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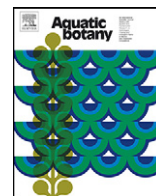
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The effect of herbivory by the mite *Orthogalumna terebrantis* on the growth and photosynthetic performance of water hyacinth (*Eichhornia crassipes*)

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ABSTRACT

Eutrophication of fresh water systems is one of the most important factors contributing to the invasion of fresh water bodies by water hyacinth, *Eichhornia crassipes*. The South American mite, *Orthogalumna terebrantis*, established on the weed in South Africa in the late 1980s, but the impact of mite herbivory on the weed has never been quantified. Water hyacinth was grown under low, medium and high nitrogen and phosphorus nutrient conditions and the effect of mite herbivory on the weed's growth was examined. Additionally, the impact of different mite herbivory intensities on the weed's photosynthetic performance was examined because herbivory may have more subtle effects on the plant than can be seen from changes in plant growth parameters. Water nutrient content had a great impact on plant growth, but growth was unaffected by mite herbivory in all levels of nutrients tested. Photosynthetic performance of water hyacinth leaves exposed to varying levels of mite herbivory was assessed by measuring net photosynthetic rate (A), leaf conductance (g_i), transpiration rate (E) and intercellular CO_2 concentration (C_i), and by measuring specific fluorescence parameters including maximal fluorescence (F_m), efficiency of photosystem II (F_v/F_m) and certain JIP-test parameters. Photosynthesis decreased as mite herbivory increased, but there was a positive correlation between g_i , E and C_i , and the amount of leaf tissue damaged through mite feeding. The efficiency of photosystem II (PSII) decreased as mite herbivory increased, as seen in the altered fluorescence emission of mite-damaged plants, but this was not the consequence of decreased chlorophyll content. Feeding by *O. terebrantis* thus decreased water hyacinth photosynthetic rate and the light reaction performance, even at relatively low mite densities. These results show that the impact of a biological control agent on its host plant may not be obvious at a plant growth level, but may nonetheless affect the plant at a physiological level.

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1. Introduction

The invasion of fresh water bodies by water hyacinth, *Eichhornia crassipes* Mart. Solms-Laubach (Pontederiaceae), has caused significant economic and ecological losses, and the plant is considered to be the worst aquatic weed in South Africa (Hill and Cilliers, 1999). The impact of biocontrol agents on water hyacinth is dependent on the plant's nutrient content and biomass, which are related to water nutrient concentrations (Moran, 2006). Nitrogen (N) and phosphorus (P) and the N:P ratio are of particular significance (Reddy et al., 1989, 1990; Koerselman and Meuleman, 1996). Numerous studies have demonstrated that the growth and biomass

of water hyacinth increases with an increase in water nutrients (Gopal, 1987; Carignan and Neiff, 1994; Xie et al., 2004; Heard and Winterton, 2000; Ripley et al., 2006; Coetzee et al., 2007; Stanley et al., 2007) which indicates that nutrient control should form a vital part of water hyacinth management plans (Hill and Olckers, 2001).

The impact of water hyacinth biocontrol agents on the size and density of individual plants has been documented in previous studies (e.g. Delfosse, 1978; Center, 1981; Coetzee et al., 2007). Plants defend themselves by tolerating or resisting insect herbivory (Schultz, 2002) and may compensate for insect herbivory by completely or partially replacing yield losses caused by the herbivory (McNaughton, 1983; Trumble et al., 1993). Plant compensatory responses are consequently considered to hamper the efficacy of biocontrol agents (Myers et al., 1990; Wirf, 2006; Watt et al., 2007).

Recent studies show that insect herbivory triggers multifaceted physiological responses in the remaining plant tissue (tissue not directly damaged by herbivore feeding) that may reduce the

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photosynthetic ability of a plant more so than simply removing photosynthetic surface area (Aldea et al., 2005; Nabity et al., 2009). However, impact studies of biocontrol agents of water hyacinth have generally focused on measuring plant growth parameters such as petiole lengths, and leaf and daughter plant (ramet) production (Coetzee et al., 2007; Ajuonu et al., 2009; Bownes et al., 2010; Byrne et al., 2010), while the more subtle effects of herbivory on plant physiology are usually not measured. Furthermore, plant responses to injury through defoliation have been extensively studied (Peterson et al., 2004), yet plant responses to injuries from other feeding guilds, i.e. leaf miners such as the mite *Orthogalumna terebrantis* Wallwork (Acari: Galumnidae), are less well studied.

O. terebrantis is one of seven biocontrol agents on water hyacinth in South Africa and is currently established at 17 out of the 66 recorded water hyacinth infestation sites across the country (D. Schlange, pers. comm.; Integrated Management of Water Hyacinth in South Africa database; Byrne et al., 2010). Field observations in South Africa indicate that during summer certain water hyacinth infestations may have more than 50% of the leaf surface area damaged by mite herbivory (Byrne et al., 2010), but to date no studies have examined the impact of the mite on water hyacinth growth parameters or physiology.

Female *O. terebrantis* oviposit on the youngest water hyacinth laminae and the eggs are deposited underneath the cuticle in small perforations made with their mandibles, in the middle layer of the parenchyma (Silveira-Guido, 1965). As the larvae and nymphs develop and feed inside the lamina, they remove plant tissue and create distinctive yellowish linear markings, called galleries, which reach a length of ± 4 mm before adults emerge (Cordo and DeLoach, 1976). Feeding by *O. terebrantis* causes visible damage to water hyacinth laminae but how this translates into overall plant fitness, growth and photosynthetic physiology remains to be determined.

Water hyacinth plants were grown in three different water nutrient conditions to determine whether herbivory by *O. terebrantis* would have an impact on plant growth, especially at low nutrient levels. In a separate experiment, plants were subjected to different intensities of mite herbivory to determine whether the herbivory impacts on the plant's physiology, and if so, whether the effects result from altered stomatal conductance and decreased supply of intercellular CO_2 , or are because of direct effects on photosynthetic metabolism. It is expected that as the mite herbivory intensifies, the greater will be the negative impact on the plants' photosynthetic performance, and that this will be seen as a direct effect on the photosynthetic metabolism.

2. Methods

2.1. Experiment 1: impact of herbivory on plant growth under different water nutrient levels

Plants were obtained from stock cultures grown in large plastic pools inside a glasshouse, at the Agricultural Research Council – Plant Protection Research Institute (PPRI), Pretoria, South Africa. The pools were topped up with tap water as needed to ensure full capacity at all times. The tap water had undetectable levels of phosphorus and nitrite, and 1.5 mg L^{-1} nitrate and 0.015 mg L^{-1} NH_4 . Nutrients were supplied in the form of a water soluble granular fertilizer (N3:P2:K1) which was placed into porous plastic bottles to allow for a slow release of nutrients. Two bottles were suspended in the water column inside each pool and were topped up with fertilizer every 6 months.

Experimental plants were grown in 48 plastic tubs ($42 \text{ cm} \times 30 \text{ cm}$ and 20 cm depth) inside a glasshouse at the PPRI, and were filled with 14 L of tap water. Two free-floating plants, with ramets, dead leaves and stems removed, were placed

into each tub. Tubs were then divided into three groups so that plants could be grown at three different nutrient concentrations. Potassium nitrate (KNO_3) and potassium dihydrogen orthophosphate (KH_2PO_4) were used as the nitrogen (N) and phosphorus (P) bases, respectively. High (eutrophic), medium (mesotrophic) and low (oligotrophic) nutrient concentrations were set up as follows: 4.52 mg N L^{-1} with 0.43 mg P L^{-1} (high), 3.02 mg N L^{-1} with 0.22 mg P L^{-1} (medium) and 2.02 mg N L^{-1} with 0.08 mg P L^{-1} (low). The nutrient levels chosen were similar to those found at three different field sites in South Africa, namely Mbozambo Swamp ($29^\circ 21' \text{S}$, $31^\circ 18' \text{E}$), Hammarsdale Dam ($29^\circ 48' \text{S}$, $30^\circ 39' \text{E}$) and Farm Dam ($26^\circ 02' \text{S}$, $27^\circ 57' \text{E}$), which were considered to be eutrophic (high), mesotrophic (medium) and oligotrophic (low), respectively (Byrne et al., 2010). Commercial iron chelate (13% Fe) was added to each tub at 1.3 g/14 L water. The high, medium and low nutrient treatments were replicated 18, 14 and 16 times, respectively, where each tub represented a replicate. The plants were grown at these nutrient levels for 4 weeks, and the nutrients and water in each tub were replaced weekly.

After 4 weeks, ramets, dead leaves and stems were again removed and the plants were weighed. Using a fine camel-hair paintbrush, mites were placed onto the plants in one half of the tubs (100 mites/plant) while the plants in the other half of the tubs were retained as controls. One hundred mites per plant were used as the maximum number of mites timeously possible to place onto the plants within 1 week. The mites were collected from stock cultures kept at the PPRI. The tubs were placed at least 0.5 m apart to prevent the mites, which are poor dispersers, from moving between tubs. The study ran for 12 weeks to allow the mite population to increase; the egg to adult stage takes between 22 and 25 days at $25\text{--}26^\circ \text{C}$ (Cordo and DeLoach, 1976). Tubs were arranged in a complete randomised block design and the average day and night temperatures in the glasshouse were $28.61 \pm 5.47^\circ \text{C}$ and $16 \pm 1.66^\circ \text{C}$, respectively.

Plants were sampled at 14-day intervals and the following parameters were measured on each plant: number of leaves, number of ramets, length of the longest petiole, and wet biomass (which included daughter plants) and leaf damage. These parameters were averaged for the two plants in each tub to obtain a mean response per tub.

Damage by mite herbivory to leaves 4 and 6 was visually estimated as the percentage of the adaxial leaf surface area damaged. Water hyacinth leaves are arranged in a rosette around the apical bud (Center, 1981), and the first unfurled leaf closest to the apical bud was numbered as leaf 0. Leaves 4 and 6 were chosen because mite damage becomes more distinct on older leaves; females oviposit on younger leaves and as the mite nymphs develop inside the galleries, while the leaves age, the galleries become longer over time, and hence more visible (Cordo and DeLoach, 1976). The lengths of five of the longest and shortest galleries, on leaf 4 and leaf 6, were measured to the nearest 0.5 mm to determine mite development over time, since gallery lengths correspond to mite developmental stages (Delfosse, 1978).

The chlorophyll content ($\text{mg chl-a} + \text{chl-b L}^{-1}$) was compared in 40 leaves, randomly selected from stocks of water hyacinth plants grown at the PPRI, half of which were kept agent free and half which had been grown with the mites for 10 years (C. Cilliers, pers. comm.) and had a well established mite population (>200 mites/plant). The plant stocks were kept as described above, inside two glasshouses (one with and one without mites) adjacent to each other, ensuring similar light and temperature conditions. Plants in the agent-free glasshouse were sprayed with water under high pressure every other day to ensure they were kept clean from insects and mites. The chlorophyll content was measured according to a modified method of Dere et al. (1998). Roughly 0.5 g of leaf material was ground up with a pestle and mortar, and extracted in 100%

acetone (20 ml per gram of wet plant material). The homogenate was filtered under suction. The supernatant absorbance was read at 663 nm and 645 nm on a UNICO® 1100 spectrophotometer, and pure acetone was used as the blank. The amounts of chlorophyll-a, chlorophyll-b and chlorophyll (a + b) were calculated using the equations of [Lichtenthaler \(1987\)](#), and were expressed per gram of fresh weight.

2.1.1. Statistical analyses

All data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene's test). Data that failed to meet the requirements of normality and homogeneity after transformation were analysed using non-parametric statistics.

Multivariate analysis of variance (MANOVA) was used to examine variation in the measured plant growth parameters (ramet production, leaf production, maximum petiole length and change in wet biomass), with nutrients (low, medium and high) and herbivory (mite damage and control) as the independent variables. Univariate analyses of variance and post hoc multiple comparisons were subsequently employed to explore significant effects identified using MANOVA. Post hoc comparisons were conducted using Fisher's Least Significant Difference test at the 5% level of significance ([Snedecor and Cochran, 1980](#)), followed by a Bonferroni correction for multiple comparisons ([Rice, 1989](#)). The wet weights of the plants were measured at the start and the end of the experiment, and the difference in wet biomass after 12 weeks was used as a measure of the relative growth rate calculated for each nutrient treatment.

A Kruskal–Wallis ANOVA was used to determine whether there were differences between the nutrient treatments in the leaf surface area damaged by mites, at the end of the sample period, using leaves 4 and 6 separately. In addition, mite damage on leaves 4 and 6 was compared at each nutrient treatment using the Kruskal–Wallis ANOVA. The lengths of galleries were combined to obtain a mean gallery length for leaves 4 and 6. One-way ANOVA was used to determine whether there were differences in gallery lengths, and therefore mite development, between the three nutrient treatments. The gallery lengths were plotted against time and fitted with logarithmic curves.

A *t*-test was used to evaluate the difference in the chlorophyll content between leaves of agent free plants and leaves that had been exposed to the long-established and large mite population. All data were analysed using STATISTICA Version 7.0 (© StatSoft, Inc., USA).

2.2. Experiment 2: impact of herbivory on plant photosynthetic performance

Water hyacinth plants were collected from stock cultures at the ARC-PPRI and also from Rhodes University. The leaf turn-over rate of water hyacinth can be as high as one leaf per week during summer ([Byrne et al., 2010](#)) and the plants are thus easy to grow. Mites had been established on the plants at PPRI for more than 10 years, and the plants were heavily damaged by the mites. In contrast, the plants at Rhodes University had only been subject to 6 months of mite herbivory and were much less damaged. The plants from the two stock cultures therefore had varying levels of mite damage and together simulated a natural water hyacinth population where plants of different ages and different levels of damage occur.

Plants from the ARC-PPRI were transported to Rhodes University a week prior to the start of the gas exchange measurements and both sets of plants with their resident mite populations were maintained in large plastic tubs inside a polythene tunnel at the university. Osmocote® (3–4 months release time) and iron chelate were used as nutrient supplies. Plants were well-fertilized and produced ramets vegetatively. Plants subject to herbivory were

compared to uninfested control plants that originated from Rhodes University stock.

Prior to measuring the gas exchange and photosynthetic rates, digital photographs were taken of 10 leaves (one leaf from each of 10 individual plants damaged by mites) so that the relative surface area damaged by mites could be measured (using the image analysis software ImageJ, National Institute of Health, USA). This ensured that differences in leaf area, which impact on calculation of gas exchange and photosynthetic rate, were accounted for. The fourth leaf was photographed for measurement of leaf area and mite damage, and thereafter used in the experiment. These leaves remained attached to the plant throughout the measurements.

The gas exchange parameters were measured on the fourth attached leaf of mite-damaged and control plants ($n=10/\text{treatment}$), using a LI-6400 portable photosynthesis system and conifer chamber (Li-Cor, Inc., Lincoln, NE). The air entering the leaf chamber had a sample CO_2 concentration of $380 \mu\text{mol mol}^{-1}$, the block temperature was set to 30°C and the light intensity at saturating photosynthetic photon flux (PPFD) of $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ was provided by an external halogen lamp. To ensure that leaf-to-air vapour pressure deficits would not exceed 1 kPa the sample relative humidity levels were kept at 75–80% by manually adjusting the H_2O scrubber ($\text{Mg}(\text{ClO}_4)_2$). Gas exchange parameters were used to calculate net photosynthetic rate (A), leaf conductance (g_l), transpiration rate (E) and intercellular CO_2 concentration (C_i), according to [Farquhar and von Caemmerer \(1982\)](#). When sample CO_2 was $>390 \mu\text{mol mol}^{-1}$ or $<370 \mu\text{mol mol}^{-1}$ the data were removed before analysis.

For the measurements of chlorophyll-a fluorescence, the photochemical efficiency of PSII was determined on the same leaves as were used for gas exchange measurements, using a Plant Efficiency Analyzer (PEA, Hansatech, UK). Five sections of each leaf were dark-adapted using leaf clips (5 mm diameter each), and the clips were kept on the leaves for a minimum of 1 h. The chl-a fluorescence parameters measured included minimal fluorescence (F_0), maximal fluorescence (F_m), the maximum quantum efficiency of PSII (F_v/F_m) and 30 s fluorescent transients. The F_0 , F_m and F_v/F_m values from the five dark-adapted sections on each leaf were combined and a mean value per leaf was calculated for each of the chl-a fluorescence parameters measured. The sequence of phases from initial (F_0) to maximal (F_m) fluorescence forms a curve with several intermediate steps which have been labelled O, J, I and P ([Strasser and Govindjee, 1992](#)). The O–J–I–P curve changes shape when plants are exposed to stress caused by changing environmental conditions, e.g. temperature and chemical influences. The method of analyzing the curve is called the JIP-test and it is a tool which can be used to analyse environmental effects on photosynthetic organisms ([Strasser et al., 2004](#)). The JIP-test calculates the relative proportions of energy that are dissipated by different processes during the light reaction, and are referred to as energy fluxes ([Strasser and Strasser, 1995](#)). The fluorescent transients were used to obtain the JIP-test parameters ([Strasser et al., 2004](#)) using BioLyzor (Version 3, Laboratory of Bioenergetics, University of Geneva, Switzerland).

Table 1 lists the JIP-test parameters that were measured, explains their biophysical and biochemical importance, and shows how they were calculated from original fluorescence data. Fluorescence readings are expressed in arbitrary units and the JIP-test parameters are expressed as relative values.

After the completion of the above measurements, the experimental leaves were cut off the plants and then sliced vertically in half, from the base to the tip of the lamina, and each half was weighed. Chlorophyll was extracted from one half of the fresh leaf, using 6 ml of 100% acetone per 1 g of fresh weight. Chlorophyll content was expressed per gram of fresh weight. Half of a leaf was used to ensure that at least one half of the area damaged by the mites was included in the extraction, since mite damage tends to be spread

Table 1

Selected JIP-test parameters used to determine the efficiency of Photosystem II (PSII) of water hyacinth plants exposed to varying levels of *O. terebrantis* herbivory. Biophysical or biochemical meanings are given, as well as equations showing how the selected energy fluxes of the JIP-test are calculated from the original fluorescence data (Strasser et al., 2004; Baker et al., 2007).

JIP-test parameters	Meaning	Calculation
<i>Energy fluxes expressed per Q_A^- reducing PSII reaction centres (RCs)</i>		
ABS/RC	Rate of photon absorption	$(TR_0/RC)/[(F_m - F_0)/F_m]$
TR ₀ /RC	Maximum rate of Q_A reduction	$(M_0/V_j) = M_0/[(F_j - F_0)(F_m - F_0)]$
ET ₀ /RC	Rate of electron transport beyond Q_A reduction	$(TR_0/RC)(1 - V_j)$
RC/CS	Density of RCs in PSII per cross section (CS)	
<i>Performance index on absorption basis</i>		
PI _{ABS}	Compound function of light energy absorption, efficiency of Q_A reduction and conversion of excitation energy to electron transport	$[RC/ABS][TR_0/ABS]/[(F_m - F_0)][(ET_0/TR_0)/V_j]$

The subscript '0' refers to the quantification of PSII behaviour at the onset of fluorescence induction. *Abbreviations:* ABS, absorption flux (occurs when photons are absorbed by the photosynthetic antenna pigment); TR, trapping flux (occurs when energy is transferred to the reaction centres); ET, electron transport flux (occurs when Q_A is reduced to Q_A^- which is then reoxidized to Q_A thereby maintaining the metabolic reactions of the PSII).

equally on both halves of a leaf. The plant material was ground up with an electronic grinder (Janke & Kunkel, Ikwerek) and removed by centrifuging at 6000 rpm for 7 min. The supernatant was poured off and the remaining pellet was re-suspended in 6 ml 100% acetone and re-centrifuged as before. The two subsequent supernatants, originating from one half of a leaf from each plant, were pooled and their absorbencies were read at 663 nm and 645 nm on a PU8670 Vis/NIR spectrophotometer (Philips, UK). Pure acetone was used as the blank. The supernatants were diluted where necessary, i.e. if the absorbencies were >1. The equations of Lichtenthaler (1987) were used to determine chlorophyll-a, chlorophyll-b and chlorophyll (a + b). Chlorophyll content was expressed per gram of fresh weight. The remaining half of each leaf was weighed to obtain fresh biomass data.

2.2.1. Statistical analyses

Linear regressions were fitted to the measured gas exchange parameters, the chl-a fluorescence parameters, the JIP-test parameters, the leaf chlorophyll contents and the wet leaf biomass, and plotted against mite damage per leaf. Data were analysed with Pearson correlation using STATISTICA Version 7.0 (© StatSoft, Inc., USA).

3. Results

3.1. Impact of nutrients and herbivory on plant growth and leaf chlorophyll

Water hyacinth growth was more affected by nutrients than by mite herbivory (MANOVA: nutrient treatment Wilks' $\lambda = 0.060$, $F_{8,28} = 10.756$, $p < 0.001$; herbivory treatment Wilks' $\lambda = 0.851$, $F_{4,14} = 0.612$, $p = 0.661$). Table 2 shows the univariate tests of significance for each growth parameter as influenced by nutrients, herbivory, and their interaction. The numbers of ramets produced, and the lengths of the longest petioles, after 12 weeks, were generally greater in the high nutrient treatments compared to the medium and low nutrient treatments (Fig. 1A–C). In contrast, there was no significant difference in the change of plant wet biomass accumulation, after 12 weeks, across the nutrient treatments (Fig. 1D).

Unlike nutrients, mite herbivory had little impact on the majority of the plant growth parameters. At the end of the experiment, there were no significant differences in the number of leaves (Fig. 1A), or ramets (Fig. 1B) produced, or in the lengths of the longest petioles (Fig. 1C) between herbivory treatments. There was also no significant difference in the change in wet biomass (Fig. 1D) between the herbivory treatments, at any of the nutrient concentrations (Table 2).

There was a significant difference (t -value = 3.236, $p = 0.003$) in the leaf chlorophyll content (total leaf chlorophyll (a + b), mean \pm S.D.) between leaves that were subjected to herbivory by a long-established and large mite population (1.47 ± 0.64) and leaves that were not exposed to any mite herbivory (2.15 ± 0.67).

3.2. Effect of nutrients on mite herbivory and development

At the end of the 12-week experiment, there was a significant difference in mite herbivory on leaf 4 between the nutrient treatments ($H_{2,22} = 7.442$, $p = 0.024$), but not on leaf 6 ($H_{2,16} = 1.4$, $p = 0.497$). On leaf 4, mite herbivory was greatest at the high nutrient treatment. However, when comparing mite herbivory between leaf 4 and leaf 6 within the individual nutrient treatments (low, medium or high), there were no significant differences (low $H_{1,12} = 0.534$, $p = 0.465$; medium $H_{1,11} = 3.213$, $p = 0.073$; high $H_{1,12} = 0.086$; $p = 0.769$).

As there were no significant differences in mite herbivory in the individual nutrient treatments between leaves 4 and 6, gallery lengths measured on the leaves were combined. These data were analysed and plotted against time up to week 8 which is approximately two weeks after the first generation of adult mites should have emerged (Delfosse, 1978) (Fig. 2). The average length of galleries was 2.37 ± 0.7 mm, and the longest gallery measured during the experiment was 6.5 mm long. Galleries lengthened most between weeks 4 and 6 in the medium and high nutrient treatments, and between weeks 4 and 8 in the low nutrient treatment (Fig. 2). Based on gallery lengths, mite development was similar in the three nutrient treatments ($F_{2,121} = 1.107$, $p = 0.334$), although mites appeared to develop slightly faster in the high nutrient treatment, where the first generation of adults emerged roughly 6 weeks after the mites were placed onto the plants, while in the medium and low nutrient treatments development was slower and the first generation of adult mites emerged a week later (Fig. 2).

3.3. Impact of herbivory on plant photosynthetic performance and chlorophyll content

Plants that had been exposed to mite herbivory had lowered photosynthetic rates (A) (Fig. 3A). There was a strong positive correlation between the leaf surface area damaged by the mites and the leaf conductance (g_l), the rate of transpiration (E) and the intercellular CO_2 concentration (C_i) (Fig. 3B–D); indicating that mite damage significantly increases g_l , E and C_i . There was a decrease in A as damage increased leaf conductance and C_i (Fig. 3E); indicating some damage to the photosynthetic apparatus.

There was no correlation between F_0 and mite herbivory ($r^2 = 0.001$, $p = 0.897$). However, there was a significant decrease

Table 2

The effect of nutrients and herbivory on water hyacinth growth, revealed by univariate tests of significance for selected growth parameters, at the end of a 12-week experiment where plants were grown in different nutrient concentrations (high, medium and low), and either subjected, or not, to herbivory by *O. terebrantis*. *p*-Values in bold are <0.05 and are statistically significant after a Bonferroni correction for multiple comparisons (Rice, 1989 but see Moran, 2003).

	SS	MS	F	p
Ramet production				
Nutrients	201.13	100.57	26.14	<0.001*
Herbivory	5.60	5.60	1.46	0.241
Nutrients × herbivory	15.38	7.69	1.99	0.160
Leaf production				
Nutrients	43.91	21.96	5.34	0.013*
Herbivory	0.30	0.30	0.07	0.789
Nutrients × herbivory	7.39	3.69	0.89	0.422
Maximum petiole length				
Nutrients	178.58	89.29	55.70	<0.001*
Herbivory	0.92	0.92	0.57	0.458
Nutrients × herbivory	10.24	5.12	3.19	0.062
Change in wet biomass				
Nutrients	0.37	0.19	1.27	0.306
Herbivory	0.27	0.27	1.86	0.191
Nutrients × herbivory	0.39	0.19	1.36	0.283

Degrees of freedom for nutrient treatment = 2, for herbivory treatment = 1 and for the interaction of nutrient treatment and herbivory treatment = 2.

in F_m with an increase in mite herbivory ($r^2 = 0.63$, $p < 0.005$), and this was translated into a decrease in maximum efficiency of PSII photochemistry (Fig. 4A), indicating that mite herbivory had a negative impact on the efficiency with which light absorbed by PSII is converted to chemical energy, i.e. the efficiency at which plastoquinone (Q_A), an electron acceptor, is reduced.

The performance index (PI_{ABS}), which incorporates light energy absorption, the efficiency of Q_A reduction and the conversion of excitation energy to electron transport, decreased as mite herbivory increased (Fig. 4B). The decrease in PI_{ABS} is attributed to the significantly lowered density of functional reaction centres in PSII per cross section of leaf area (Fig. 4C). However, the decrease in PI_{ABS} occurred even though the energy fluxes (ABS/RC , TR_0/RC

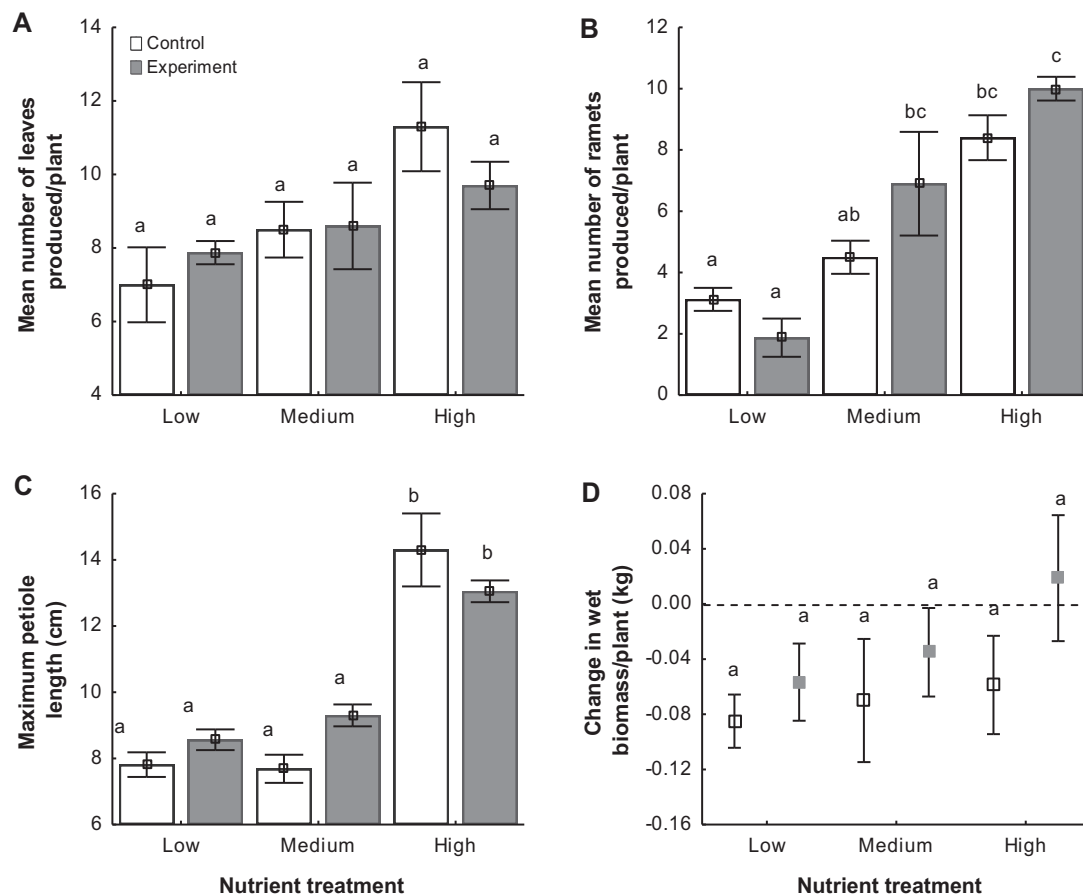


Fig. 1. Differences in water hyacinth growth parameters: (A) the number of leaves, (B) the number of daughter plants (ramets), (C) maximum petiole length and (D) the change in wet weight, at the end of a 12-week experiment, when plants were grown in low, medium or high nutrient treatments, either exposed (experiment, grey bars) or not exposed (control, white bars) to herbivory by *O. terebrantis*. Error bars represent the standard error of the mean. Error bars followed by the same letter are not significantly different (Bonferroni test, $p < 0.05$). In graph D the small box indicates the mean and the whiskers indicate the standard error. $n = 16$ (low), 14 (medium) and 18 (high).

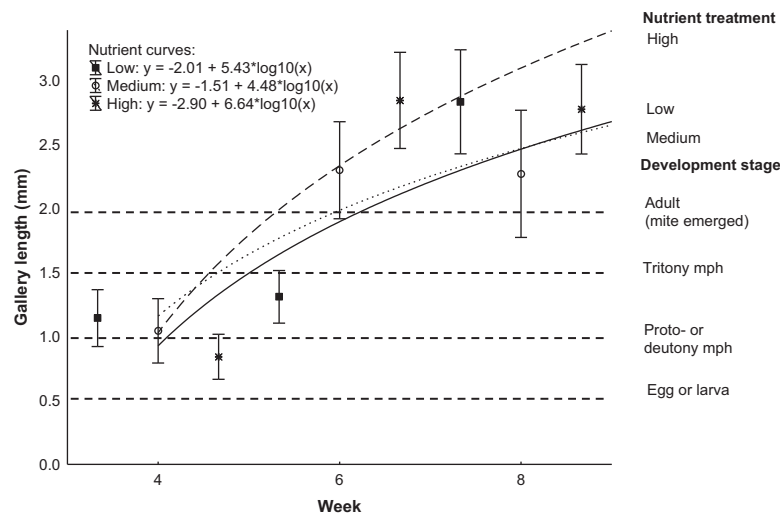


Fig. 2. Egg to adult development of *O. terebrantis*, based on the length of galleries (Delfosse, 1978) measured on water hyacinth leaves grown in low, medium or high nutrient treatments. Logarithmic curves were fitted to the data. Error bars represent the standard error of the mean. $n = 16$ (low), 14 (medium) and 18 (high).

and ET_0/RC) per PSII reaction centre increased as mite herbivory increased (ABS/RC , $r^2 = 0.33$, $p = 0.008$; TR_0/RC , $r^2 = 0.35$, $p = 0.006$; ET_0/RC , $r^2 = 0.03$, $p = 0.427$). This indicates that mite herbivory increased the rates of photon absorption (ABS/RC), the maximum rates at which excited electrons are trapped by the reaction centres resulting in Q_A reduction (TR_0/RC), and the rates of electron transport (ET_0/RC), respectively.

There was no correlation between the percentage of leaf area damaged by mite feeding and the leaf chlorophyll content ($r^2 = 0.01$, $p = 0.661$). This was surprising because leaf biomass decreased as mite damaged to leaves increased ($r^2 = 0.26$, $p = 0.017$). However, the lack of correlation is likely to be because the one measure is per unit leaf and the other per unit leaf area.

4. Discussion

These results demonstrate that water hyacinth growth is strongly influenced by water nutrient levels, in agreement with other work (Gossett and Norris, 1971; Watson and Brochier, 1988; Reddy et al., 1989, 1990; Coetzee et al., 2007). Plants grown in water with a high N and P content were healthy with tall petioles, and generally produced more leaves and daughter plants than plants grown in water with lower N and P contents. Plants grown at low nutrients had short, bulbous petioles, and did not survive the winter season following the experiment.

Herbivory by *O. terebrantis* had little effect on water hyacinth growth. Heard and Winterton (2000) and Coetzee et al. (2007) found that herbivory by the weevils *Neochetina bruchi* Hustache and *N. eichhorniae* Warner (Coleoptera: Curculionidae), and by the mirid *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae), respectively, resulted in shorter petioles and fewer ramets being produced. However, many plants are known to compensate for damage resulting from insect herbivores. For example, Briese et al. (2002) noted that slight compensation by a plant may take place where low densities of agents occur on a host plant. The densities of mites used in this study were lower than that usually found in field situations (Byrne et al., 2010) and it is therefore possible that plants damaged by mites initially compensate by increasing their leaf turn-over. Other studies have found similar plant-compensatory effects, for example, when the buds of *Salvinia molesta* (D.S. Mitchell) (Salviniaceae) are attacked by weevils *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae),

new and previously dormant buds are activated to grow, initially negating the damage (Room, 1990).

Whilst *O. terebrantis* may not decrease water hyacinth growth on its own, the use of multiple agents has been found to increase the stress on the plant and reduce its growth, more so than when a single agent is used (Delfosse, 1978; Caunter and Mohamed, 1990; Moran, 2005; Ajuonu et al., 2009). The integration of biocontrol agents with herbicides has also shown synergistic negative effects on water hyacinth growth (Charudattan, 1986; Van and Center, 1994; Center et al., 1999; Jadhav et al., 2008) and there is thus potential for *O. terebrantis* to be used as part of an integrated management plan for the control of the plant. Furthermore, the competitive ability of a plant may be influenced by agent herbivory, and the synergistic interactions between insect herbivory and plant competition should be considered when testing the efficacy of a biocontrol agent (Van et al., 1998; Center et al., 2001; Coetzee et al., 2005). Insect interaction studies between *O. terebrantis* and other water hyacinth biocontrol agents are currently underway.

The health, and therefore nutrient status of plants, has a direct impact on insect development and survival (Strong, 1984; Room, 1990; Schoonhoven et al., 1998). Mite numbers were not recorded because of the ease with which mites are knocked off plants during handling. However, the greatest number of mites was always observed on plants grown in the high nutrient treatment, which also had the greatest feeding damage. Heard and Winterton (2000) found that *N. bruchi* produced more offspring on plants grown at a high nutrient concentration. Similarly, Ripley et al. (2006) recorded a significant rise in *E. catarinensis* adult and nymph numbers with an increasing nutrient supply. In this study, the first generation of adults emerged a week earlier on plants in the high nutrient treatment than on plants in the medium and low nutrient treatments, suggesting that the mites develop faster on plants grown at high nutrients.

Despite the fact that mite herbivory had no detectable impact on water hyacinth growth parameters, the performance of photosystem II (PSII) decreased in plants with higher levels of mite damage, as indicated by the lowered CO_2 assimilation rate and altered fluorescence emission. Mite herbivory had a direct negative impact on PSII efficiency. This could be seen in the reduction in F_v/F_m below 0.83, a value below which plants are considered to be "unhealthy", indicating a negative effect on photochemistry (Baker, 2008). Since the original fluorescence data were used to work out the JIP-test parameters, the reduction in F_v/F_m was reflected in the reduction

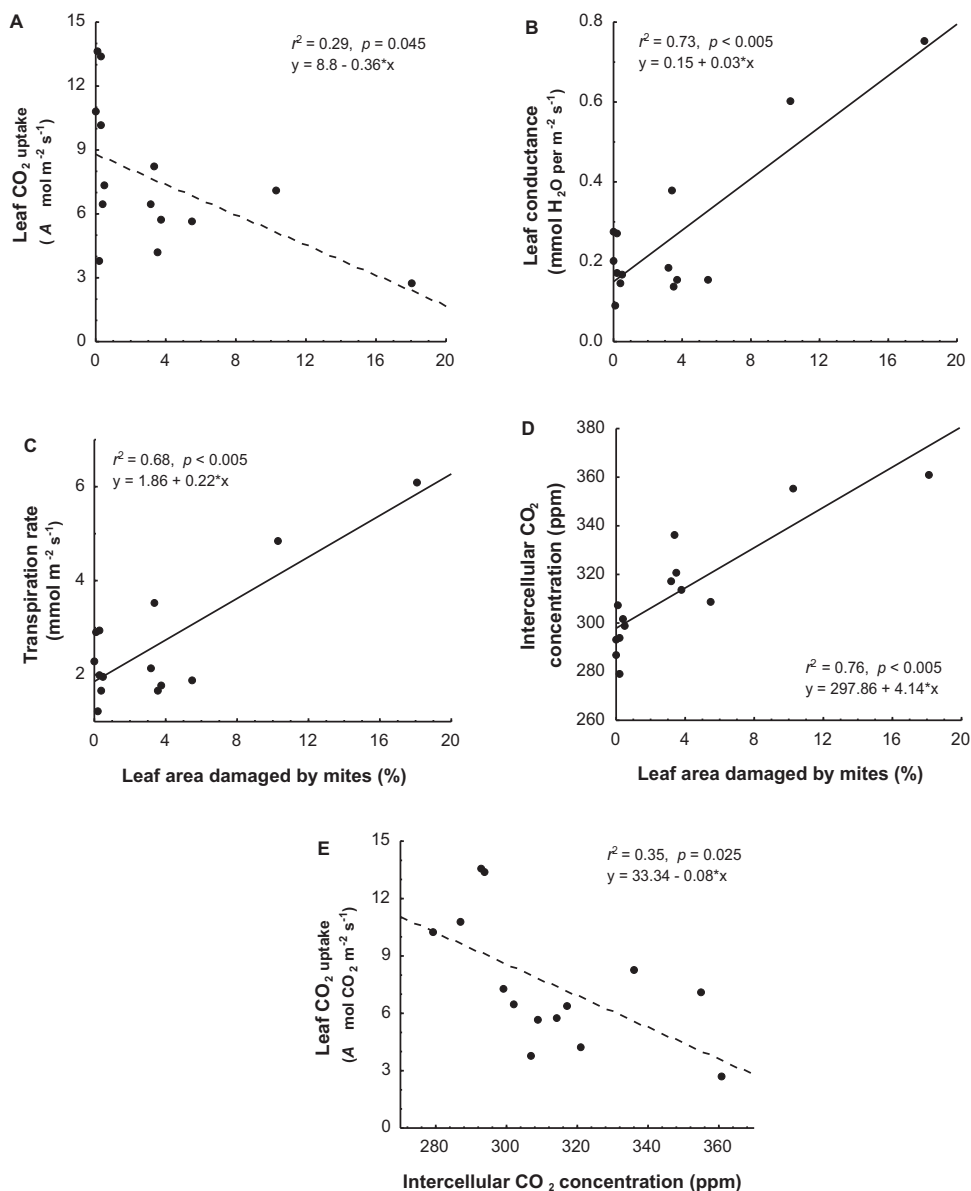


Fig. 3. Photosynthetic gas exchange parameters of water hyacinth leaves ($n = 14$) when the plants were exposed to varying levels of *O. terebrantis* feeding: (A) photosynthetic rates (A), (B) leaf conductance rates (g_l), (C) transpiration rates (E), and (D) intercellular CO_2 concentration (C_i). (E) The relationship between the uptake of CO_2 (A) and the intercellular CO_2 concentration (C_i).

of the performance index (PI_{ABS}), which is a sensitive test of the physiological state of the plant (Strasser et al., 2004). The reduction in PI_{ABS} was as a result of the reduced density of functional PSII reaction centres (RCs). A decrease in F_v/F_m is often explained by the inactivation of the RCs in PSII, since changes in F_v/F_m exhibit the same trends as changes in the density of RCs (Lu and Vonshak, 1999). These results are in agreement with those of Ripley et al. (2006) who noted a similar decrease in the density of RCs of PSII on water hyacinth exposed to *E. catarinensis* relative to insect-free plants.

The effect of mite herbivory on chl-*a* fluorescence parameters was reflected in the altered gas exchange parameters. The negative effect of arthropod herbivory on water hyacinth photosynthetic rate (A) has previously been observed on plants damaged by *N. eichhorniae* (Ripley et al., 2008), and by *E. catarinensis* (Ripley et al., 2006). Similarly, this study showed that water hyacinth plants that have been damaged by *O. terebrantis* exhibit a decreased photosynthetic rate, and this rate declined as the mite damage on the

laminae increased. However, the correlation between photosynthetic rate and the leaf surface area damaged by the mite was not strong ($r^2 = 0.29$) due to variability in the photosynthetic rates of the control leaves. This may be as a result of the leaves being taken from plants from different populations. Reductions in net photosynthesis may be attributed to stomatal limitations, i.e. when stomata impose resistance to the diffusion and the uptake of CO_2 , for example when plants are water-stressed (Thomas and Turner, 2001; Souza et al., 2004), saline-stressed (Jiang et al., 2006) or temperature-stressed (Fan et al., 2010). This is in contrast to the present study where A decreased despite a great increase in leaf stomatal conductance (g_l) and intercellular CO_2 concentration (C_i) as mite herbivory increased. This is not surprising because of the way in which the mites damage the laminae – the oviposition holes created by the females, and specifically the emergence holes created by the mites as they exit the galleries once they reach the adult stage, create large openings in the cuticle, which explains the increase in g_l , which in turn explains the observed increase in transpiration (E) and C_i .

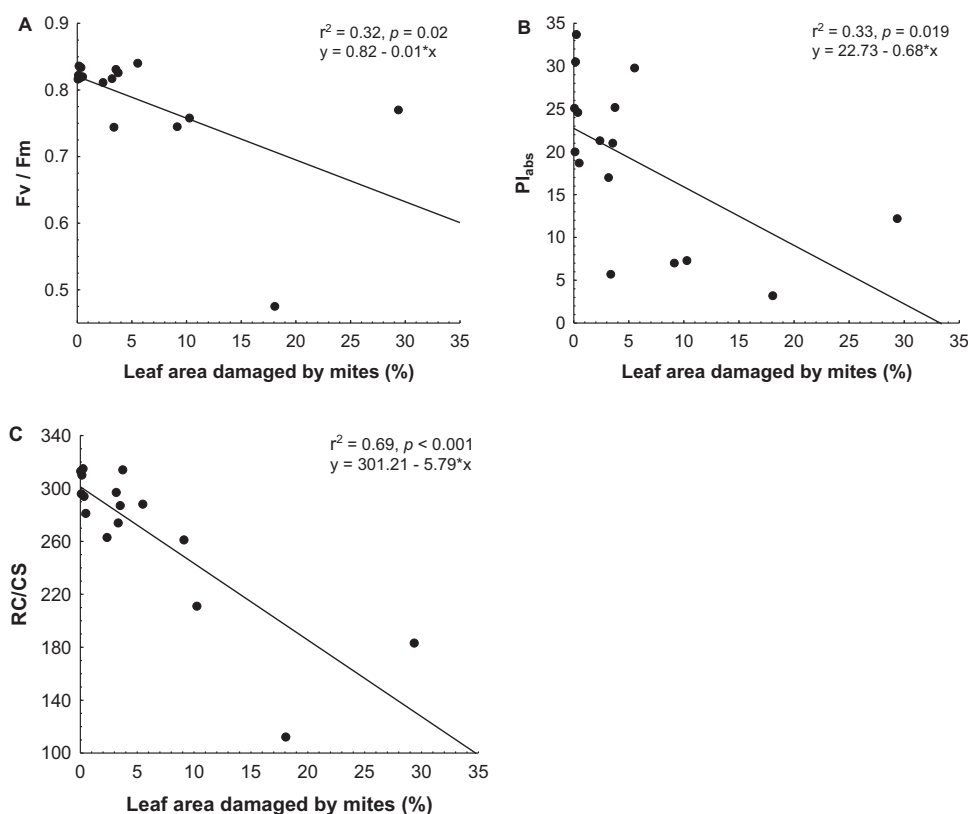


Fig. 4. Fluorescence parameters of dark-adapted water hyacinth leaves ($n = 14$) when the plants were exposed to varying levels of *O. terebrantis* feeding: (A) efficiency of photosystem II (F_v/F_m), (B) the performance index (PI_{ABS}) and (C) the density of functional reaction centres in photosystem II found per cross section of leaf area.

in this study. Consequently, the decline in photosynthesis implies direct effects on photosynthetic metabolism that are independent of g_i and the supply of CO_2 . In addition, as the mite nymphs eat away at the inner leaf tissue during their development, they create openings in the tissue and make the leaf more porous, thus making the movement of CO_2 inside the laminae less restricted. This could explain the observed increase in C_i in the leaves damaged by mites.

The chlorophyll content of leaves did not change with the varying levels of mite herbivory examined in the second part of this study, and this is not surprising considering that the average amount of leaf area that was damaged by the mites was only about 15% of the entire leaf area. At relatively low levels of mite herbivory, the chlorophyll content of water hyacinth leaves was not affected, but damage caused by mite feeding did have an effect on the light reaction and photosynthetic rate. In contrast, herbivory by a long-established and large mite population greatly decreased the chlorophyll content of leaves. This suggests that a mite population that is left to multiply undisturbed for a number of years can reach high enough numbers to significantly impact water hyacinth leaf chlorophyll content. In South Africa as many as 200 mites per leaf are found during summer months at sites such as Mbozambo Swamp (Marlin, 2011). In general, however, water hyacinth normally exhibits no significant correlation between photosynthetic rate and chlorophyll content (Patterson and Duke, 1979), and this was also observed at low mite densities. These results are similar to those of Macedo et al. (2003) who found that the aphid, *Aphis glycines* Mutsamura (Hemiptera: Aphididae), at densities of >20 individuals per leaflet had an effect on the photosynthetic rate of soybean but not on the chlorophyll content. Similarly, Tomczyk and Kropczyńska (1984) found that changes in the chlorophyll content of chrysanthemum plants damaged by the mite *Tetranychus urticae* Koch

(Acari: Tetranychidae) were not proportional to changes in photosynthesis. A reduction in chlorophyll content due to herbivory has been related to reduced photosynthetic rates in other studies (Cockfield et al., 1987; Buntin et al., 1993; Haile and Higley, 2003; Ripley et al., 2006) but the damage caused by the insects or mites in those studies was usually greater than that caused by *O. terebrantis* in the present study. The fact that there was no correlation between herbivory and F_0 , or herbivory and chlorophyll content, suggests that the plant is compensating for mite feeding by increasing the chlorophyll content of the leaf area not damaged by mites.

In this study, *O. terebrantis* had little effect on water hyacinth at a plant growth level (e.g. petiole lengths, leaf production). However, plant growth level changes in water hyacinth are usually only negatively impacted under very severe herbivory stress, low temperatures (Byrne et al., 2010) or low water nutrient conditions (Coetzee et al., 2007). The subtle effects of herbivory may not be seen at the 'whole-plant' level, but can be detected at the cellular level by examining the plant's physiology. It is therefore important to quantify the plant's physiological status to better understand the mechanism behind plant responses to agent herbivory. It then becomes possible to correlate changes in the physiology of the plant, as caused by herbivory, to potential plant level changes. Water hyacinth physiological parameters (i.e. net photosynthetic rate and functioning of PS II) were negatively affected by mite herbivory as predicted, even at the very low mite densities used in the lab experiments. Consequently, the negative impact of *O. terebrantis* herbivory on water hyacinth's photosynthetic efficiency impacts plant health and, at high levels of mite herbivory, is likely to impact plant growth. Interestingly, the reduction in plant performance appears to be independent of leaf chlorophyll content, implying that the reduction of the photosynthetic rate and the light reaction performance is not simply due to the reduced leaf area, i.e. reduced

chlorophyll content. The results of this study therefore add to recent literature which supports the hypothesis that herbivory decreases a plant's photosynthetic ability more so than can be explained by the direct removal of photosynthetic surface area (Zangerl et al., 2002; Aldea et al., 2005; Ripley et al., 2008; Nabity et al., 2009).

In conclusion, at high densities *O. terebrantis* might have an impact on water hyacinth populations, and particularly if used in combination with other biocontrol agents.

Contributors

Conceived and designed the experiments: MPH, MJB and DM. *Performed experiments:* DM and AJS. *Analysed the data:* DM, BSR and AJS. *Prepared the article:* DM, MPH, MJB and BSR. All authors have approved the final article.

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References

- Ajuonu, O., Byrne, M., Hill, M., Neuenschwander, P., Korie, S., 2009. The effect of two biological control agents, the weevil *Neochetina eichhorniae* and the mirid *Ecritotarsus catarinensis* on water hyacinth, *Eichhornia crassipes*, grown in culture with water lettuce, *Pistia stratiotes*. *BioControl* 54, 155–162.
- Aldea, M., Hamilton, J.G., Resti, J.P., Zangerl, A.R., Berenbaum, M.R., DeLucia, E.H., 2005. Indirect effects of insect herbivory on leaf gas exchange in soybean. *Plant, Cell and Environment* 28, 402–411.
- Baker, N.R., 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annual Review of Plant Biology* 59, 89–113.
- Baker, N.R., Harbinson, J., Kramer, D.M., 2007. Determining the limitations and regulation of photosynthetic energy transduction in leaves. *Plant, Cell and Environment* 30, 1107–1125.
- Bownes, A., Hill, M.P., Byrne, M.J., 2010. Assessing density-damage relationships between water hyacinth and its grasshopper herbivore. *Entomologia Experimentalis et Applicata* 137, 246–254.
- Briese, D.T., Thomann, T., Vitou, J., 2002. Impact of the rosette crown weevil *Trichosirocalus briesei* on the growth and reproduction of *Onopordum thistles*. *Journal of Applied Ecology* 39, 688–698.
- Buntin, G.D., Gilbert, D.A., Oetting, R.D., 1993. Chlorophyll loss and gas exchange in tomato leaves after feeding injury by *Bemisia tabaci* (Homoptera: Aleyrodidae). *Journal of Economic Entomology* 86, 517–522.
- Byrne, M., Hill, M., Robertson, M., King, A., Jadhav, A., Katembo, N., Wilson, J., Brudvig, R., Fisher, J., 2010. Integrated management of water hyacinth in South Africa: development of an integrated management plan for water hyacinth control, combining biological control, herbicidal control and nutrient control, tailored to the climatic regions of South Africa. Water Research Council Report No. TT 454/10.
- Carignan, R., Neiff, J.J., 1994. Limitation of water hyacinth by nitrogen in Subtropical lakes of the Paraná floodplain (Argentina). *Limnology and Oceanography* 39, 439–443.
- Caunter, I.G., Mohamed, S., 1990. The effect of *Neochetina eichhorniae* and *Myrothecium roridum* alone and in combination for the initial biological control of water hyacinth. *Journal of Plant Protection in the Tropics* 7, 133–140.
- Center, T.D., 1981. Biological control and its effects on production and survival of water hyacinth leaves. In: Delfosse, E.S. (Ed.), *Proceedings of the 5th Symposium on the Biological Control of Weeds*, Brisbane, Australia, July 22–29, 1980. Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia, pp. 393–410.
- Center, T.D., Van, T.K., Hill, M.P., 2001. Can competition experiments be used to evaluate the potential efficacy of new water hyacinth biological control agents? In: Julien, M.H., Hill, M.P., Center, T.D., Ding, J. (Eds.), *Proceedings of the Second Global Working Group Meeting for the Biological and Integrated Control of Water Hyacinth*, Beijing, China, October 9–12, 2000, pp. 77–81.
- Center, T.D., Dray, F.A., Jubinsky, G.P., Grodowitz, M.J., 1999. Biological control of water hyacinth under conditions of maintenance management: can herbicides and insects be integrated? *Environmental Management* 23, 241–256.
- Charudattan, R., 1986. Integrated control of water hyacinth (*Eichhornia crassipes*) with a pathogen, insects, and herbicides. *Weed Science* 34, 26–30.
- Cockfield, S.D., Potter, D.A., Houtz, R.L., 1987. Chlorosis and reduced photosynthetic CO₂ assimilation of *Euonymus fortunei* infested with *Euonymus scale* (Homoptera: Diaspididae). *Environmental Entomology* 16, 1314–1318.
- Coetzee, J.A., Center, T.D., Byrne, M.J., Hill, M.P., 2005. Impact of the biocontrol agent *Ecritotarsus catarinensis*, a sap-feeding mirid, on the competitive performance of water hyacinth, *Eichhornia crassipes*. *Biological Control* 32, 90–96.
- Coetzee, J.A., Byrne, M.J., Hill, M.P., 2007. Impact of nutrients and herbivory by *Ecritotarsus catarinensis* on the biological control of water hyacinth, *Eichhornia crassipes*. *Aquatic Botany* 86, 179–186.
- Cordo, H.A., DeLoach, C.J., 1976. Biology of the water hyacinth mite *Orthogalumna terebrantis* in Argentina. *Weed Science* 24, 245–249.
- Delfosse, E.S., 1978. Effect on waterhyacinth of *Neochetina eichhorniae* (Col.: Curculionidae) combined with *Orthogalumna terebrantis* (Acari: Galumnidae). *Entomophaga* 23, 379–387.
- Dere, S., Günes, T., Sivaci, R., 1998. Spectrophotometric determination of chlorophyll – a, b and total carotenoid content of some algae species using different solvents. *Turkish Journal of Botany* 22, 13–17.
- Fan, P.G., Li, L.S., Duan, W., Li, W.D., Li, S.H., 2010. Photosynthesis of young apple trees in response to low sink demand under different air temperatures. *Tree Physiology* 30, 313–325.
- Farquhar, G.D., von Caemmerer, S., 1982. Modelling of photosynthetic response to environmental conditions. In: Lange, O.L., Nobel, P.S., Osmond, C.B., Zeigler, H. (Eds.), *Encyclopedia of Plant Physiology*, new series 12A, pp. 549–587.
- Gopal, B., 1987. *Water Hyacinth*. Aquatic Plant Studies, vol. 1. Elsevier Science Publishing Co., New York.
- Gossett, D.R., Norris Jr., W.E., 1971. Relationship between nutrient availability and content of nitrogen and phosphorus in tissues of the aquatic macrophyte, *Eichhornia crassipes* (Mart.) Solms. *Hydrobiologia* 38, 15–28.
- Haile, F.J., Higley, L.G., 2003. Changes in soybean gas-exchange after moisture stress and spider mite injury. *Environmental Entomology* 32, 433–440.
- Heard, T.A., Winterton, S.T., 2000. Interactions between nutrient status and weevil herbivory in the biological control of water hyacinth. *Journal of Applied Ecology* 37, 117–127.
- Hill, M.P., Cilliers, C.J., 1999. A review of the arthropod natural enemies, and factors that influence their efficacy, in the biological control of the water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae) in South Africa. In: Olckers, T., Hill, M.P. (Eds.), *Biological control of weeds in South Africa (1990–1998)*. African Entomology Memoir 1. The Entomological Society of South Africa, Pretoria, pp. 103–112.
- 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., Ding, J. (Eds.), *Proceedings of the Second Global Working Group Meeting for the Biological and Integrated Control of Water Hyacinth*, Beijing, China, October 9–12, 2000, pp. 33–38.
- Jadhav, A., Hill, M.P., Byrne, M.J., 2008. Identification of a retardant dose of glyphosate with potential for integrated control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach. *Biological Control* 47, 154–158.
- Jiang, Q., Roche, D., Monaco, T.A., Durham, S., 2006. Gas exchange, chlorophyll fluorescence parameters and carbon isotope discrimination of 14 barley genetic lines in response to salinity. *Field Crops Research* 96, 269–278.
- Koerselman, W., Meuleman, A.F.M., 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* 33, 1441–1450.
- Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* 148, 350–382.
- Lu, C., Vonshak, A., 1999. Photoinhibition of outdoor *Spirulina platensis* cultures assessed by polyphasic chlorophyll fluorescence transients. *Journal of Applied Phycology* 11, 355–359.
- Macedo, T.B., Bastos, C.S., Higley, L.G., Ostlie, K.R., Madhavan, S., 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. *Journal of Economic Entomology* 96, 188–193.
- Marlin, D., 2011. The role of the mite *Orthogalumna terebrantis* in the biological control programme for water hyacinth, *Eichhornia crassipes*, in South Africa. Unpublished Ph.D. Thesis, Rhodes University, Grahamstown, South Africa.
- McNaughton, S.J., 1983. Compensatory plant growth as a response to herbivory. *Oikos* 40, 329–336.
- Moran, M.D., 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* 100, 403–405.
- Moran, P.J., 2006. Water nutrients, plant nutrients, and indicators of biological control on water hyacinth at Texas field sites. *Journal of Aquatic Plant Management* 44, 109–114.
- Moran, P.J., 2005. Leaf scarring by the weevils *Neochetina eichhorniae* and *N. bruchi* enhances infection by the fungus *Cercospora piaropi* on waterhyacinth, *Eichhornia crassipes*. *BioControl* 50, 511–524.
- Myers, J.H., Risley, C., Eng, R., 1990. The ability of plants to compensate for insect attack: why biological control of weeds with insects is so difficult. In: Delfosse,

- E.S. (Ed.), Proceedings of the 7th International Symposium on the Biological Control of Weeds. Rome, Italy, March 6–11, 1988, pp. 67–73.
- Nabity, P.D., Zavala, J.A., DeLucia, E.H., 2009. Indirect suppression of photosynthesis on individual leaves by arthropod herbivory. *Annals of Botany* 103, 655–663.
- Patterson, D.T., Duke, S.O., 1979. Effect of growth irradiance on the maximum photosynthetic capacity of water hyacinth [*Eichhornia crassipes* (Mart.) Solms]. *Plant and Cell Physiology* 20, 177–184.
- Peterson, R.K.D., Shannon, C.L., Lenssen, A.W., 2004. Photosynthetic responses of legume species to leaf-mass consumption injury. *Environmental Entomology* 33, 450–456.
- Reddy, K.R., Agami, M., Tucker, J.C., 1990. Influence of phosphorus on growth and nutrient storage by water hyacinth (*Eichhornia crassipes* (Mart.) Solms) plants. *Aquatic Botany* 37, 355–365.
- Reddy, K.R., Agami, M., Tucker, J.C., 1989. Influence of nitrogen supply rates on growth and nutrient storage by water hyacinth (*Eichhornia crassipes*) plants. *Aquatic Botany* 36, 33–43.
- Rice, W.R., 1989. Analyzing tables of statistical tests. *Evolution* 43, 223–225.
- 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. *African Entomology* 16, 140–142.
- 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 (*Eccritotarsus catarinensis*) biocontrol. *Biological Control* 39, 392–400.
- Room, P.M., 1990. Ecology of a simple plant–herbivore system: biological control of *Salvinia*. *Trends in Ecology and Evolution* 5, 74–79.
- Schoonhoven, L.M., Jermy, T., van Loon, J.J.A., 1998. *Insect–Plant Biology*. Chapman and Hall, London.
- Schultz, J.C., 2002. How plants fight dirty. *Nature* 416, 267–268.
- Silveira-Guido, A., 1965. Natural enemies of weed plants. Final report on PL-480 project S9-CR-1 (January 1962 to November 1965), Universidad de la Republica, Facultad de Agronomia, Montevideo, Uruguay.
- Snedecor, G.W., Cochran, W.G., 1980. *Statistical Methods*, 7th ed. Iowa State University Press, Ames.
- Stanley, J.N., Julien, M.H., Center, T.D., 2007. Performance and impact of the biological control agent *Xubida infusella* (Lepidoptera: Pyralidae) on the target weed *Eichhornia crassipes* (waterhyacinth) and on a non-target plant, *Pontederia cordata* (Pickerelweed) in two nutrient regimes. *Biological Control* 40, 298–305.
- Strasser, R.J., Govindjee, 1992. The F_0 and the O–J–I–P fluorescence rise in higher plants and algae. In: Argyroudi-Akoyunoglou, J.H. (Ed.), *Regulation of Chloroplast Biogenesis*. Plenum Press, New York.
- Strasser, B.J., Strasser, R.J., 1995. Measuring fast fluorescence transients to address environmental questions: the JIP test. In: Mathis, P. (Ed.), *Photosynthesis: From Light to Biosphere*, vol. 5. Kluwer Academic Publishers, The Netherlands.
- Strasser, R.T., Tsimilli-Michael, M., Srivastava, A., 2004. Analysis of the chlorophyll-a fluorescence transient. In: Papageorgiou, G.C., Govindjee (Eds.), *Chlorophyll Fluorescence: A Signature of Photosynthesis*, vol. 19. Kluwer Academic Publishers, The Netherlands.
- Strong Jr., D.R., 1984. Exorcising the ghost of competition past: phytophagous insects. In: Strong Jr., D.R., Simberloff, D., Abele, L.G., Thistle, A.B. (Eds.), *Ecological Communities: Conceptual Issues and the Evidence*. Princeton University Press, Surrey, United Kingdom.
- Souza, R.P., Machado, E.C., Silva, J.A.B., Lagôa, A.M.M.A., Silveira, J.A.G., 2004. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. *Environmental and Experimental Botany* 51, 45–56.
- Thomas, D.S., Turner, D.W., 2001. Banana (*Musa* sp.) leaf gas exchange and chlorophyll fluorescence in response to soil drought, shading and lamina folding. *Scientia Horticulturae* (Amsterdam) 90, 93–108.
- Tomczyk, A., Kropczyńska, D., 1984. Feeding effects of *Tetranychus urticae* Koch on the physiology of some plants. In: Griffiths, D.A., Bowman, C.E. (Eds.), *Acarology VI*, vol. 2. Ellis Horwood, Ltd., Chichester, England, pp. 740–746.
- Trumble, J.T., Kolodny-Hirsch, D.M., Ting, I.P., 1993. Plant compensation for arthropod herbivory. *Annual Review of Entomology* 38, 93–119.
- Van, T.K., Center, T.D., 1994. Effect of paclobutrazol and waterhyacinth weevil (*Neochetina eichhorniae*) on plant growth and leaf dynamics of waterhyacinth (*Eichhornia crassipes*). *Weed Science* 42, 665–672.
- Van, T.K., Wheeler, G.S., Center, T.D., 1998. Competitive interactions between *Hydrilla* (*Hydrilla verticillata*) and *Vallisneria* (*Vallisneria americana*) as influenced by insect herbivory. *Biological Control* 11, 185–192.
- Watson, M.A., Brochier, J., 1988. The role of nutrient levels on flowering in water hyacinth. *Aquatic Botany* 31, 367–372.
- Watt, M.S., Whitehead, D., Kriticos, D.J., Gous, S.F., Richardson, B., 2007. Using a process-based model to analyse compensatory growth in response to defoliation: simulating herbivory by a biological control agent. *Biological Control* 43, 119–129.
- Wirf, L.A., 2006. The effect of manual defoliation and *Macaria pallidata* (Geometridae) herbivory on *Mimosa pigra*: implications for biological control. *Biological Control* 37, 346–353.
- Xie, Y., Wen, M., Yu, D., Li, I., 2004. Growth and resource allocation of water hyacinth as affected by gradually increasing nutrient concentrations. *Aquatic Botany* 79, 257–266.
- Zangerl, A.R., Hamilton, J.G., Miller, T.J., Crofts, A.R., Oxborough, K., Berenbaum, M.R., de Lucia, E.H., 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. *Proceedings of the National Academy of Sciences of the United States of America* 99, 1088–1091.