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INVASIVE SPECIES II

Plant–herbivore–parasitoid interactions in an experimental freshwater tritrophic system: higher trophic levels modify competitive interactions between invasive macrophytes

Grant Douglas Martin · Julie Angela Coetzee · Stephen Compton

Abstract Natural enemies are known to modify competitive hierarchies among terrestrial plants. Here we examine whether the same applies to freshwater systems. *Lagarosiphon major* (Hydrocharitaceae) is a submerged aquatic macrophyte, indigenous to South Africa. Outside its native range, it outcompetes with indigenous submerged species and degrades aquatic habitats. *Hydrellia lagarosiphon* (Diptera: Ephydridae) is the most abundant and ubiquitous herbivore associated with *L. major* in South Africa and is a potential biological control agent elsewhere. *Chaenusa anervata* (Hymenoptera: Braconidae: Alysiinae) is its main parasitoid. We generated an experimental system involving one, two or three trophic levels to monitor variation in the competitive ability of *L. major* relative to that of *Myriophyllum spicatum* (Haloragaceae), a second submerged macrophyte that can also be invasive. Using inverse linear models to monitor competition, we found that herbivory by *H. lagarosiphon* greatly reduced the competitive ability of *L. major*. Addition of the wasp at typical field densities halved the impact of herbivory and re-established the competitive advantage of *L. major*. Our results demonstrate how multitrophic interactions modify relative competitive abilities among aquatic plants, emphasize the significance of higher tropic levels in these systems and illustrate how parasitoids can reduce the effectiveness of insects released as biocontrol agents.

Keywords Competition · Herbivory · *Lagarosiphon major* · *Myriophyllum spicatum* · Parasitoid · Tritrophic interactions

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Introduction

Aquatic macrophytes and their associated taxa are fundamental to the structure and functioning of freshwater ecosystems (Jones et al., 1997). Aquatic plants alter the chemical and physical conditions of their surroundings (Duarte, 2000; Kufel & Kufel, 2002), they form the basis of herbivorous and detrital

food webs, and their physical structure provides shelter and microhabitats for numerous other organisms (Carpenter & Lodge, 1986; Cyr & Downing, 1988; Cheruvelil et al. 2002; Ferreiro et al. 2011). The composition and structure of aquatic macrophytes within freshwater systems are driven by a number of top-down and bottom-up or combination of influences (Speight et al., 2008). The bottom-up abiotic factors driving aquatic macrophyte assemblages are usually associated with the limnology of the water body, including geomorphology, climate and hydrology (Titus & Adams, 1979; Smart & Barko, 1985; Dawson, 1988; Barko et al., 1991; Champion & Tanner, 2000; Lacoul & Freedman 2006; Mackay, 2007; Loo et al., 2009), whereas top-down biotic factors include herbivory, competition and disease (Hofstra et al., 1999; Van et al., 1998; Lacoul & Freedman 2006). Harvey et al. (2010) argue that plant community structure and function are also influenced by the biotic effect of natural enemies of herbivores, particularly parasitoids of herbivorous insects; however, this interaction has received very little attention in aquatic ecology studies.

Parasitoids of herbivorous insects are almost ubiquitous and parasitoid–herbivore–plant tritrophic interactions are an integral, though not self-contained, part of most terrestrial food webs (Rosenheim, 1998). Parasitoids kill their host insects and have a role in the regulation of herbivorous insect populations, but do not necessarily directly benefit their plant associates, because many parasitoids do not immobilize their hosts immediately and this allows the herbivores to continue damaging the plants (Van Loon et al., 2000). Consequently, and depending on parasitoid feeding behaviour and the responses of their hosts, parasitoids may increase or decrease the extent of feeding by parasitized hosts (Guillot & Vinson, 1973; Gómez & Zamora, 1994; Hoballah & Turlings, 2001; Hasan & Ansari, 2012; Gols et al., 2015; Xi et al., 2015), and the extent of any benefits from parasitoids can also vary according to the status of individual plants (Wilson & Woods, 2015). Although parasitoids do not necessarily reduce the damage generated by the individual hosts they have parasitized, they do nonetheless reduce the numbers of herbivores in subsequent generations, and across larger temporal and spatial scales, contribute to the regulation of herbivorous insect populations at levels that would otherwise be more damaging to their host plants (Murdoch, 1994;

Balmer et al., 2013). Parasitoids of herbivorous insects feeding on aquatic macrophytes are taxonomically diverse and often highly specialized, especially if their hosts develop on submerged plant parts (Corbet, 1999; Querino & Hamada, 2009; Kula, 2009). Although the influence of parasitoids on submerged macrophyte herbivores has been recorded in the field, the resulting influence on plant community structure has not been quantified.

For example, in the USA in the absence of herbivores, the invasive aquatic macrophyte *Hydrilla verticillata* (L.f.) (Royle) (Hydrocharitaceae) outcompetes an indigenous species (*Vallisneria americana* Michx (Hydrocharitaceae)), but its competitive dominance is greatly reduced in the presence of an introduced leaf-mining fly, *Hydrellia pakistanae* Deonier (Diptera: Ephydridae) (Van et al., 1998). However, a native parasitoid *Trichopria columbiana* (Ashmead) (Hymenoptera: Diapriidae) has expanded its host range to include introduced *Hydrellia* species, including *H. pakistanae* (Harms & Grodowitz, 2011). This parasitoid can have a significant impact on *Hydrellia* spp. populations (Coon et al., 2014) and seems likely to reduce the impact of these biological control agents on *H. verticillata* in North America, but the extent of this has not been measured.

Lagarosiphon major (Ridley) Moss (Hydrocharitaceae) is a widely invasive submerged macrophyte native to South Africa. It is a strong competitor that can displace species such as *Myriophyllum spicatum* L. (Haloragaceae) in its adventive range (Titus et al., 1975; Agami & Waisel, 1985; Rattray et al., 1994; James et al., 1999; Hofstra et al., 1999). In herbivore-free environments, intraspecific competition has a far greater impact on the productivity of *L. major* than competition with other species such as *M. spicatum* (Martin & Coetzee, 2014). Its competitive dominance is a result of rapid growth, which allows the plant to form a dense canopy that denies other species access to nutrients and light (Rattray et al., 1994; Caffrey et al., 2010).

Like *H. verticillata*, *L. major* is a host plant for *Hydrellia* spp. leaf-mining flies. In an attempt to investigate a submerged macrophyte tritrophic system, we generated an experimental system comprising *L. major*, its leaf-mining fly, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae) and an associated parasitoid wasp, *Chaenusa anervata* Achterberg (Hymenoptera: Braconidae). We then compared the

competitive ability of *L. major* when growing with a second macrophyte, *M. spicatum,* in the presence or absence of the herbivore and the parasitoid.

Study species

Lagarosiphon major is a submerged macrophyte native to sub-Saharan Africa (Symoens & Triest, 1983). Although indigenous in southern Africa, it is often regarded as a noxious weed because it proliferates in man-made impoundments (Obermeyer, 1964). Widely introduced outside Africa, dense infestations of the plant readily outcompete indigenous submerged species and can alter the ecology of freshwater systems (Rattray et al., 1994; Caffrey et al., 2010).

Myriophyllum spicatum is a submerged macrophyte present in South Africa, but is indigenous to Europe, Asia and North Africa (Smith & Barko, 1990; Weyl et al., 2016). *Myriophyllum spicatum* can also outcompete other macrophytes for light and nutrients and negatively affects aquatic biodiversity (Smith & Barko, 1990; Madsen et al., 1991). It is considered the most important North American waterweed, where millions of dollars are spent annually on its control (Smith & Barko, 1990).

Hydrellia lagarosiphon is the most widespread and abundant invertebrate herbivore associated with *L. major* in South Africa (Baars et al., 2010). Adults move on the surface of the water where they lay eggs on protruding shoot tips of the plant (Martin et al., 2013). The larvae move between leaves, where they feed between the upper and lower leaf epidermal layers, and produce mines. Pupation occurs within the leaves. Larval feeding reduces the plant's ability to photosynthesize and a single *H. lagarosiphon* larva can destroy approximately 20 leaves, with the result that fewer side branches are formed (Baars et al., 2010; Martin et al., 2013). Up to 10 larvae per 20 cm of stem have been recorded in South Africa and larvae can be found in leaves throughout the water column (Martin et al., 2013). *Hydrellia lagarosiphon* does not feed on *M. spicatum*.

Chaenusa is a nearly cosmopolitan genus of koinobiont endoparasitoids (Kula, 2009) and *C. anervata* is the most abundant of the three recorded parasitoids of *H. lagarosiphon* on *L. major* in South Africa (Martin et al., 2013; van Achterberg & Prinsloo, 2012). Adult females of this species walk down the plant beneath the water surface in search of host larvae (Baars et al., 2010). In natural populations, up to 30% of *H. lagarosiphon* larvae can be parasitized by *C. anervata* in summer and this rises to over 50% during the winter months (Martin et al., 2013).

Methods

The experimental design used in this study was initially developed in agricultural systems (Spitters, 1983) but has been modified and used in submerged aquatic plant competition experiments (Van et al., 1998; Mony et al., 2007; Martin & Coetzee, 2014), and developed further to determine the subtle effect of herbivory on plant competition outcomes where regular linear models cannot (Coetzee et al., 2005; Van et al., 1998). The design uses an addition series, which allows for the relative competitive ability of the two plant species in the experiment to be determined using reciprocal yield models of mean plant mass under the various treatments.

Initial plant growth

The experiments were carried out between the end of summer (January) and the beginning of winter (April) in an unheated greenhouse at Rhodes University, Grahamstown in the Eastern Cape Province of South Africa using 16 polypropylene plastic pools (215 cm diam. × 40 cm deep, 1452 l) referred to from here as pools, filled with clean locally obtained borehole water (pH 7.7, Total Dissolved Solids (TDS) 235 mg l^{-1}, Electrical Conductivity (EC) 329 AS cm^{-1}). Twelve of the 16 pools were covered with fine-mesh netting (0.8 mm × 0.5 mm) and four were uncovered controls. The netting reduced photosynthetically active radiation by 63% (recorded using a Li-COR LI 1 88B Integrating Quantum Radiometer).

Sixteen 12-L plastic tubs (42 cm diam. × 14 cm deep) were placed within each pool with enough space between them to avoid overlap between plants from different tubs. The tubs were filled with sediment collected from Jameson Dam, Eastern Cape, South Africa (−33.319073 S; 26.444206 N), which had physical and chemical characteristics that are typical of *L. major* and *M. spicatum* sites across South Africa (Martin & Coetzee, 2014). Ten grams of a controlled 15-7-15 N:P:K slow-release fertilizer (Haifa, Multicote 8; 15-7-15 + 2MgO + Micronutrients

formulated for an 8-month release rate at 21°C or 5- to 6-month release at 30°C) was added to the sediment in each tub. The sediment was covered with a thin layer of silica sand to reduce algal growth. Twelve-centimetre growth tips from the two test species were planted in the sediment in the tubs at varying densities and proportions in planting ratios (L. major: M. spicatum) of 0:3, 0:9, 3:3, 3:9, 3:0, 9:0, 9:3 and 9:9 per pool, in accordance with Spitters (1983) addition series, with the tubs placed in the same predetermined order, but with starting points for the sequence varying in location between pools (supplementary data). Therefore, each of the sixteen pools contained two sets (i.e. 2 × 0:3, 0:9, 3:3, 3:9, 3:0, 9:0, 9:3, 9:9) of planted tubs, respectively. The plants were not moved once the experiment had started. The initial mass per shoot of L. major and M. spicatum were 1.3 g ± 0.20 (mean ± S.E.; $n = 1152$) and 1.7 g ± 0.24 ($n = 1152$), respectively. After one week, any plants that had become detached or appeared unhealthy were replaced. Plants used in this experiment were collected from the field and then maintained in an unheated greenhouse at Rhodes University. Lagarosiphon major was collected from Wriggleswade Dam (32.586 S, 27.464 E), near Stutterheim in the Eastern Cape. Myriophyllum spicatum was collected from the Vaal River (28.115 S, 24.925 E) near Warrenton in the Northern Cape.

To determine relative competitive ability between the two species in the absence of herbivory or parasitism, at the start of the herbivory component of the study, the plants from half the tubs from each pool were harvested at 35 days, by which time they had grown up to the water surface (i.e.1 × 0:3, 0:9, 3:3, 3:9, 3:0, 9:0, 9:3, 9:9 planted tubs were removed). The species were separated where necessary, washed and cleaned, and then dried in a Heraeus drying oven for 96 h at 60°C. Dry biomass (g) was measured using an Ohaus® Adventurer™ balance.

Preparatory insect rearing

Approximately 900 adult H. lagarosiphon were collected from L. major plants in January from a small impoundment near Rosetta, Kwa-Zulu Natal, South Africa (29°18′18″S 29°58′28″E). The flies were divided into groups of 15 with sex ratios of approximately 1:1, placed in 25 × 20 × 15 cm containers with short lengths of L. major stems and sealed with a fine-mesh netting lid. A yeast hydrolysate and sugar mixture (4 g yeast hydrolysate: 7 g sugar) was provided as a food source. The flies were allowed to oviposit for 24 h and were then transferred to other similar containers. This was repeated until 800 eggs were available.

Experimental treatments

The sixteen pools were equally divided into four treatments (i.e. four pools for each treatment): an uncovered procedural control where there were plants but no insects (uncovered control treatment); an insect-free control with netting-covered plants (covered control treatment); netting-covered plants with the leaf-mining fly (herbivore treatment) and netting-covered plants with both the fly and its parasitoid (parasitoid treatment). Water levels were lowered to expose L. major stems, and in two of the treatments (herbivore and parasitoid treatments), H. lagarosiphon eggs were distributed evenly on their exposed tips using a fine paintbrush, at a density of approximately 2–3 eggs on each large L. major sprig (equivalent to a stocking density of 327 eggs m^{-2}). This density replicated that recorded under natural conditions (Martin et al., 2013), rather than attempting to maximize damage to the plants (Van et al., 1998). Once the eggs had hatched, the water level was once again raised. A yeast hydrolysate and sugar mixture placed on two floating polystyrene foam (2 × 2 cm) feeding stations in each pool provided a food source for the adult flies when they appeared. Seven weeks after the introduction of the fly eggs, late instar fly larvae and pupae were available for parasitoid oviposition. Adult C. anervata were collected with nets from the same field site as the H. lagarosiphon adults and five female parasitoids, imitating approximate field densities, were released into the parasitoid treatment. The plants from each treatment were harvested 21 weeks after planting, and then dried and weighed. To summarize, after 40 days, half of the initial plants from each of the 16 pools were destructively sampled, and competition determined, whereafter the flies were added to eight pools (herbivore and parasitoid treatments) and allowed to establish for approximately 55 days, at which point the parasitoid was added to the parasitoid treatment and left for a further 55 days, before the final destructive sampling event.

Throughout the duration of the experiment, temperatures were monitored every 2 h using Thermochron iButtons (Climastats Environmental Monitoring software, Version 4) placed in water-tight containers floating on the water surface and placed within the sediment at the base of the plants.

Statistical analysis

Multiple regressions were conducted on the mean final dry total biomass under each planting density, for each treatment, using inverse linear models (Spitters, 1983), a well-established method used to analyse relative competitive abilities between competing plant species. The magnitude of the relationship was analysed using the reciprocal model of Spitters (1983). This model involves multiple linear regressions of the form:

$$1/W_l = a_{l0} + a_{ll}d_l + a_{lm}d_m$$

$$1/W_m = a_{m0} + a_{mm}d_m + a_{ml}d_l,$$

where $1/W_l$ and $1/W_m$ are the inverse dry biomass of individual *L. major* and *M. spicatum* and d_l and d_m represent the respective planting densities for *L. major* and *M. spicatum*, respectively. Intraspecific competition was estimated by the partial regression coefficients a_{ll} and a_{mm} and interspecific competition by the coefficients a_{lm} and a_{ml} in terms of their effects on the reciprocal biomass of *L. major* or *M. spicatum* masses, where each *L. major* plant has an effect of $1/W_l$ equal to a_{ll}/a_{lm} of *M. spicatum* plants. In other words, *L. major* is a_{ll}/a_{lm} times as important as *M. spicatum* in terms of its impact on *L. major* mean plant biomass. The coefficient a_{lm} is defined as the effect on *L. major* by *M. spicatum* and a_{ml} is the effect on *M. spicatum* by *L. major*. Similarly, a_{ll} and a_{mm} are the intraspecific effects of *L. major* and *M. spicatum,* respectively. Competitive interactions were analysed for total dry mass and root, shoot and total plant biomass. The intercepts (a_{l0} and a_{m0}) measure the reciprocal of the maximum mass of isolated plants. Interspecific and intraspecific competition by one species on its own biomass, as well as the biomass of the other species, was measured using the ratio of the coefficients (a_{ll}/a_{lm} and a_{mm}/a_{ml}).

F-tests determined whether competition coefficients were significantly different in different treatments. Differences between mean daily temperatures on the surface and sediment were compared using Student's *t* tests. All statistical analyses were conducted in STATISTICA ver. 8.0.

Results

Temperatures

Mean daily temperatures and daily maxima and minima declined over time because the experiment ran from late summer into early winter (see supplementary data). The sediment temperatures in the covered treatments were significantly lower than the uncovered control treatment, but there was no significant difference between the surface temperatures ($F_{(3, 444)} = 3.7393$, $P = 0.011$).

Initial plant growth

After 40 days, both *L. major* and *M. spicatum* had grown to reach the water surface. There were no significant differences in the dry biomasses between the four treatments at each of the planting densities (*L. major*: $F_{(3, 88)} = 0.54$, $P = 0.66$; *M. spicatum*: $F_{(3, 88)} = 0.34$, $P = 0.80$) so the treatments were grouped together. After 5 weeks in the absence of herbivory, *L. major* had already established a competitive advantage over *M. spicatum*. The ratio of coefficients, based on dry biomass, comparing intraspecific to interspecific competition, a_{ml}/a_{mm}, showed *L. major* to be 9.8 times more competitive than *M. spicatum*, and a_{ll}/a_{lm} showed *M. spicatum* to be only 0.19 times as competitive as *L. major* (Table 1).

Competition in the absence of herbivory

Twenty-six weeks after initial planting, in the absence of herbivory, *L. major* remained a stronger competitor than *M. spicatum*. In the uncovered control treatment based on dry biomass, the relative competition coefficient a_{mm}/a_{ml} was approximately 8.18 for *L. major*. This equates to the addition of a single *L. major* plant having the same impact on mean *L. major* biomass as adding 8.18 *M. spicatum* individuals (Table 1). Conversely, the ratio of coefficients comparing *M. spicatum* to *L. major* a_{lm}/a_{ll} was 1.11 (Table 1), indicating that *M. spicatum* had very little effect on the dry biomass of *L. major*. Neither inter- nor intraspecific

Table 1 Multiple regression analysis of the impact of insect herbivory, parasitism and plant density on the reciprocals of the mean plant mass of *Lagarosiphon major* and *Myriophyllum spicatum*

Regression coefficients

Species	Intercept	Intraspecific competition[a]	Interspecific competition[b]	Ratio of competition coefficients[c]	R^2; P
Uncovered control-Pre-release					
L. major	0.141	0.094	0.010	9.853	0.470; 0.001
M. spicatum	1.55	0.017	0.085	0.196	0.133; 0.001
Uncovered control					
L. major	0.236	0.218	0.027	8.180	0.627; 0.001
M. spicatum	0.261	0.264	0.237	1.110	0.482; 0.002
Insect-free control					
L. major	0.693	0.297	0.019	15.524	0.508;0.001
M. spicatum	1.393	0.022	0.196	0.114	0.479;0.001
Herbivory treatment					
L. major	1.166	0.290	0.093	3.12	0.337; 0.013
M. spicatum	0.857	0.073	0.061	1.19	0.372; 0.207
Parasitoid treatment					
L. major	0.415	0.221	0.035	6.34	0.296; 0.025
M. spicatum	0.937	0.098	0.061	1.6	0.323; 0.017

[a]Intraspecific competition is represented by the regression coefficients a_{ll} for *L. major* and a_{mm} for *M. spicatum*

[b]Interspecific competition represents the regression coefficient a_{lm} for *L. major* and a_{ml} for *M. spicatum*

[c]The ratio of the competition coefficients measures the effect of intraspecific competition by one species on its own weight relative to the effects of interspecific competition by the other species, a_{mm}/a_{ml} for *M. spicatum* and a_{ll}/a_{lm} for *L. major*

competition influenced dry biomass of *M. spicatum* (Table 1). In the covered control treatment, where light levels were 63% lower than in the uncovered treatment, a_{ml}/a_{mm} showed *L. major* had become 15.5 times more competitive than *M. spicatum* (Table 1). *Myriophyllum spicatum* showed correspondingly increased competition from *L. major* and was only 0.11 times as competitive as *L. major* (Table 1).

Intraspecific competition significantly reduced the overall biomass of *L. major* in both the covered and uncovered control treatments (Fig. 1a, c). The steep slope in one direction indicates the strong effect of the density of *L. major* on the biomass of *L. major*, whereas the gentler opposing slope illustrates the weak effects of *M. spicatum* density on the biomass of *L. major* (Fig. 1a, c). The flat slopes of the *M. spicatum* density regression planes confirm that interspecific competition from *M. spicatum* was negligible in both the covered and uncovered control treatments (Fig. 1b, d). In both control treatments, increasing planting densities of *L. major* and *M. spicatum*

significantly decreased the final dry biomass of *M. spicatum*, as indicated by the increased gradients in both directions (Fig. 1b, d). Therefore, both inter- and intraspecific competition influenced the final biomass of *M. spicatum*.

Competition in combination with herbivory

Leaf damage resulting from feeding by *H. lagarosiphon* larvae was concentrated in the top 30 cm of the water column, where most of the *L. major* stems showed signs of damage. A total of 55 fly pupae, 13 ± 0.85 (mean $\pm$ SE) per pool, were collected from the four pools. The competitive advantage of *L. major* in the covered control treatment (15.5) was reduced to 3.12 in the presence of *H. lagarosiphon* (Table 1), but despite the influence of herbivory, interspecific competition continued to have a more important impact than intraspecific competition on the biomass of *M. spicatum* (Table 1). Intraspecific competition also continued to have a far greater effect on *L. major*
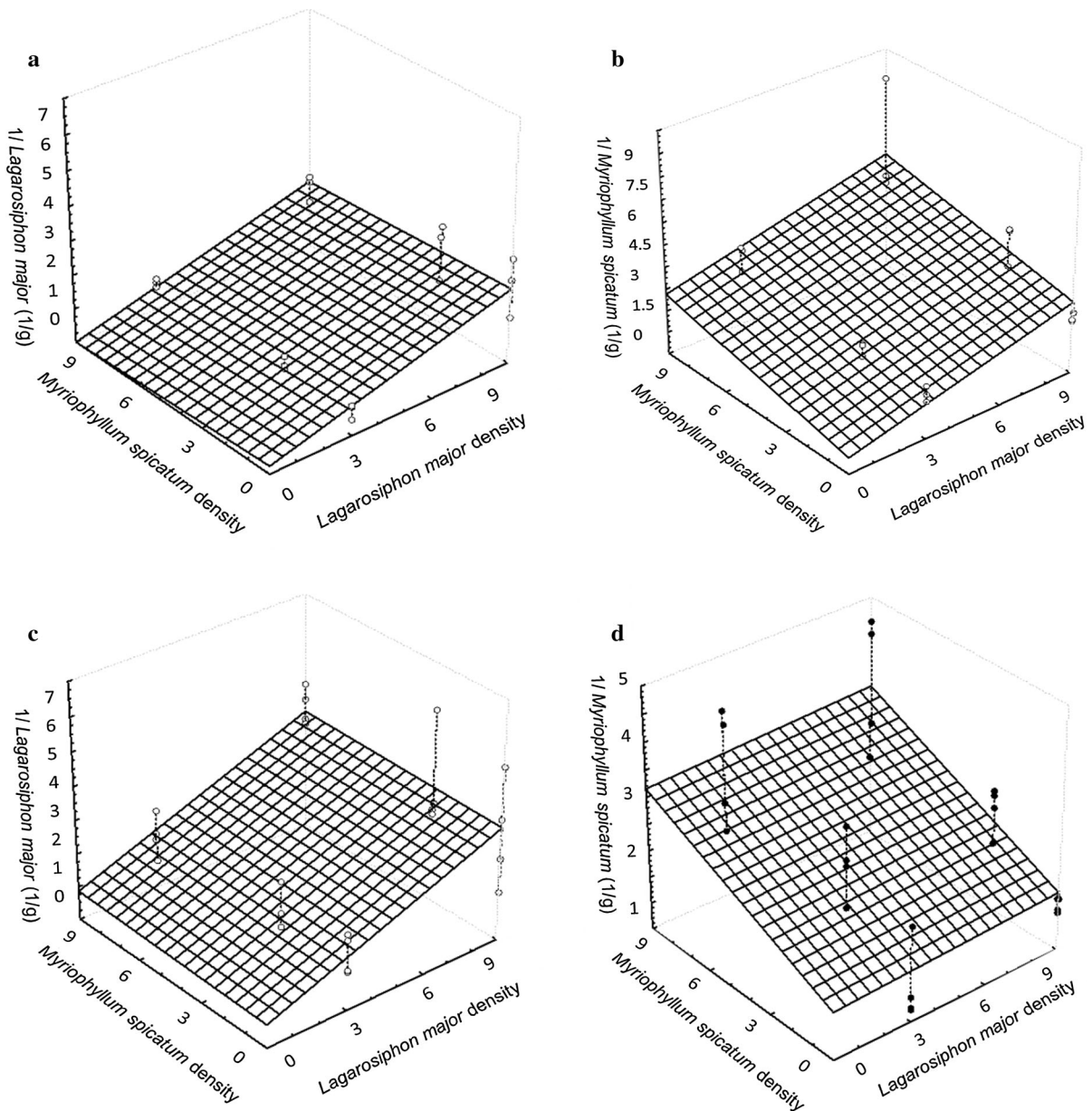
Fig. 1 Multiple regression planes indicating the combined effect of *Lagarosiphon major* and *Myriophyllum spicatum* on the reciprocal of the mean wet biomass (1/g) of one *L. major* plant (**a**, **c** uncovered control experiment), and the combined effect of *L. major* and *M. spicatum* on the reciprocal of the mean wet biomass (1/g) of one *M. spicatum* plant (**b**, **d** insect-free control) (i.e. higher values represent lower yields). Points indicate observations ($n = 24$) and vertical lines between data points represent the residuals. Values on *X* and *Y* axes represent *L. major* and *M. spicatum* planting densities at the start of the experiment, respectively

than interspecific competition, despite the presence of its herbivore (Fig. 2). The three-dimensional surface plot for the biomass of *M. spicatum* (Fig. 2b) nonetheless displays a steeper slope compared to the covered control treatment and reflects an increase in the impact of intraspecific competition on *M. spicatum* as a result of the herbivory on *L. major* (estimated as increasing from 0.11 to 1.19; Table 1 and Fig. 2).
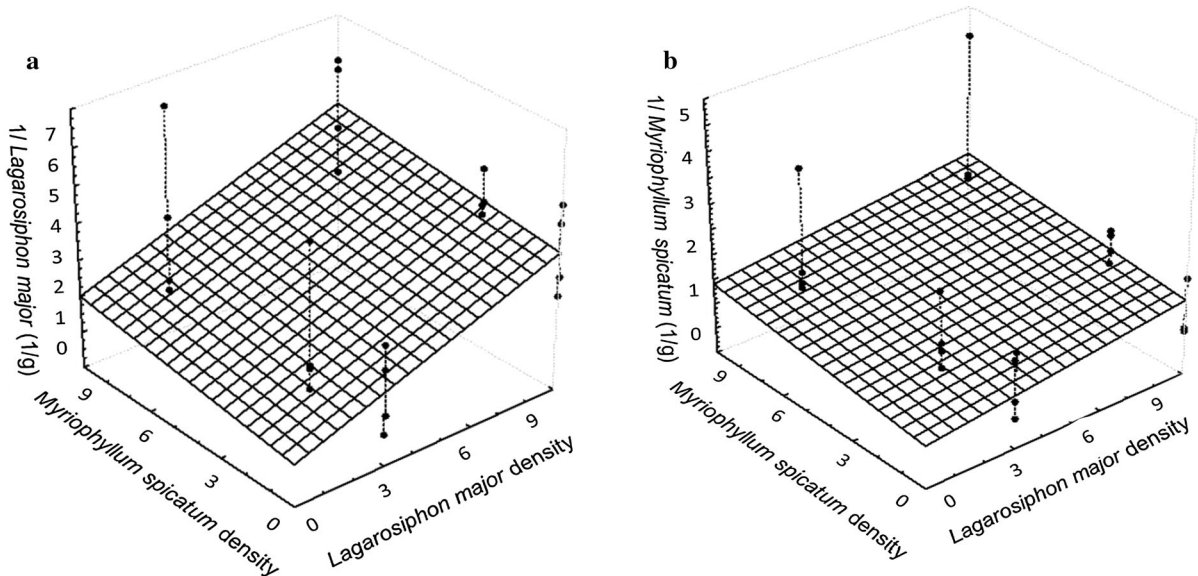
a

b

Fig. 2 Multiple regression planes indicating the combined effect of *Lagarosiphon major* and *Myriophyllum spicatum* on the reciprocal of the mean wet biomass (1/g) of one *L. major* plant (**a**) and one *M. spicatum* plant (**b**), respectively, in the presence of herbivory by *Hydrellia lagarosiphon*. Higher values represent lower yields. Points indicate observations ($n = 24$) and vertical lines between data points represent the residuals. Values on X and Y axes represent *L. major* and *M. spicatum* planting densities at the start of the experiment, respectively

Competition in combination with herbivores and parasitoids

Parasitism rates at the end of the experiment were estimated at 51 ± 3.23 (mean ± S.E., $n = 32$ fly puparia). The importance of intraspecific competition on *L. major* was doubled in the presence of the parasitoid of its herbivore, from 3.12 to 6.34 (Table 1). A steeper slope of the *L. major* regression plane also indicates that the presence of the parasitoid further increased the competitive advantage of *L. major* over *M. spicatum* (Fig. 3). However, the slope associated with *M. spicatum* impact on *L. major* was unchanged, suggesting that the presence of the parasitoid had not affected the competitive ability of *M. spicatum* in relation to *L. major.* This was also reflected in the similar competition coefficients of 1.19 in the absence and 1.6 in the presence of the parasitoid (Fig. 3). The influence of the parasitoid on the dry biomass of *M. spicatum* was therefore related to increased interspecific competition with *L. major* rather than intraspecific competition (Fig. 3).

Discussion

Under natural conditions in South Africa, the native aquatic macrophyte *L. major* supports populations of the leaf-mining fly *H. lagarosiphon*, which in turn are host to the parasitoid *C. anervata*. Under experimental conditions that replicated field insect densities, we found that feeding by the leaf-mining fly reduced, but did not eliminate, the competitive superiority of *L. major* over a second macrophyte, *M. spicatum,* which is itself invasive in some of the countries where it has been introduced (Smith & Barko, 1990; Madsen et al., 1991; Caffrey et al., 2010). When it was present, the parasitoid reduced the number of flies by about half, and this had the effect of greatly reducing the fly's impact on its host and restoring the strong competitive advantage of *L. major*. The experimental insect densities were representative of known field densities (Martin et al., 2013). Our experimental results suggest that under field conditions in South Africa, where all three trophic levels are usually represented, tritrophic interactions contribute to the strong competitive ability of *L. major* because they largely negate the negative effects of *H. lagarosiphon* on its host. The significance of tritrophic interactions in terrestrial
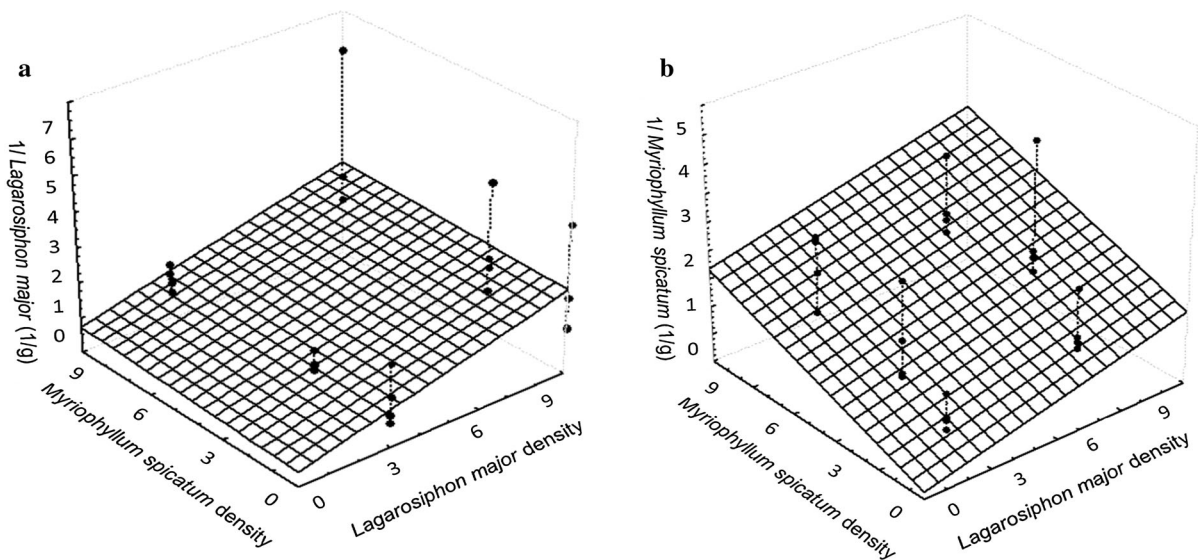
Fig. 3 Multiple regression planes indicate the combined effect of *Lagarosiphon major* and *Myriophyllum spicatum* on the reciprocal of the mean wet mass (1/g) of one *L. major* plant (**a**) and one *M. spicatum* plant (**b**) under the influence of herbivory and parasitism. Higher values represent lower yields. Points indicate observations ($n = 24$) and vertical lines between data points represent the residuals. Values on *X* and *Y* axes represent *L. major* and *M. spicatum* planting densities at the start of the experiment, respectively

ecosystems was emphasized by Harvey et al. (2010) and our results extend this conclusion to freshwater systems.

The superior competitive ability of *L. major* over other submerged aquatic species has been reported previously, both in the field and in common garden experiments (Rattray et al., 1994; James et al., 1999; Caffrey et al., 2010; Martin & Coetzee, 2014). Physiologically, *L. major* outcompetes other submerged macrophytes through rapid shoot production and shoot biomass accumulation (at the expense of root growth), effectively creating a dense, light-excluding canopy layer on the water surface that allows it to outcompete other aquatic macrophytes for light (Rattray et al., 1994). This competitive advantage is enhanced under certain water conditions, such as elevated pH and O_2 and lower free CO_2 levels (James et al., 1999).

In our experiments, when there was no herbivory, *L. major* was the superior competitor relative to *M. spicatum* irrespective of the presence or absence of netting to exclude insects, but this competitive advantage was increased greatly by the netting. The change in competitive ratios between the two treatments is likely to have resulted from the reduction in solar radiation reaching the plants caused by the

netting. Barko and Smart (1981) showed that under lower light intensities *M. spicatum* invests less in shoot elongation and new shoot production, and more into root formation. In contrast, *L. major* growing in lower light conditions rapidly increases shoot lengths (at the expense of root development), using stored reserves (Rattray et al., 1994). These responses combine to increase the competitive advantage of *L. major* over *M. spicatum* when they are growing together under shade and will have influenced the results of the experiments where insects were present, all of which took place under netting.

Strong (1992), in his review on whether 'trophic cascades are all wet', elucidated that when plant taxa are impacted by an increase in herbivore pressure, they are often replaced by plants that are not as affected. This trend was clearly shown within our experimental system where the introduction of *H. lagarosiphon* on *L. major* resulted in increased competition from *M. spicatum*. The impact of leaf mining by larvae of *H. lagarosiphon* on the competitive ability of *L. major* is similar to that of its congener *H. pakistanae* when feeding on *Hydrilla verticillata* growing together with *V. americana* (Van et al., 1998). Our results are also comparable to previous studies comparing the impact of other insect herbivores on the competitive ability of

submerged macrophytes (Van et al., 1998; Cabrera Walsh et al., 2013), floating aquatic species (Coetzee et al., 2005) and also terrestrial plants (Bentley & Whittaker, 1979; Brown & Gange, 1992; Carson & Root, 1999, 2000; Engelkes et al., 2016).

From an applied perspective, our results demonstrate that *H. lagarosiphon* has the potential to contribute to the management of *L. major* in countries where the plant is a problem, but they also suggest that the fly may be ineffective if locally occurring parasitoids of other *Hydrellia* species extend their home ranges to include *H. lagarosiphon*. *Hydrellia* is a species-rich cosmopolitan genus associated with many different plants and has a rich fauna of associated parasitoids (Deonier, 1971), some of which are themselves valuable for the control of species such as *Hydrellia griseola* Fallén, a significant pest of rice (*Oryza sativa* L.) (Hesler, 1995). *Hydrellia pakistanae* and *H. balciunasi* Bock have been shown to help suppress *H. verticillata*, in the USA, where it is a significant weed (Doyle et al., 2002; Owens et al., 2008), despite records of the flies being attacked by indigenous parasitoids (Harms & Grodowitz, 2011). The impact of the parasitoids on *H. verticillata* control has not been assessed. It seems likely that similar colonization by local parasitoids will occur wherever other *Hydrellia* agents are introduced, though in the case of *H. verticillata* in the USA, the host switches by local parasitoids onto the introduced *Hydrellia* species may have been facilitated by two native congeners that now also feed on the same plant and may have brought their parasitoids with them. Clearly, the apparent ease with which parasitoids of *Hydrellia* species switch hosts, and the resulting absence of 'agent release', needs to be considered in future biocontrol projects involving this genus.

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