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Weevil borne microbes contribute as much to the reduction of photosynthesis in water hyacinth as does herbivory

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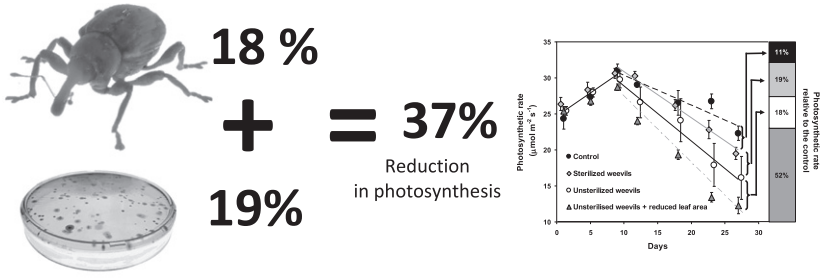
HIGHLIGHTS

- Water hyacinth weevils were demonstrated to be vectors for phyopathogens.
- Weevils carried both fungi and bacteria and transferred these to leaves on which they fed.
- These pathogens contributed as much to the decrease in photosynthetic productivity as did biomass removal.
- Hence, the selection and use of biocontrol agents needs to include their role as pathogen vectors to maximise efficiency.

GRAPHICAL ABSTRACT



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ABSTRACT

Arthropods released for weed biocontrol can have effects other than simply removing biomass and frequently decrease photosynthetic rate more than can be attributed to the mere loss of photosynthetic surface area. Some of this effect may result because biological control agents facilitate the transfer and ingress of deleterious microbes into plant tissues on which they feed. We evaluated this facilitation effect using water hyacinth (*Eichhornia crassipes*) and a weevil (*Neochetina eichhorniae*) and compared the reductions in photosynthetic rates between leaves subject to herbivory by adult weevils sterilized with 3.5% chlorine bleach, to those that were unsterilized. The results showed that weevils carried both fungi and bacteria, transferred these to leaves on which they fed, and that microbes and biomass removal contributed almost equally to the 37% decrease in photosynthetic productivity. Hence, maximising the effectiveness of using arthropods that damage leaf surfaces for biocontrol requires the presence of microorganisms that are deleterious to plants.

1. Introduction

Water hyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae), is native to South America (Bechara, 1996), but has become an invasive in many parts of the tropics and subtropics forming dense mats on waterways that impact biodiversity, fisheries, transport and hydroelectric production (Mailu, 2001; Midgley et al., 2006). The only effective long-term control of this weed has been with biological control, particularly using the weevils *Neochetina eichhorniae* (Warner) and *N. bruchi* (Hustache) (Coleoptera: Curculionidae) (Hill and Olckers, 2001).

These weevil species reduce water hyacinth vigour by decreasing plant size, vegetative reproduction, and flower and seed production (Del Fosse, 1978; Coetzee et al., 2005). The weevil larvae tunnel in the petioles and crown of water hyacinth (Bashir et al., 1984), while adults chew small rectangular patches from the photosynthetic surfaces. This combined damage decreases the

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photosynthetic rate of leaves. However the effect is larger than can be attributed to the loss of photosynthetic surface area alone (Ripley et al., 2008), and suggests that weevils could facilitate the transfer and ingress of saprophytic and other deleterious microorganisms to plant leaves.

These introduced microbes may be airborne and enter via herbivory feeding scars, or may be carried on insect cuticles and in frass and therefore enter directly into the feeding scars (Paine et al., 1997; Moran, 2005). Introductions resulting from herbivory have been demonstrated for *N. eichhorniae* feeding on water hyacinth and for Scolytidae beetles feeding on conifers, both of which introduce pathogenic fungi into plants on which they feed (Charudattan et al., 1978; Paine et al., 1997; De Nooij, 1988). Microorganism infections elicit general symptoms that include increased respiration rates, increased permeability of plasma membranes, decreased photosynthetic rates, water and nutrient deficiencies (de Nooij et al., 1992; Lambers et al., 2008), all of which can contribute to decreased growth and cause necrosis of plant tissue (Del Fosse, 1978; Charudattan et al., 1978; Agrios, 2005; Hatcher, 1997).

This study aimed to determine the contribution of weevil-borne microbes to the decline in photosynthetic rate of water hyacinth and compared the effect of herbivory by unsterilized weevils with that of weevils that were externally sterilized of bacteria and fungi. This tests the hypothesis that these biocontrol agents decrease plant vigour, not only by removing biomass, but also by facilitating the transfer and entry of deleterious microbes to plant tissues.

2. Materials and methods

2.1. Plant and insect material

Water hyacinth plants were sourced from a wild population at New Years Dam, Eastern Cape Province, South Africa (33°17′35, 89″ S 26°07′18, 85″ E) and were maintained insect free in 70 L containers in a clear polythene tunnel. During the experimental period mean day/night tunnel temperatures were 40/21.5 °C, and humidity ranged between 34% and 90%. Containers were filled with tap water and supplied with 0.68 g L^{-1} of controlled release NPK fertiliser (Osmocote®) and 11.2 g L^{-1} of iron chelate to ensure healthy growth and vegetative production of daughter plants. Similar sized daughter plants, were selected from stock plants and four or five individuals were maintained in 25 L tubs under the same growth conditions. Weevils (*N. eichhorniae*) used for herbivory treatments were sourced from colonies maintained in a polythene tunnel at Rhodes University, which had originated from individuals collected from *E. crassipes* plants growing naturally on New Years Dam (33°17′35,89″ S 26°07′18,85″ E).

2.2. Weevil sterilization

Weevils were externally sterilized by placing four individuals in a plastic centrifuge tube with 15 ml of 3.5% sodium hypochlorite and vortex mixed for one minute under sterile conditions, followed by another two vortex mixes with sterilized distilled water. Weevils that were not subject to this procedure but sourced directly from the stock culture are referred to as unsterilized weevils.

The effectiveness of this sterilization procedure was determined by vortex mixing individual sterilized or unsterilized weevils in 1 ml of sterile deionised water. 100 µl aliquots of this aqueous extract were then spread onto potato dextrose agar (PDA) and nutrient agar (NA) plates under sterile conditions. Both types of plates were used in order to allow a broad spectrum of microorganisms to be cultured. Plates were incubated for 72 h at 25 and 32 °C, respectively, and the number of bacterial and fungal colonies that developed was counted. This was replicated three times. A similar procedure was used to determine the microorganisms associated with control leaves and leaves that were exposed to herbivory by unsterilized and sterilised weevils. Herbivory treatments were imposed by confining four weevils to an individual leaf, on five separate plants, for 4 days. Weevils were then removed from the leaves and these, and control leaves, were excised from plants and vortex mixed in 10 ml of sterile deionised water. 100 µl aliquots of these aqueous extract were then plated and analysed as above.

2.3. Herbivory treatments

The effect of herbivory by sterilized and unsterilized weevils on water hyacinth photosynthetic rates and gas exchange was compared to control leaves that were not subject to herbivory. Herbivory treatments were imposed by enclosing a single fully expanded leaf in a fine mesh bag with four weevils and this was replicated on five plants. Control leaves were enclosed in mesh bags without any weevils. Treatments were maintained on the same leaves for a period of 27 days and at intervals of between 3 and 5 days, leaf photosynthetic and gas exchange parameters were measured. On each of these occasions the weevils were removed from the leaves, and after gas exchange measurements, were replaced with four

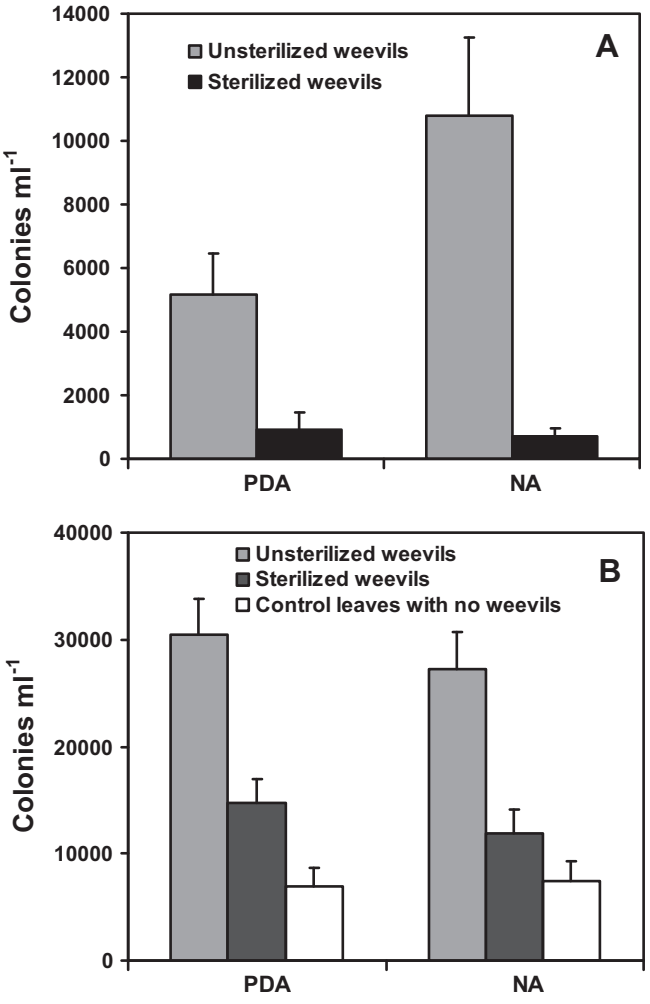

Fig. 1. Mean (SE) number of bacterial and fungal colonies washed from unsterilized or sterilized weevils (A; $n = 3$) or from control leaves and leaves subject to herbivory by sterilized, or unsterilised weevils (B; $n = 5$), and cultured on potato dextrose agar (PDA) or nutrient agar (NA).

freshly-prepared weevils. The mesh bags were sterilised on each occasion with 3.5% chlorine bleach.

2.4. Gas exchange measurements and leaf area calculations

Gas exchange parameters were determined using a Li-6400 (Li-COR, Inc., Lincoln, Nebraska) with a 6400-05 conifer chamber that enclosed the entire experimental leaf. Prior to gas exchange measurements, plants were placed under a sodium vapour light source for approximately 2 h in the laboratory to acclimate to a photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1}, which was the saturating intensity used for gas exchange measurements. During measurements, leaf temperature was kept at 25 °C, leaf to air VPD was below 1.0 kPa and the CO_2 partial pressure in the leaf chamber was set at 38 Pa.

The leaf area and extent of adaxial and abaxial leaf scarring were determined for each leaf by digital photography and image analysis (Windias, Delta-T Devices Ltd., UK). Measured gas exchange parameters were used to calculate rates of photosynthetic CO_2 assimilation (A), the conductance to CO_2 transfer from the atmosphere to leaf interior (g_{st}) and the intercellular CO_2 concentrations (P_i), according to the equations of von Caemmerer and Farquhar (1981). These calculations include leaf area as an input and can be used to calculate parameters for entire leaves that include weevil feeding scars, or calculated accounting for the reduction in leaf area because of herbivory. This allowed the direct effect of microbes to be distinguished from effects resulting from reductions in leaf area.

2.5. Statistical analysis

Linear increases and then subsequent decreases in photosynthetic rates observed after day nine of the experiment were compared separately between treatments using a General Linear model (GLM) with time set as the continuous predictor (Statistica, Tulsa, USA). When significant interactions ($P \leqslant 0.05$) of treatment and time indicated differences in slope, *post hoc* Tukey tests comparisons were used to compare treatments. Similarly, leaf conductance, intercellular CO_2 concentrations and reductions in leaf area were compared between treatments using a GLM with time and treatment set as categorical predictors and *post hoc* Tukey tests were conducted where appropriate. The differences in the numbers of microbial colonies cultured from the various treatments were compared with a GLM, Univariate Test.

3. Results

3.1. Effectiveness of sterilization

Sterilization with 3.5% bleach significantly reduced the number of microorganism colonies that were cultured from weevils on both the PDA ($F_{1,\ 4} = 9.3$, $P < 0.04$) and NA plates ($F_{1,\ 4} = 16.6$, $P < 0.02$; Fig. 1A). This correlated with the increase in the number of colonies that were cultured from leaves after they had been exposed to unsterilized weevils. Relative to control leaves and leaves exposed to sterilised weevils, leaves exposed to unsterilized weevils produced significantly more colonies on both the PDA ($F_{2,\ 12} = 22$, $P < 0.0001$) and NA plates ($F_{2,\ 12} = 16$, $P < 0.0004$; Fig. 1B).

3.2. Gas exchange response to herbivory

Over the initial nine days, leaf photosynthesis increased linearly and rates of increase were not significantly different between treatments ($F_{3,\ 49} = 1.8$, $P > 0.15$; Fig. 2). Subsequently, photosynthetic rates decreased linearly and the rate of decline was significantly different amongst treatments ($F_{3,\ 87} = 33.6$, $P < 0.0001$). The rate of decrease was least for the control leaves and greatest in leaves that were subject to herbivory by unsterilized weevils, where photosynthetic rates were calculated for entire leaves not accounting for the reduction in area caused by feeding scars (Fig. 2). If photosynthetic rates were recalculated accounting for the reduction in leaf area, then the rate of decline was decreased but remained higher than that observed for control leaves. In contrast, the rate of decline for leaves with sterilised weevils and

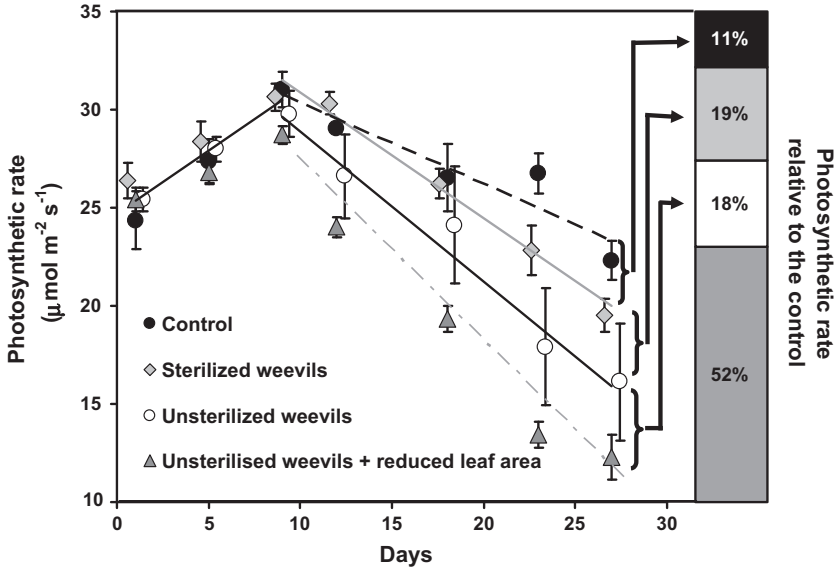

Fig. 2. Change in average photosynthetic rates for the control leaves, leaves subjected to herbivory by sterilized and unsterilized weevils, and of leaves with unsterilized weevils and uncorrected for reductions in leaf area over the course of the 27 day experiment. For the sake of presentation, values at each time point have been staggered on the time axis. Initial linear increase in photosynthetic rate was not different between treatments and was fitted with a single line, while the subsequent declines in photosynthetic rate were not homogenous between treatments and each was fitted with an individual line. ($n = 5$, means ± SE). The bar indicates the proportional differences in photosynthetic rate resulting from each of the treatments relative to rates for the control plants.

corrected leaf areas was not significantly different to that of control leaves.

When the GLM analyses were repeated excluding the data from day 23 and 27, the differences between slopes were not significant ($F_{3,\ 58} = 1.7$, $P > 0.16$), indicating that the effect of the treatments was only apparent from day 23. The proportional effects of these treatments after day 23 are shown in Fig. 2.

Leaf conductance (g_{st}) and intercellular CO_2 partial pressures (P_i) for the control leaves remained relatively constant over the course of the experiment, while the values for both herbivory treatments increased (Fig. 3A and B). Consequently, after day nine, the values for control leaves were significantly lower than those of leaves subject to herbivory (g_{st}: $F_{12,\ 74} = 7.2$, $P < 0.0001$; P_i: $F_{12,\ 74} = 4.2$, $P < 0.0001$), but differences between the two herbivory treatments were not significant. Increases in g_{st} and P_i are likely due to greater permeability of the leaf cuticle and epidermis, and were correlated to increasing leaf damage which was similar (Fig. 3C), irrespective of whether the weevils had been sterilized or not ($F_{6,\ 49} = 0.8$, $P > 0.6$).

4. Discussion

Our results show that there are microorganisms associated with *N. eichhorniae* feeding and that unsterilized adults feeding on water hyacinth leaves have a greater effect on photosynthetic rates than happens in the absence of these microorganisms. The microbes reduced leaf photosynthetic rate by 19%, a value that was similar to the 18% decrease caused by the leaf area reduction due to herbivory. Rates of decrease between controls and leaves eaten by sterilised weevils were not significantly different, but were lowered by 11%. This same result was evident when this experiment was repeated (data not shown), and as in both experiments sterile herbivory decreased rates relative to controls, a type-II statistical error cannot be ruled out (Rolf and Sokal, 1994). Such decreased rates could possibly be due to imperfect sterilization and because of the presence of microorganisms that cannot be cultured on PDA and NA plates (Purcell and Almedia, 2005; Narayanasamy, 2011). The effect of leaf age contributes to the observed responses, as is evident in the controls, and such unimodal productivity responses are typical for most leaves (e.g. Field and Mooney, 1983; Suzuki et al., 1987).

Plant responses that result in reduced photosynthetic rate are frequently associated with decreased leaf conductance and limitations to the supply of CO_2 to photosynthesis (Hsiao, 1973; Chaves et al., 2002). Arthropod feeding has been shown to disrupt vasculature and alter leaf hydraulics with consequential effects on stomatal conductance and photosynthesis (Nabity et al., 2009). However in *E. crassipes*, this was not the case and the herbivory treatments increased leaf conductance and intercellular CO_2 partial pressures (P_i). Increasing P_i in healthy leaves stimulates photosynthesis, as has been demonstrated for well-fertilised hyacinth leaves (Ripley et al., 2006), and the observed increase in P_i from 31 to 35.5 Pa at day 27, should have increased photosynthesis by ~8%. Hence, the observed decreases in photosynthetic rates relative to controls, points to mechanisms at the level of CO_2 utilisation rather than supply. These findings corroborate other work showing that arthropod herbivory has direct effects on the light reactions of photosynthesis (Ripley et al., 2006; Marlin et al., in press) and on photosynthetic enzymes (Portis, 1995; Hermsmeier et al., 2001; Hui et al., 2003).

These findings, like those of Caesar (2005) and Charudattan et al. (1978), highlight the significant role that microbes play in contributing to the decline of arthropod damaged plants. This has important implications for studies that simulate the effects of herbivory by clipping (e.g. Bossdorf et al., 2004; Schutzenhofer and Knight, 2007). Clipping is unlikely to facilitate the transfer and ingress of microbes and hence would underestimate the impact of herbivores like weevils. Furthermore, it demonstrates that to maximise the effectiveness of using chewing arthropods for biocontrol requires the presence of the microorganisms that are deleterious to plants. Conceptually, this is no different to the release of multiple biocontrol agents where additive interactions can enhance their effectiveness (Charudattan et al., 1978; Caesar 2005; Moran, 2005; Turner et al., 2010; Buccellato et al., 2012).

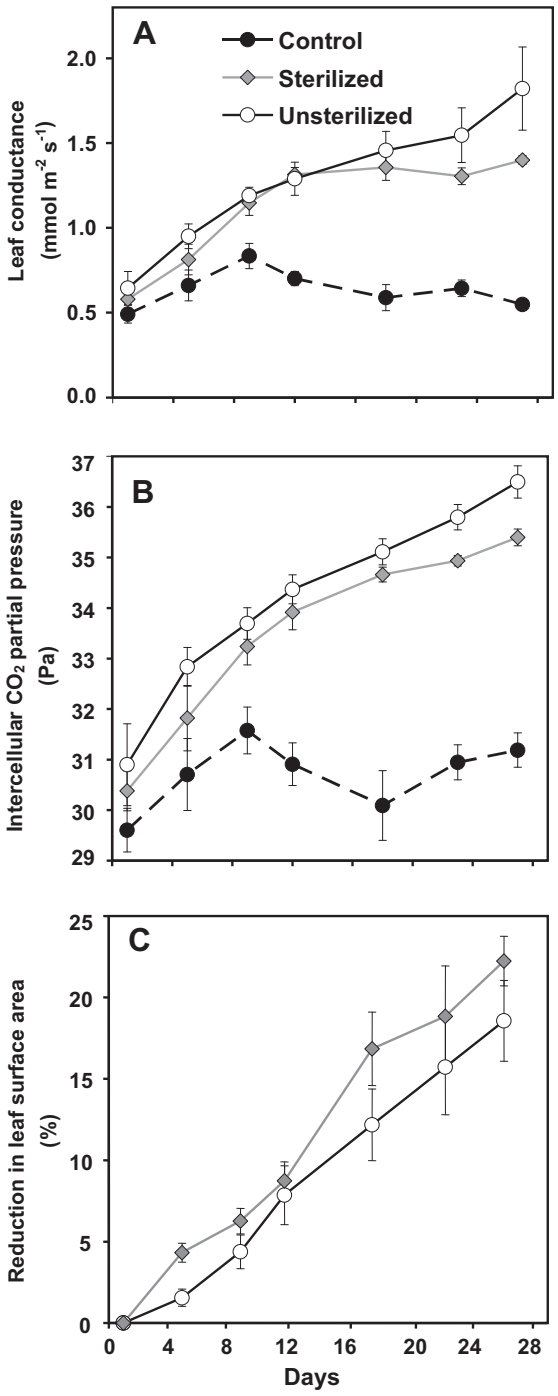

Fig. 3. Change in average leaf conductance (A), intercellular CO_2 partial pressures (B) and reduction in leaf surface area (C) for the control leaves, leaves subjected to herbivory by sterilized and unsterilized weevils over the course of the 27 day experiment. ($n = 5$, means ± SE).

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References

Agrios, G.N., 2005. Plant Pathology, fifth ed. Academic Press, Burlington, USA.
Bashir, M.O., El Abjar, Z.E., Irving, N.S., 1984. Observations on the effect of the weevils *Neochetina eichhorniae* Warner and *Neochetina bruchi* hustache on the growth of water hyacinth. Hydrobiologia 110, 95–98.
Bechara, J.A., 1996. The relative importance of water quality, sediment composition and floating vegetation in explaining the macrobenthic community structure of floodplain lakes (Paraná River, Argentina). Hydrobiologia 333, 95–104.
Bossdorf, O., Schroder, S., Prati, D., Auge, H., 2004. Palatability and tolerance to simulated herbivory in native and introduced populations of *Alliaria petiolata* (brassicaceae). Am. J. Bot. 91, 856–862.
Buccellato, L., Byrne, M.J., Witowski, E.F.T., 2012. Interactions between a stem gall fly and a leaf-spot pathogen in the biological control of *Ageratina adenophora*. Biol. Control 61, 222–229.
Caesar, A., 2005. Melding ecology, weed biocontrol and plant microbial ecology can inform improved practices in invasive plant species biocontrol. Biol. Control 35, 240–246.
Charudattan, R., Perkins, B.D., Littell, R.C., 1978. The effects of fungi and bacteria on the decline of arthropod damaged waterhyacinth in Florida. Weed Sci. 26, 101–107.
Coetzee, J.A., Center, T.D., Byrne, M.J., Hill, M.P., 2005. Impact of the biocontrol agent *Eccritotarsus catarinensis*, a sap-sucking mirid, on the competitive performance of waterhyacinth *Eichhornia crassipes*. Biol. Control 32, 90–96.
Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T., Pinheiro, C., 2002. How plants cope with water stress in the field? Photosynthesis and growth. Ann. Bot. 89, 907–916.
De Nooij, M.P., 1988. The role of weevils in the infection process of the fungus *Phomopsis subordinaria* in *Plantago lanceolata*. Oikos 52, 51–58.
De Nooij, M.P., Biere, A., Linders, E.G.A., 1992. Interaction of pests and pathogens through host disposition. In: Ayres, P.G. (Ed.), Pests and Pathogens: Plant Responses to Foliar Attack. BIOS Scientific Publishers, Oxford, UK, pp. 142–160.
Del Fosse, E.S., 1978. Effect on waterhyacinth of *Neochetina eichhorniae* [*col.: Curculionidae*] combined with *Orthogalumna terebrantis* [*Acari: Galumnidae*]. Entomophaga 23, 379–387.
Field, C., Mooney, H.A., 1983. Leaf age and seasonal effects on light, water, and nitrogen use efficiency in a California shrub. Oecologia 56, 348–355.
Hatcher, P.E., 1997. Indirect interactions between insect herbivores and pathogenic fungi on leaves. In: Gange, A.C., Brown, V.K. (Eds.), Multitrophic Interactions in Terrestrial Systems. Blackwell, London, UK, pp. 133–149.
Hermsmeier, D., Schittko, U., Baldwin, I.T., 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. Plant Physiol. 125, 683–700.
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.H., Hill, M.P., Center, T.D., Jianqing, D., (Eds.) Biological and Integrated Control of Water Hyacinth, ACAIR Proceedings 102, pp. 33-38.
Hsiao, T.C., 1973. Plant responses to water stress. Ann. Rev. Plant Physiol. 24, 519–570.
Hui, D., Javeed, I., Lehmann, K., Gase K., Saluz, H.P., Baldwin, I.T., 2003.
Lambers, H., Stuart III, F., Pons, T.L., 2008. Plant Physiological Ecology. Springer, New York, pp. 604.
Marlin, D., Hill, M.P., Ripley, B.S., Strauss, A.J., Byrne, M.J., in press. The effect of herbivory by the mite *Orthogalumna terebrantis* on the growth and photosynthetic performance of water hyacinth (*Eichhornia crassipes*). Aquat. Bot., http://dx.doi.org/10.1016/j.aquabot.2012.09.005.
Mailu, A.M., 2001. Preliminary assessment of the social, economic and environmental impacts of Water Hyacinth in Lake Victoria Basin and status of control biological and integrated control of Water Hyacinth, Eichhornia crassipes, in: Julien, M.H., Hill, M.P., Center, T.D., Jianqing, D., (Eds.) Biological and Integrated Control of Water Hyacinth, ACAIR Proceedings 102, pp. 130–139.
Midgley, J.M., Hill, M.P., Villet, M.H., 2006. The effect of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae), on benthic biodiversity in two impoundments on the New Year's River, South Africa. Afr. J. Aquat. Sci. 31, 25–30.
Moran, P.J., 2005. Leaf scarring by the weevils *Neochetina eichhorniae* and *N. bruchi* enhances infection by the fungus *Cercospora piaropi* on Water Hyacinth *Eichhornia crassipes*. Biol. Control 50, 511–524.
Nabity, P.D., Zavala, J.A., DeLucia, E.H., 2009. Indirect suppression of photosynthesis on individual leaves by arthropod herbivory. Ann. Bot. 103, 655–663.
Narayanasamy, P., 2011. Microbial Plant Pathogens-Detection and Disease Diagnosis: Fungal Pathogens, vol. 1. Springer, New York, pp. 291.
Paine, T.D., Raffa, K.F., Harrington, T.C., 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annu. Rev. Entomol. 42, 179–206.
Portis Jr, A.R., 1995. The regulation of Rubisco by Rubisco activase. J. Expt. Bot. 46, 1285–1291.
Purcell, A.H., Almedia, R.P.P., 2005. Insects as vectors of disease agents. In: Dekker, M. (Ed.), Encyclopedia of Plant and Crop Science. Taylor and Francis, London, W1T 3JH, p. 1329.
Ripley, B.S., Muller, E., Behenna, M., Whittington-Jones, G.M., Hill, M.P., 2006. Biomass and photosynthetic productivity of water hyacinth (*Eichhornia crassipes*) as affected by nutrient supply and mirid (*Eccritotarus catarinensis*) biocontrol. Biol. Control 39, 392–400.
Ripley, B.S., DeWet, L., Hill, M.P., 2008. Herbivory-induced reduction in photosynthetic productivity of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae), is not directly related to reduction in photosynthetic leaf area. Afr. Entomol. 16, 140–142.
Rolf, R.R., Sokal, F.J., 1994. Biometry: the principals and practice of statistics in biological research, third edition. W.H. Freeman, San Francisco, pp. 199.
Schutzenhofer, M.R., Knight, T.M., 2007. Population-level effects of augmented herbivory on *Lespedeza cuneata*: implications for biological control. Ecol Appl. 17, 965–971.
Suzuki, S., Nakamoto, H., Ku, M.S.B., Edwards, G.E., 1987. Influence of leaf age on photosynthesis, enzyme activity, and metabolite levels in wheat. Plant Physiol. 84, 1244–1248.
Turner, P.J., Morin, L., Williams, D.G., Kriticos, D.J., 2010. Interactions between a leafhopper and rust fungus on the invasive plant *Asparagus asparagoides* in Australia: a case of two agents being better than one for biological control. Biol. Control 54, 322–330.
von Caemmerer, S., Farquhar, G.D., 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153, 376–387.