supressing growth parameters of water

hyacinth plants maintained at two contrasting nutrient regimes. Healthy water hyacinth plants were obtained from the stock cultures and groups of five plants were placed into 18 100 L plastic tubs (40 cm x 40 cm x 60 cm) filled with 50 L of tap water. The tubs were divided into two nutrient treatments, eutrophic (high nutrient) and oligotrophic (low nutrient). Nutrient regimes were applied in accordance with Reddy et al. (1989), which were deemed suitable for growth of water hyacinth, and within the range of nutrients of South African waterbodies (Coetzee and Hill, 2012). The commercial controlled-release fertilizer Multicote^{TM} 8 (15 N: 3 P: 12 K) (Haifa Chemicals Ltd., Cape Town, South Africa) was applied at 8.0 mg N L^{-1} (high nutrient treatment) and 0.5 mg N L^{-1} (low nutrient treatment). An initial treatment of KNO_3 was added to the high nutrient tubs at 40 mg N L^{-1} (Saarchem, uniLAB©, Gauteng, South Africa) along with KH_2PO_4 at 1.55 mg P L^{-1}. Commercial iron chelate (13 % Fe) was added to both nutrient regimes at 1.69 mg Fe L^{-1} water to reduce chlorosis of the plants. The nutrient medium was replaced weekly. After three weeks any daughter plants, dead leaves and stems were removed. Wet weight biomass was measured using a digital bench-top kitchen scale (Clicks©, South Africa) and chlorophyll content was measured using an Apogee CCM-200 plus chlorophyll meter (ADC BioScientific Ltd., Hoddeson, United Kingdom).

The impact of M. scutellaris herbivory on water hyacinth and its ability to facilitate fungal pathogen infection was examined by placing groups of 10 brachypterous adults onto single expanded leaves with approximately 5 cm of petiole inside a fine mesh bag (mesh size: 0.5 mm x 1 mm). Leaf age was standardised by selecting leaf two (the second youngest leaf) (Center & Spencer 1981). The combinations of sterile and unsterile treatments of both E. crassipes and M. scutellaris were employed to highlight the role of each organism's pathogen load in

supressing E. crassipes. The herbivory and leaf sterilisation treatments applied were: (i) sterile insect/sterile plant (IS x PS); (ii) sterile insect/unsterile plant (IS x PU), (iii) unsterile insect/sterile plant (IU x PS) and (iv) unsterile insect/unsterile plant (IU x PU). Control leaves were enclosed in mesh bags which did not receive any M. scutellaris adults or sterilisation. Each plant in every tub received a single treatment, equating to nine replicates for the five treatments at both nutrient regimes.

The experiment ran for five weeks, with leaf production, daughter plant production, maximum petiole length and the length of petiole two recorded at weekly intervals. Leaf production by the plant meant that the longest and leaf two petioles measured each week were not necessarily the same as before. The chlorophyll content index was recorded at end of the experiment, rather than at weekly intervals, to minimise disruption and contamination of the leaf surfaces. At the end of the five weeks, wet weight biomass was measured as before, and the percentage of each abaxial and adaxial leaf surface displaying chlorosis was estimated through visual inspection (Coetzee et al., 2007). Insect performance indicators were recorded upon completion of the experiment by recording M. scutellaris adult abundance (survival) and presence of eggs and nymphs (reproductive capacity).

Two-way ANOVA followed by Tukey's HSD post hoc analysis examined differences in plant growth parameters across nutrient and sterility treatments at the start and end of the experiment, together with nutrient x treatment interactions.

2.3. Isolation and identification of pathogens

Upon completion of the experiment, water hyacinth leaves inoculated with M. scutellaris or control leaves displaying symptoms of fungal infection, such as necrotic flecks, necrotic lesions, leaf spots, zonate lesions and leaf blight, were removed and

223 wrapped in paper towelling to absorb excess moisture (to reduce unwanted
224 secondary microbial growth). Diseased leaf material (4 mm^2) was then excised from
225 sites of fungal infection, rinsed first with tap water to remove unwanted particulate
226 matter, and then with sterile distilled water before being immersed sequentially for 30
227 seconds in each of 70% ethyl alcohol, sodium hypochlorite (3.5% w/v) and three
228 times in distilled, sterile water (Ray and Hill, 2012b). The sterilised leaf pieces were
229 individually transferred onto PDA, RBCA and Malt Extract Agar (MEA) and cultured
230 under sterile conditions at 27 ± 2 ˚C (mean ± S.D.).
231 The fungal samples isolated from diseased water hyacinth were aseptically
232 purified by streak plating and sub-culturing protocols as outlined in Agrawal and
233 Hasija (1986). The margins of growing colonies were isolated and serially transferred
234 onto fresh growth media (PDA, MEA and RBCA) until a pure culture was obtained.
235 Preparation of fungal specimens for identification was performed using a modified
236 tape mount technique (Harris, 2000). A piece of transparent tape (4 cm x 1.5 cm)
237 was pressed against the fungal isolate, radiating from the centre to the edge of the
238 culture. A drop of lactophenol blue was placed onto the tape, and mounted onto a
239 microscope slide with a coverslip. The slide preparation was then examined under
240 high power (400 X magnification). A preliminary identification of fungal isolates was
241 obtained using morphological, structural and growth characteristics and the ability of
242 the fungi to produce pigmentation on the culture media (Gilman, 1959; Barnett, 1960;
243 Mpofu, 1995; Shabana et al., 1995; Domsch et al., 2007).

244

245 **3. Results**

246 3.1. Sterilisation of insects and plants

The number of CFU/ml obtained from sterilised M. scutellaris was not significantly different when media were incubated at 27 ℃ and 32 ℃ (PDA medium: 216 ± 37.78 vs 256 ± 30.25; RBCA medium: 80 ± 9.42 vs 170 ± 40.73), on both PDA ($F_{1,28}$ = 1.18, P = 0.188) and RBCA media ($F_{1,28}$ = 1.46, P = 0.238). Similarly, the number of CFU/ml from sterilised E. crassipes leaves did not differ when media were incubated at 27℃ and 32 ℃ (PDA: 352 ± 28.51 vs 616 ± 36.49; RBCA: 152 ± 25.55 vs 308 ± 53.34), on both PDA ($F_{1,28}$ = 2.57, P = 0.120) and RBCA media ($F_{1,28}$ = 2.05, P = 0.162). Temperature treatments were therefore pooled in subsequent analyses.

Sterilisation of M. scutellaris adults was partially effective, resulting in a significant reduction in the number of CFU/ml cultured on both PDA (sterile: 236 ± 16.27 vs unsterile: 616 ± 30.47) ($F_{2,27}$ = 23.36, P < 0.001) and RBCA media (sterile: 116 ± 12.29 vs unsterile: 224 ± 16.67) ($F_{2,27}$ = 7.73, P = 0.002). Sterilisation of E. crassipes leaves was also partially effective, with a significant reduction in the number of CFU/ml on both PDA (sterile: 484 ± 20.78 vs unsterile: 2212 ± 89.68) ($F_{2,27}$ = 47.16, P < 0.001) and RBCA media (sterile: 230 ± 31.26 vs unsterile: 1312 ± 65.21) ($F_{2,27}$ = 39.92, P < 0.001).

3.2. Nutrient regime effects on plant growth

Water hyacinth growth responded more to water nutrient regime than to herbivory and leaf sterilisation treatments (Table 1) with consistently more growth under high nutrient conditions. Leaf production (Fig. 1a), increased by 45%, daughter plant production by 69% (Fig. 1b), longest petiole length by 18% (Fig. 1c), second petiole length by 24% (Fig. 1d), chlorophyll content index by 23% (Fig. 1e); and wet weight biomass by 57%).

3.3. Herbivory and fungal pathogen effects on plant growth

Herbivore and leaf sterilisation treatments had no appreciable impact on mean leaf production (Fig. 1a) and longest petiole length (Fig. 1c) after five weeks. However, these treatments resulted in significant differences in daughter plant production (Fig. 1b), second petiole lengths (Fig. 1d), relative chlorophyll content (Fig. 1e) and wet weight biomass (Fig. 1f). In combination with M. scutellaris herbivory, the unsterilised leaf treatment resulted in a greater reduction in water hyacinth vigour than the sterilised leaf treatments. Significant interactions between nutrient regime, and herbivore and leaf sterilisation treatments were observed for mean daughter plant production and wet weight biomass (Table 1). These interactions indicate greater absolute reductions of both plant parameters when they were cultivated in eutrophic, rather than oligotrophic water nutrient conditions, although the proportional reduction of plant parameters were comparable between nutrient regimes.

3.4. Herbivore and pathogen performance

The number of M. scutellaris adults recovered at the end of the experiment was greater in the high nutrient treatment (93%) than the low nutrient treatment (83%) ($F_{1,52}$ = 9.86, P = 0.003) (Fig. 2a), but there were no herbivore and leaf sterilisation treatment effects on adult insect survival across treatments ($F_{3,52}$ = 0.41, P = 0.744) (Fig. 2a). Megamelus scutellaris reproductive output was also greater on plants growing under high nutrient conditions, with 6 out of the 7 tubs (86%) containing nymphs, whereas no nymphs were present on any of the plants grown under low nutrient conditions. These findings suggest that the presence of fungal

296 pathogens did not impact M. scutellaris reproduction and survival. The highest extent
297 of leaf chlorosis was observed under high nutrient treatments (15%, compared with
298 8%), when both plants and insects were unsterilised (Fig. 2b), with a significant
299 interaction between nutrient regime and treatment observed ($F_{4,65}$ = 6.50, P = 0.013).
300

301 3.5. Fungal pathogens isolated from water hyacinth

302 Eichhornia crassipes leaves displayed various disease symptoms at the end
303 of the five week experiment. These were cultured to obtain a baseline estimate of
304 fungal pathogen community structure in the presence of M. scutellaris. A total of 35
305 isolates were cultured from the leaves, of which 17 could not be identified further
306 because of contamination, failure to grow, sterility or a lack of useful morphological
307 characteristics (Table 2).
308 The most frequently isolated genus was Alternaria Nees, with three species
309 obtained from eight isolates. Alternaria eichhorniae Nag Raj & Ponappa was the
310 most abundant species within this genus, with five isolates, followed by A.
311 tenuissima (Nees ex Fr.) Wiltshire with two isolates and lastly A. alternata with a
312 single isolate (Fr.) Keissler. The remaining isolates comprised Fusarium moniliforme
313 Sheldon with three isolates, Cladosporium sp. with two isolates and single isolates
314 from the genera Acremonium (Link ex. Fr) and Ulocladium Preuss.
315

316 **4. Discussion**

317 One reason that water hyacinth is so invasive is that it directs the majority of
318 its resources into growth and maintenance of photosynthetic tissues rather than
319 sexual reproduction (Coetzee and Hill, 2012), which allows the plant to respond

320 rapidly to changes in nutrient regimes (Coetzee et al., 2007). Our study
321 demonstrated that water hyacinth growth was significantly impacted by water nutrient
322 status, which is in accordance with a large body of literature (Gossett and Norris
323 1971, Reddy et al., 1989, Coetzee et al., 2007, Marlin et al., 2013). Under low
324 nutrient conditions, our experimental plants were less healthy and productive than
325 those grown under high nutrient conditions, which corroborates the findings of
326 Coetzee et al. (2007), who showed that plants cultivated under low nutrient
327 conditions displayed a short-petioled, bulbous growth form.
328 Herbivory by M. scutellaris did not have as appreciable an effect on water
329 hyacinth growth as water nutrient status. Our results indicate that leaf chlorosis was
330 the sole parameter that was significantly influenced by M. scutellaris herbivory alone
331 (treatment: IS x PS), although reductions in several plant growth parameters were
332 observed across the remaining treatments. This implies that although herbivory
333 alone can impact water hyacinth productivity parameters, our findings highlight the
334 role of other less conspicuous factors required for a deleterious impact on water
335 hyacinth vigour.
336 Fungal pathogens have been implicated as a factor that can contribute to a
337 reduction in water hyacinth growth and proliferation (Charudattan et al., 1978;
338 Moran, 2005). The fungal pathogens harboured on M. scutellaris adults in
339 combination with herbivory (treatment: IU x PS) resulted in a significant reduction in
340 relative chlorophyll content and leaf chlorosis. Unsterilised leaf treatments,
341 regardless of whether insects were sterilised (treatment: IS x PU) or unsterilised
342 (treatment: IU x PU), resulted in reductions in the length of the longest petiole and
343 wet weight biomass. Further, the cumulative effect of herbivory, M. scutellaris-borne
344 fungal pathogens and fungal pathogens harboured on water hyacinth (treatment: IU

x PU), was required to reduce daughter plant production and length of the second petiole. This highlights the deleterious impact of fungal pathogens associated with water hyacinth leaves, and to a lesser extent pathogens harboured on the herbivore. Our results are in accordance with Venter et al. (2013) who demonstrated that pathogens associated with the weevil N. eichhorniae effected a significant reduction in water hyacinth leaf photosynthetic rate, and Ray and Hill (2015) who showed that the mirid E. catarinensis facilitated disease initiation of A. zonatum on water hyacinth, but we explicitly highlight the deleterious impact of water hyacinth-borne fungal pathogens when in the presence of the herbivore M. scutellaris.

Avocanh et al. (2003) examined the efficacy of applications of the fungal pathogen A. eichhorniae on water hyacinth, and found that disease incidence and severity were significantly lower on plants growing under high nutrient conditions. This led Muniappan et al. (2009) to argue that mycoherbicidal applications are likely to be more effective against water hyacinth in low nutrient systems. Our results suggest that this is not necessarily the case when fungal pathogens are present in combination with insects that are feeding on the plants. When M. scutellaris was inoculated onto unsterilised water hyacinth leaf material there was a greater absolute reduction in mean daughter plant production and wet weight biomass at high nutrient conditions, although the size of the effect was similar between nutrient levels. Phloem-feeding insects such as M. scutellaris are likely to be particularly effective at facilitating fungal phytopathogen infection because their stylets pierce the epidermis, creating feeding scars that can act as entry sites for opportunistic pathogens (Galbraith, 1987). Pathogens associated with chewing insects such as the weevil N. eichhorniae can nonetheless significantly reduce rates of photosynthesis in water hyacinth leaves (Venter et al., 2013). Moran (2005) similarly showed that

370 augmentation of the weevils N. eichhorniae and N. bruchi with the fungus
371 Cercospora piaropi resulted in greater reductions in water hyacinth leaf production
372 and plant densities in relation to control plots. Mode of feeding therefore does not
373 appear to limit the insects that can facilitate the spread of pathogens.
374 A multi-faceted, integrated approach has been proposed as the most effective
375 management strategy for controlling aquatic weeds (Pieterse, 1977; Charudattan,
376 2001) and the synergy between insect herbivores and plant pathogens has been
377 highlighted as a potential management tool (Charudattan et al., 1978). Our results
378 suggest that fungal pathogens may indeed contribute to reductions in water hyacinth
379 growth and proliferation (Charudattan et al., 1978; Moran, 2005; Venter et al., 2013;
380 Ray and Hill 2015). Surface sterilisation of M. scutellaris and the leaves of water
381 hyacinth neither increased nor decreased the insect's growth, survival and
382 reproduction. This suggests that M. scutellaris has a casual, rather than a
383 mutualistic, relationship with the fungi that it was transmitting, and that plant nutrient
384 status, rather than plant disease is the major determinant of host plant suitability.
385 It can be concluded that M. scutellaris herbivory facilitates fungal pathogen
386 infection. Unlike either herbivory or fungal phytopathogens alone, this has a
387 deleterious impact on water hyacinth fitness. Mycoherbicidal applications on water
388 hyacinth appear unlikely to impact negatively on M. scutellaris, although this needs
389 to be tested explicitly, preferably with whole-plant and field trials. Mycoherbicide
390 effects on other water hyacinth-feeding insects also need to be considered, but our
391 results suggest that an integrated approach utilising M. scutellaris and
392 mycoherbicide formulations may represent an effective control strategy against water
393 hyacinth when it is growing in eutrophic waters, where this weed is currently most
394 problematic (Coetzee & Hill 2012).

Acknowledgements

Philip Weyl and Martin Hill are thanked for providing valuable comments on an earlier manuscript, as are two anonymous reviewers whose suggestions significantly improved this paper. The authors acknowledge the funding provided by the South African Research Chairs Initiative of the Department of Science and Technology, The Department of Environmental Affairs of South Africa, The Natural Resources Management Programme, Working for Water Programme and the National Research Foundation of South Africa. Any opinion, finding, conclusion or recommendation expressed in this material is that of the authors and the NRF does not accept any liability in this regard.

References

Agrawal, G.P., Hasija, S.K., 1986. Microorganisms in the laboratory: a laboratory guide for mycology, microbiology and plant pathology. Print House Lukhnow, India.

Avocanh, A., Senouwa, V., Diogo, R., Beed, F.D., 2003. Use of Alternaria eichhorniae to control the invasive aquatic weed water hyacinth in Africa. Proceedings of the 8th International Congress of Plant Pathology. Christchurch, New Zealand. pp. 52.

Barreto, R.W., Evans, H.C., 1996. Fungal pathogens of some Brazilian aquatic weeds and their potential use in biocontrol. In: Moran, V.C., Hoffmann, J.H. (Eds.), Proceedings of the IX International Symposium on Biological Control of Weeds. University of Cape Town, Cape Town, pp. 121-126.

417 Barnett, H.L., 1960. Illustrated genera of imperfect fungi, second ed. Burgess,
418 Minneapolis, Minnesota.

419 Bechara, J.A., 1996. The relative importance of water quality, sediment composition
420 and floating vegetation in explaining macrobenthic community structure of floodplain
421 lakes (Parana River, Argentina). Hydrobiologia 333, 95-109. DOI:
422 10.1007/BF00017572

423 Bownes, A., Hill, M.P., Byrne, M.J., 2010. Evaluating the impact of herbivory by a
424 grasshopper, Cornops aquaticum (Orthoptera: Acrididae), on the competitive
425 performance and biomass accumulation of water hyacinth, Eichhornia crassipes
426 (Pontederiaceae). Biol. Control 53, 297-303. DOI: 10.1016/j.biocontrol.2010.02.013

427 Center, T.D., Spencer, N.R., 1981. The phenology and growth of water hyacinth
428 (Eichhornia crassipes (Mart. Solms) in a eutrophic north-central Florida lake. Aquat.
429 Bot. 10, 1-32. DOI: 10.1016/0304-3770(81)90002-4

430 Center, T.D., Van, T.K., Dray, F.A., Franks, S.J., Rebelo, M.T., Pratt, P.D.,
431 Rayamajhi, M.B., 2005. Herbivory alters competitive interactions between two
432 invasive aquatic plants. Biol. Control 33, 173-185. DOI:
433 10.1016/j.biocontrol.2005.02.005

434 Charudattan, R., 1984. Role of Cercospora rodmanii and other pathogens in the
435 biological and integrated controls of waterhyacinth. In: G. Thyagarajan (Ed.),
436 Proceedings of the International Conference on Water Hyacinth. United Nations
437 Environment Programme, Nairobi, Kenya. pp. 823-833.

438 Charudattan, R. 2001. Biological control of weeds by means of plant pathogens:
439 significance for integrated weed management in modern agro-ecology. Biocontrol
440 **46,** 229-260. DOI: 10.1023/A:1011477531101

441 Charudattan, R., Perkins, B.D., Littell, R.C., 1978. Effects of fungi and bacteria on
442 the decline of arthropod-damaged waterhyacinth (Eichhornia crassipes) in Florida.
443 Weed Sci. 26,101-107.

444 Cilliers, C.J., 1991. Biological control of water hyacinth, Eichhornia crassipes
445 (Pontederiaceae), in South Africa. Agric. Ecosyst. Environ. 37, 207-217. DOI:
446 10.1016/0167-8809(91)90149-R

447 Coetzee, J.A., Hill, M.P., 2012. The role of eutrophication in the biological control of
448 water hyacinth, Eichhornia crassipes, in South Africa. BioControl 57, 247-261. DOI:
449 10.1007/s10526-011-9426-y

450 Coetzee, J.A., Byrne, M.J., Hill, M.P., 2007. Impact of nutrients and herbivory by
451 Eccritotarsus catarinensis on the biological control of water hyacinth, Eichhornia
452 crassipes. Aquat. Bot. 86, 179–186. DOI: 10.1016/j.aquabot.2006.09.020

453 Coetzee, J.A., Jones, R.W., Hill, M.P., 2014. Water hyacinth, Eichhornia crassipes
454 (Pontederiaceae), reduces benthic macroinvertebrate diversity in a protected
455 subtropical lake in South Africa. Biodivers. Conserv. 23, 1319-1330.
456 DOI: 10.1007/s10531-014-0667-9

457 Coetzee, J.A., Center, T.D., Byrne, M.J., Hill, M.P., 2005. Impact of the biocontrol
458 agent Eccritotarsus catarinensis, a sap-feeding mirid, on the competitive
459 performance of waterhyacinth, Eichhornia crassipes. Biol. Control 32, 90-96. DOI:
460 10.1016/j.biocontrol.2004.08.001

461 Coetzee, J.A., Hill, M.P., Byrne, M.J., Bownes, A., 2011. A review of the biological
462 control programmes on Eichhornia crassipes (C. Mart.) Solms (Pontederiaceae),
463 Salvinia molesta D.S. Mitch. (Salviniaceae), Pistia stratiotes L. (Araceae),

464 Myriophyllum aquaticum (Vell.) Verdc. (Haloragaceae) and Azolla filiculoides Lam.
465 (Azollaceae) in South Africa. Afr. Entomol. 19, 451-468. DOI: 10.4001/003.019.0202

466 Conway, K.E., 1976. Cercospora rodmanii, a new pathogen of water hyacinth with
467 biological control potential. Can. J. Bot. 54, 1079-1083. DOI: 10.1139/b76-115

468 Cordo, H.A., 1996. Recommendations for finding and prioritizing new agents for
469 biological control of water hyacinth control. In: Charudattan, R., Labrada, R., Center,
470 T.D., Kelly-Begazo, C. (Eds.), Strategies for Water Hyacinth Control, Report of a
471 Panel of Experts Meeting. Institute of Food and Agricultural Sciences, University of
472 Florida, United States. pp. 181-185.

473 Dean, R., Van Kan, J.A.L., Pretorious, Z.A., Hammond-Kosack, K.E., Di Pietro, A.,
474 Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J., Foster, G.D., 2012.
475 The top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13,
476 414–430. DOI: 10.1111/j.1364-3703.2011.00783.x

477 Denno, R.F., Roderick, G.K., 1990. Population biology of planthoppers. Annu. Rev.
478 Entomol. 35, 489-520. DOI: 10.1146/annurev.en.35.010190.002421

479 Domsch, H., Gams, W., Anderson, T.H., 2007. Compendium of soil fungi, second ed.
480 IHW-Verlag, Eching.

481 El-Morsy, E.M., 2004. Evaluation of microfungi for the biological control of water
482 hyacinth in Egypt. Fungal Divers. 16, 35-51.

483 Galbraith, J.C., 1987. The pathogenicity of an Australian isolate of Acremonium
484 zonatum to water hyacinth, and its relationship with the biological control agent,
485 Neochetina eichhorniae. 38, 219-229. Crop Pasture Sci. DOI: 10.1071/AR9870219

Gilman, J.C., 1959. A manual of soil fungi, second ed. Oxford and IBH Publishing, Calcutta.

Gopal, B., 1987. Water hyacinth. Elsevier Science Publishers, Amsterdam. DOI: 10.1017/S0266467400002571

Gossett, D.R., Norris Jr, W.E., 1971. Relationship between nutrient availability and content of nitrogen and phosphorus in tissues of the aquatic macrophyte, Eichornia crassipes (Mart.) Solms. Hydrobiologia 38, 15-28. DOI: 10.1007/BF00036789

Harris, J.L., 2000. Safe low-distortion tape touch method for fungal slide mounts. J. Clin. Microbiol. 38, 4683-4684.

Harris, K.F., Maramorosch, K., 1980. Vectors of plant pathogens. Academic Press, New York, United States of America.

Hill, M.P., Olckers, T., 2001. Biological control initiatives against water hyacinth in South Africa: constraining factors, success and new courses of action. In: Julien, M., Hill, M.P., Center, T., Ding, J. (Eds.), Proceedings of the Second Global Working Group Meeting for the Biological and Integrated Control of Water Hyacinth. Australian Centre for International Agricultural Research, Canberra, Australia. pp. 33-38.

Holm, L.G., Pluchnet, D.L., Pancho, J.V., Herberger, J.P., 1977. The World's Worst Weeds: Distribution and Biology. Hawaii University Press, Honolulu.

Krokene, P., Roux, J., Solheim, H., Wingfield, M.J., 2010. Pathogenicity of Ceratocytis resinifera to Norway spruce. For. Path. 40, 458-464. DOI: 10.1111/j.1439-0329.2009.00623.x

508 Lambers, H., Chapin, F.S., Pons, T.L., 2008. Plant physiological ecology, second ed.
509 Springer, New York, United States of America.

510 Mailu, A., 2001. Preliminary assessment of the social, economic and environmental
511 impacts of water hyacinth in the Lake Victoria basin and the status of control. In:
512 Julien, M., Hill, M.P., Center, T., Ding, J. (Eds.), Proceedings of the Second Global
513 Working Group Meeting for the Biological and Integrated Control of Water Hyacinth.
514 Australian Centre for International Agricultural Research, Canberra, Australia. pp.
515 130-139.

516 Malik, A., 2007. Environmental challenge vis a vis opportunity: The case of water
517 hyacinth. Environ. Int. 33, 122-138. DOI: 10.1016/j.envint.2006.08.004

518 Marlin, D., Hill, M.P., Ripley, B.S., Strauss, A.J., Byrne, M.J., 2013. The effect of
519 herbivory by the mite Orthogalumna terebrantis on the growth and photosynthetic
520 performance of water hyacinth (Eichhornia crassipes). Aquat. Bot. 104, 60–69. DOI:
521 10.1016/j.aquabot.2012.09.005

522 Martínez Jiménez, M., Charudattan, R., 1998. Survey and evaluation of Mexican
523 native fungi for potential biocontrol of Waterhyacinth. J. Aquat. Plant Manage. 36,
524 145-148.

525 Martínez Jiménez, M., Gomez Balandra, M.A., 2007. Integrated control of Eichhornia
526 crassipes by using insects and plant pathogens. Crop Prot. 26, 1234-1238. DOI:
527 10.1016/j.cropro.2006.10.028

528 Midgley, J.M., Hill, M.P., Villet, M.H., 2006. The effect of water hyacinth, Eichhorniae
529 crassipes Solms-Laubach (Pontederiaceae), on benthic biodiversity in two
530 impoundments on the New Years River, South Africa. Afr. J. Aquat. Sci. 31, 25-30.
531 DOI: 10.2989/16085910609503868

532 Mpofu, B., 1995. Biological control of water hyacinth in Zimbabwe. Ph.D. Thesis,
533 McGill University, Canada.

534 Moran, P.J., 2005. Leaf scarring by the weevils Neochetina eichhorniae and N.
535 bruchi enhances infection by the fungus Cercospora piaropi on waterhyacinth,
536 Eichhornia crassipes. BioControl 50, 511-524. DOI: 10.1007/s10526-004-4254-y

537 Muniappan, R., Reddy, G.V.P., Raman, A., 2009. Biological control of tropical weeds
538 using arthropods. Cambridge University Press, Cambridge.

539 Peay, K.G., Kennedy, P.G., Bruns, T.D., 2008. Fungal community ecology: a hybrid
540 beast with a molecular master. BioScience 58, 799-810. DOI: 10.1641/B580907

541 Pieterse, A.H., 1977. Biological control of aquatic weeds: perspectives for the
542 tropics. Aquat. Bot. 3, 133-141. DOI: 10.1016/0304-3770(77)90013-4

543 R Core Team (2013). R: A language and environment for statistical computing. R
544 Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

545 Ray, P., Hill, M.P., 2012a. Impact of feeding by Neochetina weevils on pathogenicity
546 of fungi associated with waterhyacinth in South Africa. J. Aquat. Plant Manage. 50,
547 79-84.

548 Ray, P., Hill, M.P., 2012b. Fungi associated with Eichhornia crassipes in South
549 Africa and their pathogenicity under controlled conditions. Afr. J. Aquat. Sci. 37, 323-
550 331. DOI: 10.2989/16085914.2012.712912

551 Ray, P., Hill, M.P. 2015. More is not necessarily better: the interaction between
552 insect population density and culture age of fungus on the control of invasive water
553 hyacinth. Hydrobiologia 766, 189-200. DOI: 10.1007/s10750-015-2454-3

554 Ray, P., Sushilkumar, Pandey, A.K., 2008. Efficacy of pathogens of water hyacinth
555 (Eichhornia crassipes), singly and in combinations for its biological control. J. Biol.
556 Control 22, 173-177.

557 Reddy, K.R., Agami, M., Tucker, J.C., 1989. Influence of nitrogen supply rates on
558 growth and nutrient storage by water hyacinth (Eichhornia crassipes) plants. Aquat.
559 Bot. 36, 33-43. DOI: 10.1016/0304-3770(89)90089-2

560 Ripley, B.S., De Wet, L., Hill, M.P., 2008. Herbivory-induced reduction in
561 photosynthetic productivity of water hyacinth, Eichhornia crassipes (Martius) Solms-
562 Laubach (Pontederiaceae), is not directly related to reduction in photosynthetic leaf
563 area. Afr. Entomol. 16, 140-142. DOI: 10.4001/1021-3589-16.1.140

564 Sanders, D.R., Theriot, R.F., Theriot, E.A., 1982. Organisms impacting water
565 hyacinth in the Panama Canal. J. Aquat. Plant Manage. 20, 22-29.

566 Shabana, Y.M., Charudattan, R., Elwakil, M.A., 1995. Identification, pathogenicity
567 and safety of Alternaria eichhorniae from Egypt as a bioherbicide agent for water
568 hyacinth. Biol. Control 5**,** 123-135. DOI: 10.1006/bcon.1995.1015

569 Sosa, A.J., Cordo, H.A., Sacco, J., 2007. Preliminary evaluation of Megamelus
570 scutellaris Berg (Hemiptera: Delphacidae), a candidate for biological control of
571 waterhyacinth. Biol. Control 42, 129-138. DOI: 10.1016/j.biocontrol.2007.04.012

572 Sosa, A.J., Marino De Remes Lenicov, A.M., Mariani, R., Cordo, H.A., 2004.
573 Redescription of Megamelus scutellaris Berg (Hemiptera: Delphacidae), a candidate
574 for biological control of water hyacinth. Ann. Entomol. Soc. Am. 97, 271-275. DOI:
575 10.1603/0013-8746(2004)097[0271:ROMSBH]2.0.CO;2

576 Tipping, P.W., Center, T.D., 2010. Planthopper released against water hyacinth in
577 the USA. BioControl News and Information 31, 19-20.

578 Tipping, P.W., Center, T.D., Sosa, A.J., Dray, F.A., 2011. Host specificity
579 assessment and potential impact of Megamelus scutellaris (Hemiptera: Delphacidae)
580 on waterhyacinth Eichhornia crassipes (Pontederiales: Pontederiaceae). Biocontrol
581 Sci. Techn 21, 75-87. DOI: 10.1080/09583157.2010.525739

582 Venter, N., Hill, M.P., Hutchinson, S.L., Ripley, B.S., 2013. Weevil borne microbes
583 contribute as much to the reduction of photosynthesis in water hyacinth as does
584 herbivory. Biol. Control 64, 138-142. DOI: 10.1016/j.biocontrol.2012.10.011

585 Wilson, J.R.U., Ajuonu, O., Center, T.D., Hill, M.P., Julien, M.H., Katagira, F.F.,
586 Neuenschwander, P., Njoka, S.W., Ogwang, J., Reeder, R.H., Van, T., 2007. The
587 decline of water hyacinth on Lake Victoria was due to biological control by
588 Neochetina spp. Aquat. Bot. 87, 90-93. DOI: 10.1016/j.aquabot.2006.06.006

589

590

591

592

593

594

595

596

597

598 **Table. 1.** Differences in water hyacinth growth parameters across Megamelus
599 scutellaris herbivory and insect/leaf sterilisation treatments at high (n = 7) and low
600 nutrient (n = 8) regimes upon completion of the five week experiment. F-statistics
601 were obtained from univariate tests of significance. Significant effects on plant
602 parameters due to nutrient, treatment and nutrient x treatment interactions are
603 highlighted in bold. Degrees of freedom and sample sizes were (1,65) for nutrient
604 regime, (4,65) for treatments and (4,65) for nutrient x treatment interactions.
605
606
607
608
609
610
611
612
613
614
615
616
617
618

Table. 2. Fungal isolates identified morphologically from diseased water hyacinth plant tissues exposed to various sterilisation treatments of Megamelus scutellaris and water hyacinth.

Figure. 1. Differences in water hyacinth growth parameters in relation to Megamelus scutellaris herbivory and insect/leaf sterilisation treatments upon completion of the five week experiment under high (n = 7) and low nutrient (n = 8) regimes for: (a) leaf production, (b) daughter plant production, (c) maximum petiole length, (d) second petiole length, (e) chlorophyll content index and (f) wet weight biomass. Treatments applied were: sterile insect/sterile plant (IS x PS); sterile insect/unsterile plant (IS x PU), unsterile insect/sterile plant (IU x PS), unsterile insect/unsterile plant (IU x PU) and control (which did not receive any M. scutellaris adults or sterilisation). Error bars indicate standard errors of the mean, those followed by the same letter are not significantly different from one another (Tukey's HSD, $P > 0.05$).

Figure. 2. Differences in herbivore and fungal pathogen performance across treatments upon completion of the five week experiment under high (n = 7) and low nutrient (n = 8) regimes for: (a) Megamelus scutellaris adult survival percentages and (b) combined herbivore/fungal pathogen inductions of leaf chlorosis. For the figure legend refer to figure. 1. Error bars indicate standard errors of the mean. Those followed by the same letter are not significantly different from one another (Tukey's HSD, $P > 0.05$).