Hydrellia lagarosiphon Deeming (Diptera: Ephydridae), a Potential Biological Control Agent for the Submerged Aquatic Weed, Lagarosiphon major (Ridl.) Moss ex Wager (Hydrocharitaceae)

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Hydrellia lagarosiphon Deeming (Diptera: Ephydridae), a potential biological control agent for the submerged aquatic weed, *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae)

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The leaf-mining fly, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), was investigated in its native range in South Africa, to determine its potential as a biological control agent for *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae), an invasive submerged macrophyte that is weedy in many parts of the world. The fly was found throughout the indigenous range of the plant in South Africa. High larval abundance was recorded at field sites with nearly all *L. major* shoots sampled ontaining larvae, with densities of up to 10 larvae per shoot. Adults laid batches of up to 15 eggs, usually on the abaxial sides of *L. major* leaves. The larvae mined internally, leaving the epidermal tissues of the upper and lower leaves intact. The larvae underwent three instars which took an average of 24 days and pupated within the leaf tissue, from which the adults emerged. Impact studies in the laboratory showed that *H. lagarosiphon* larval feeding significantly restricted the formation of *L. major* side branches. Based on its biology and damage caused to the plant, *Hydrellia lagarosiphon* could be considered as a useful biological control candidate for *L. major* in countries where the plant is invasive.

Key words: submersed aquatic macrophyte, native range survey, natural enemy, leaf damage, weed biological control.

INTRODUCTION

Lagarosiphon major (Ridl.) Moss ex Wager (Hydrocharitaceae) is a submerged aquatic macrophyte indigenous to southern Africa (Cook 2004), but has been introduced to Australia, New Zealand, the United Kingdom (including Ireland), and mainland Europe (Howard-Williams & Davies 1988; Bowmer *et al*. 1995; Baars *et al*. 2010; ISSG 2011), probably through the aquarium and horticultural trade. It has become invasive in many waterways in these countries, and is particularly difficult to control using traditional methods of mechanical removal and chemical application (Caffrey *et al*. 2011). Biological control using insect natural enemies of *L. major* could be a suitable alternative to complement other control methods.

Lagarosiphon major can grow to a depth of 7 m in clear water; it thrives in shallow, muddy, alkaline waters but is capable of establishing under most aquatic conditions (Caffrey & Acevedo 2007). It can be distinguished from other similar looking species of Hydrocharitaceae by its recurved leaves arranged spirally around the stem (Bowmer *et al*. 1995) and the small blunt unicellular spines with 2–3 rows of fibres on their margin, only seen under a microscope (Symoens & Triest 1983). The roots vary in length but may grow up to 50 cm and are capable of penetrating deep into the substrate. Single stems arise from the roots and branch repeatedly as they rise through the water column, producing an extremely dense surface canopy (Caffrey *et al*. 2010). The stems are more robust than other *Lagarosiphon* spp. (Cook 2004), but they nevertheless break easily under the influence of wind or wave action, which aids in the natural spread of the plant as these stem fragments can root (Caffrey & Acevedo 2007). Under favourable conditions, dense growth of the plant can block light penetration into waterways, eliminating growth of native water plants and negatively affecting aquatic fauna (Rattray *et al.*1994). Large mats may choke shallow dams and rivers (Cook 2004), thereby restricting the passage of boats, limiting recreational activities such as swimming and angling, and blocking intakes to hydro-electric generators (Bowmer *et al*. 1995; James *et al*. 1999; McGregor & Gourlay 2002).

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Weed biological control programmes have focused largely on terrestrial and free-floating aquatic plant invaders with comparatively little attention given to the majority of submerged invasive macrophytes, including *L. major* (Bennett & Buckingham 2000; Schutz 2007; Baars *et al*. 2010). However, other Hydrocharitaceae species have been targeted with biological control. The closely related hydrilla, *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), an invasive species in the United States, has been surveyed extensively in Asia and Australia and more than 25 species of herbivorous insects, from five orders, have been identified. Of these, four have been released in the United States as biological control agents (Bennett & Buckingham 2000) and include two weevils, *Bagous hydrillae* O'Brien and *B. affinis* Hustache (Coleoptera: Curculionidae) (Buckingham & Bennett 1998; Balciunas *et al*. 1996), and two flies, *Hydrellia balciunasi* Bock and *Hydrellia pakistanae* Deonier (Diptera: Ephydridae). *Hydrellia pakistanae* has been successful in the long-term management of *H.verticillata* in controlled experimentation and at field sites (Doyle *et al*. 2002; Grodowitz *et al*. 2003), and is being considered for release in South Africa (Coetzee *et al.* 2011) where *H. verticillata* was discovered in 2006 (Henderson 2006). An unidentified species of *Hydrellia* (Diptera: Ephydridae), recently identified from Brazil, has shown promise as a candidate agent for Brazilian water weed, *Egeria densa* Planch (Hydrocharitaceae), another submerged species that is becoming problematic in several countries worldwide (Cabrera Walsh *et al*. 2012). *Lagarosiphon major* may thus also be a suitable target for biological control.

As yet, no biological control programmes have been implemented against *L. major* anywhere in the world. Thus, field surveys in the plant's native range in South Africa were undertaken to identify and screen potential control agents for use elsewhere in the world. Several phytophagous species were recorded on *L. major* for the first time (Baars *et al*. 2010), including an ephydrid fly, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae) (Deeming 2012), which was found to have a wide distribution and cause significant leaf damage. This study describes the results of field surveys and laboratory studies on the biology and impact of *H. lagarosiphon* and assesses its potential as a biological control agent of *L. major.*

MATERIAL AND METHODS

Field surveys

Botanical records from the South African National Biodiversity Institute (SANBI) and Rhodes University were used to determine the distribution of *L. major* in South Africa (Baars *et al*. 2010). Surveys for potential biological control agents across the distribution of *L. major* were undertaken in November 2008 (Baars *et al*.2010), December 2009 and May 2010. Sites in the Eastern Cape Province were regularly surveyed between 2008 and 2011.

The holotype (specimen number TYPH01958) of *H. lagarosiphon*, housed at the National Collection of Insects, Biosystematics Division, Agricultural Research Council-Plant Protection Research Institute, South Africa,was collected from Featherstone Farm Dam, near Stutterheim in the Eastern Cape (32.57746S 27.49583E). Before being submitted to the National Museum of Wales, Cardiff, for description, a culture of the fly collected from Featherstone Farm was established at University College Dublin, Ireland. To ensure that the same species of *Hydrellia* was feeding on *L. major* throughout South Africa, *Hydrellia* spp. were collected from the water surface above *L. major* mats at selected sites in South Africa, reared to F3 generation on *L. major,* and then sent for comparison with the holotype specimen (Deeming 2012).

Where *H. lagarosiphon* was found in the field, 100 *L. major* shoots, approximately 15–20 cm long, were examined for the presence of larvae and puparia of *H. lagarosiphon*. All infested material was returned to the laboratory and placed in emergence chambers. Any adults emerging from the material were identified. Distribution records of *H. lagarosiphon* arising from the field surveys were overlaid onto a map incorporating mean daily minimum temperature data during the coldest months in South Africa (Schulze 1997), using ArcView v.9. (ESRI 2007), to estimate temperatures typically experienced by fly populations in the field during the coldest months of the year.

Parasitism of Hydrellia lagarosiphon

Parasitoid populations of *H. lagarosiphon* were monitored at two field sites in the Amatola region of the Eastern Cape: Site 1, Featherstone Farm Dam (32.57746S 27.49583E), a small, shallow, sheltered farm dam; and Site 2, Wriggleswade Dam, with two sampling stations (32.58618S 27.46415E and 32.36531S 27. 33223E), a compara-

tively large and deep impoundment. Both dams had large healthy beds of *L. major* with both *H. lagarosiphon* and parasitoids present. At each site, a 1 m² quadrat was randomly thrown onto the *L. major* mat and all plant material within the quadrat was collected. Three quadrats were sampled monthly at each site for eight months (March–October 2010). The *L. major* material from each quadrat was then sorted into stems with larval damage. All leaves containing larvae or puparia were removed from the stems and placed individually into 5 ml Eppendorf™ tubes half-filled with water and sufficient undamaged leaves of *L. major*, which were replaced when required. The tubes were left under a growth light and checked daily for fly pupariation and eclosion or parasitoid emergence.

Biology of Hydrellia lagarosiphon

The initial laboratory colony of *H. lagarosiphon* was started from approximately 200 adults collected from a farm dam near Stutterheim, Eastern Cape (32.58618S 27.46415E) in 2009.

In order to establish a culture, insect-free populations of *L. major* were grown in polypropylene pools (267 cm 65 cm, 3300 l), fitted with a steel frame for support. When needed, additional plant material was collected from the field to replenish the laboratory stock. The laboratory population of *H. lagarosiphon* was reared in a greenhouse in a similar polypropylene pool (see above) that was covered with fine gauze mesh to prevent the flies from escaping. The pool was stocked with fresh *L. major* and the flies were allowed to complete their life cycle within the pool. The adults were supplemented with a combination of yeast hydrolysate and sugar (4 g: 7 g) (Buckingham & Okrah 1993) to promote oviposition. Larvae and adults were harvested from the pool when required for experiments. Larvae were dissected from infested shoots under a stereomicroscope while adults were collected from the water surface using aspirators.

Lifespan and fecundity of Hydrellia lagarosiphon

Seven to eight newly eclosed flies were confined in polystyrene containers (12 cm × 8 cm, 500 ml), containing 100 ml of water. Water was provided to maintain humidity. A yeast hydrolysate and sugar mixture was provided as food on a 2 cm × 2 cm plastic float. The flies were allowed to mate for 24 hours and the females were separated and placed individually in a Petri-dish with moist filter paper, yeast hydrolysate/sugar mixture and a shoot of *L. major*. The females were confined and allowed to oviposit until death. This study was conducted under controlled temperatures of 21 ± 1.5 °C and fluorescent plant growth lights (85 W OSRAM Plant lighting) at 12:12 day:night regimes.

Larval and pupal development of Hydrellia lagarosiphon

Sixty apical shoots (10 cm length) of insect-free *L. major* were exposed to recently mated flies. Each apical shoot was kept in an individual polystyrene container (12 cm × 8 cm, 500 ml) and exposed to 12 flies with an approximately sex ratio of 1:1 for 12 h at 21 °C, to ensure oviposition on the shoots. The sex ratio was confirmed by sexing all adults after death. Once the flies had been removed, the apical shoots were checked for oviposition, indicated by the presence of clusters of eggs on the leaves. The eggs were then checked daily to determine incubation time. Larvae were also monitored daily to determine when pupariation occurred. Once the larvae had pupated within the *L. major* leaves, the leaves were removed from the main stem and placed in 5 ml Eppendorf™ tubes containing 2 ml water. The number of days until adult eclosion was then monitored.

Measurements of eggs, larvae and puparia were made at ×50 or ×25 magnifications with an ocular micrometer in a stereomicroscope. Egg length was determined as the distance between the posterior and anterior ends of the eggs, and the width as the maximum transverse extent of the egg. Larval length was measured from the anterior edge of the head lobe to the posterior end ofthe spiracular peritremes, with the larvae outstretched, and the width as the maximum transverse extent, measured in the dorsal view. Puparia were measured similarly and were weighed on a CAHN C-31 microbalance. Means ± S.E. were calculated for each parameter.

Impact on Lagarosiphon major

In order to determine the effect of feeding by *H. lagarosiphon* on *L. major*, 60 apical shoots, 20 cm in length, were placed individually in clear plastic containers (7.5 cm × 25 cm, 900 ml), filled with tap water and allowed to grow for two weeks under controlled temperatures of 25 ± 2 °C, and a 12:12 day:night regime provided by fluorescent lights (85W OSRAM Plant lighting). After two

weeks, eggs laid on the same day by recently mated flies, were placed on *L. major* shoots in a random block design at densities of 0 (control), 1, 2, 4 and 8. The emerging larvae were then allowed to feed and develop until pupariation. Each egg density treatment was replicated 12 times. Once pupariation had occurred, puparia were removed and counted. The root length, change in original shoot length, number of branches, number of damaged leaves and the number of puparia were recorded.

The influence of egg density on plant parameters was analysed using Kruskal-Wallis ANOVA and *post hoc* multiple comparison of mean rank tests. All statistical analyses were conducted in STATISTICA ver. 8.0.

RESULTS

Field surveys

Water bodies where *H. lagarosiphon* was recorded included impoundments, natural lakes, perennial streams and rivers. Stands of *L. major* infested with *H. lagarosiphon* ranged from small clumps amongst beds of other submerged macrophytes along the edges of dams to large beds occupying the entire water column of small dams and natural lakes.

Sites where *L. major* and *H. lagarosiphon* were recorded were limited to the colder areas of South Africa, namely the Amatola Region of the Eastern Cape, Drakensberg regions of KwaZulu-Natal, the Eastern Cape, and the Mpumalanga highlands. Only one site was found outside these areas at Reitz, in the Free State (Fig. 1). Minimum winter ambient temperatures at these sites ranged from –2 to +4 °C (Schulze 1997) (Fig. 1). The mean minimum temperature in these areas can drop to well below 0 °C (Schulze, 1997). Even at the coldest sites, *H. lagarosiphon* adults and larvae were found in abundance.

At the sites where *H. lagarosiphon* was found, 1 to 100 % of *L. major* stems collected contained either fly larvae or puparia. Other submerged species investigated at these sites were infrequently damaged by *Hydrellia* spp.; however, a small number of puparia were found on adjacent plant species, such as *Stuckenia pectinata* (= *Potamogeton pectinatus* L.) (Potamogetonaceae) and *Lagarosiphon muscoides* Harv. (Hydrocharitaceae). Since these puparia were all parasitized by braconid wasps, no *Hydrellia* spp. were reared through to adulthood and identifications could thus not be confirmed from these non-target plants.

Parasitism of *Hydrellia lagarosiphon*

Three different braconid parasitoids were recovered from *H. lagarosiphon* collected in the field as well as from stock cultures. Adult parasitoids were observed in the field searching and probing underwater for larvae or pupae; this was achieved by holding a bubble of air in the wings and walking over the plant material while searching for larvae or pupae (Baars *et al*. 2010). Investigations at Featherstone Farm Dam showed that over the eight months studied, 32 ± 14 % (S.E.) of *H. lagarosiphon* larvae were parasitized by the wasps. The highest mean % parasitism in summer was 28.9 ± 19.8 % at Wriggleswade Dam (Site 1),while the highest mean % parasitism in winter was 52.8 ± 2.3 % at Featherstone Farm Dam (Table 1). Specimens reared from the three study sites were identified as *Ademon lagarosiphonae* (Opiinae), *Chaenusa luteostigma* and *C. nigristigma* (Alysiinae: Dacnusini) (van Achterberg & Prinsloo 2012).

Table 1. Mean percentage of wasp parasitism of *Hydrellia lagarosiphon* at three sites for the entire sampling period and during summer and winter.

Site	Mean (±S.E.) parasitism (%)		
	Annual	Summer	Winter
Featherstone Farm Dam	32 ± 14.4	18 ± 14.8	52 ± 2.3
Wriggleswade 1 Dam Site	17 ± 16.7	28 ± 19.8	0
Wriggleswade 2 Dam Site	8 ± 8.3	13 ± 10.2	0

Biology of *Hydrellia lagarosiphon*

Eggs

Females laid up to 25 eggs on average (mean = 18.8 ± 1.9 (S.E.), n = 18 females). Eggs were 0.68 ± 0.01 mm long and 0.20 ± 0.003 mm wide (n = 22), white in colour and had longitudinal ridges running along their length. Eggs were mostly deposited one day after mating and hatched two days after oviposition. In the laboratory, egg-laying depended upon the plant structures available for oviposition; when shoots of *L. major* were available, the eggs were deposited on parts that protruded from the water such as exposed leaves or growth tips. When leaves protruded, eggs were often laid on the abaxial sides of the recurved leaves. The eggs were laid singly or in clusters of

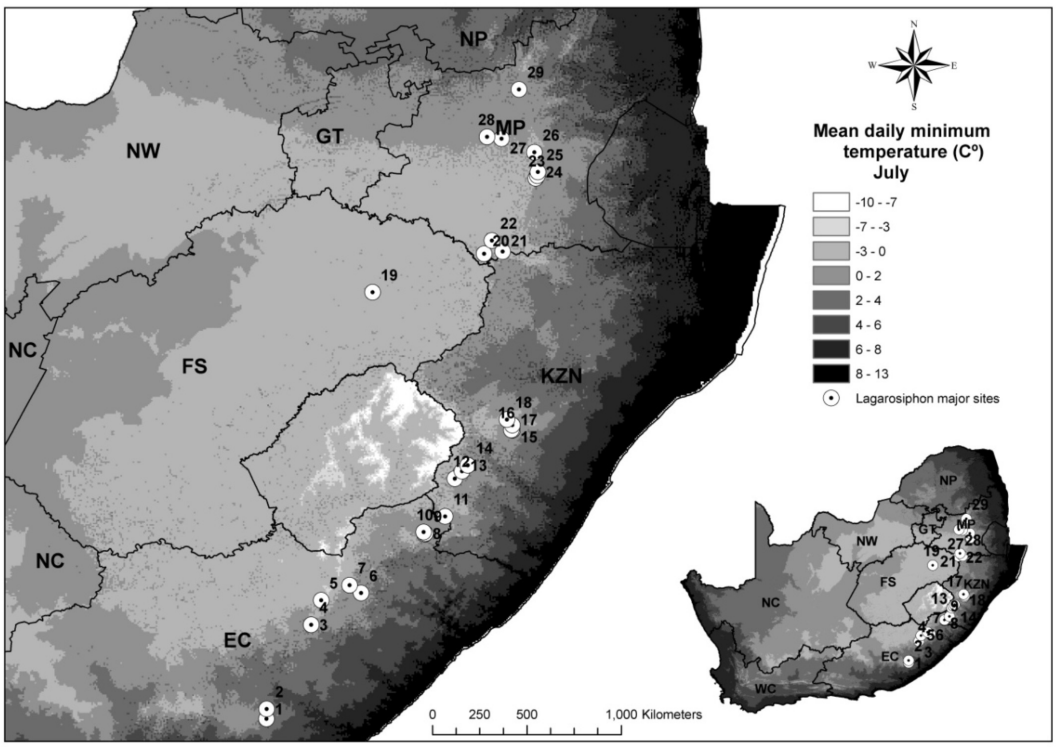

Fig. 1. Distribution of *Lagarosiphon major* sites and *Hydrellia lagarosiphon* occurrence in South Africa, in relation to the mean daily minimum temperatures during July (winter). The plant and the fly were limited to the colder higher regions of the Eastern Cape, KwaZulu-Natal and Mpumalanga. Data generated from the South African Atlas of Agrohydrology and Climatology (Schulze 1997). GT, Gauteng; MP, Mpumalanga; NW, North West; KZN, KwaZulu-Natal; EC, Eastern Cape; WC, Western Cape; NC, Northern Cape; FS, Free State.

up to 15. Even when fresh shoots were available, eggs were found floating on the surface of the water as well as on any other available structures, particularly slight indentations or ridges, such as the rim of the lid of the holding containers. Similar observations were made on *H. pakistanae* which feeds on *H. verticillata* (Buckingham & Okrah 1993). It has been shown in other *Hydrellia* spp. that newly hatched larvae are highly mobile and are able to leave the egg site to search for the host plant, and thus the oviposition substrate is not necessarily important to larval survival (Buckingham & Okrah 1993).

Larvae

The larvae were translucent yellow/white in colour and were usually visible within the leaf by their conspicuously dark feeding apparatus, the cephalopharyngeal skeleton. Neonates were found between the epidermal layers of the leaves. Most early instars were found within the youngest leaves of the growth tips. There was very little difference in appearance between the three larval instars other than the change in shape of the feeding apparatus and slight darkening of the larval spiracular peritremes. The spiracular peritremes became reduced in size and changed colour as the larvae developed from first to third instar. Beside this, the three instars differed morphologically in size only (Table 2). First instars were 0.48 ± 0.02 mm (S.E.) long by 0.1 ± 0.08 mm wide. Third instars were 3.3 ± 0.1 mm long by 0.7 ± 0.03 wide just before pupation. The larval stage lasted around 26 ± 0.7 days (n =16) (Table 2); slight mortality was noted.

Neonates moved quite freely after hatching in search of fresh young leaves in the growth tips. The larvae mined between the epidermal layers of the leaf and removed the leaf mesophyll tissue. Larvae damaged on average 19.2 ± 1.1 (S.E.) leaves, ranging from 6–37 leaves; the majority of the leaf contents were usually consumed before

Table 2. Mean larval developmental time at 21 °C and mean body lengths and widths of the three larval instars of *Hydrellia lagarosiphon* (n = 25 for each instar).

	Time (days)	Length (mm)	Width (mm)
1st instar	1	0.48 ± 0.02 (S.E.)	0.1 ± 0.004 (S.E.)
2nd instar	5	1.0 ± 0.08 (S.E.)	0.2 ±0 .02 (S.E.)
3rd instar	11	1.6 ± 0.10 (S.E.)	0.3 ± 0.02 (S.E.)
Pre-pupa	26	3.3 ± 0.10 (S.E.)	0.7 ± 0.03 (S.E.)

the larva moved on (n = 38). Larvae predominantly moved down the stem, from the tip towards the roots, in search of new leaves and are able to move below the stem epidermis to reach new leaves.

Puparia

Puparia were usually located within the epidermal layers of the tunnelled leaf, predominantly 1–5 cm below the growth tip, or occasionally within the growth tip. On average, they were 3.42 ± 0.03 mm (S.E.) long and 1.07 ± 0.02 mm wide, and weighed 0.31 ± 0.01 g (n = 50). They were initially yellow to white in colour but gradually turned dark brown to black as the flies developed. Puparia eclosed in 14 ± 0.2 days (n = 17).

Adults

Hydrellia spp. adults were sent for identification and were confirmed as *H. lagarosiphon* (Deeming 2012). Adults were dark, with a lighter shiny face. The knob of the haltere was yellow. All hairs and bristles were black apart from some on the dorsal surface being greyish. The wings were greyish. The morphology and colouration of *H. lagarosiphon* closely resembled that of other African *Hydrellia* spp., such as *H. bicolorithorax* Giordani Soika, from Rwanda, and *H. varipes* Lamb, from the Seychelles Islands (Deeming 2012). The sexes were easily distinguishable by their genitalia. The females had a uniformly flat abdomen with clearly visible sternum segments separated by an intersegmental membrane. The males had a very apparent cavity in the abdomen covered with small dark bristles; this cavity is protected by the cercus which pulls open to expose the inner copulatory organ during mating (Deeming 2012).

Impact studies of *Hydrellia lagarosiphon* on *Lagarosiphon major*

Hydrellia lagarosiphon was found at all 29 sites sampled around South Africa. Repeated visits to dams in the Eastern Cape Province showed that flies and larvae were always present. Some 1–100 % of damaged shoots produced at least one fly or parasitoid, or when dissected contained at least one live larva or puparium. In the field, the highest number of larvae recorded per 20 cm of stem was 10. Larvae were also not restricted to growth tips near the surface and were sometimes found throughout the water column. Damage to the plant was clearly visible where high densities of *H. lagarosiphon* were present.

Laboratory experiments showed that all shoots infested with *H. lagarosiphon*, independent of density, were significantly longer than uninfested shoots ($H_{(4,49)}$ =14.38, P <0.05). This was an unexpected result, since one would have expected more growth from the control shoots. There was no significant difference in shoot length at different egg densities (Fig. 2a). Infested shoots grew to over 30 cm, but averaged 26 cm, whereas shoots with no *H. lagarosiphon* eggs rarely grew over 23 cm. Uninfested shoots produced significantly more branches than those infested with *H. lagarosiphon* ($H_{(4,49)}$ = 24.32, P < 0.05) (Fig. 2c); however, the number of branches produced was not influenced by egg density, *i.e.* there were no differences between shoots exposed to 1, 2, 4 or 8 eggs (Fig. 2c). Up to four branches were produced on uninfected *L. major* shoots whereas significantly fewer branches were found on *L. major* that was infested with *H. lagarosiphon* (Fig. 2c). There were significant differences in damage between shoots exposed to 1 and 4 eggs, and between those exposed to 1–2 eggs and 8 eggs (Fig. 2b). Larvae from 8 eggs per shoot caused significantly more damage than 1 or 2 eggs per shoot ($H_{(4,n\,=\,49)}$ = 39.6, P < 0.05) (Fig. 2c).

Failure of eggs to hatch and of larvae to pupate did occur. There was 100 % survival at the single egg stocking density, whereas at densities of 2 or 4 eggs, approximately 60 % of eggs produced larvae that reached pupation. At the highest egg density (8 eggs), there was an increase in mortality and a reduction in larval survival to pupation (42 %), probably due to larval competition. There was no significant difference in survival between 1 and 2 eggs; however, there was significantly greater survival at a density of 1 compared to 4 and 8 eggs. There was no significant difference in survival between 2, 4 and 8 eggs ($H_{(3,34)}$ =13.15, P < 0.05) (Fig. 2d).

Hydrellia lagarosiphon did not affect the number

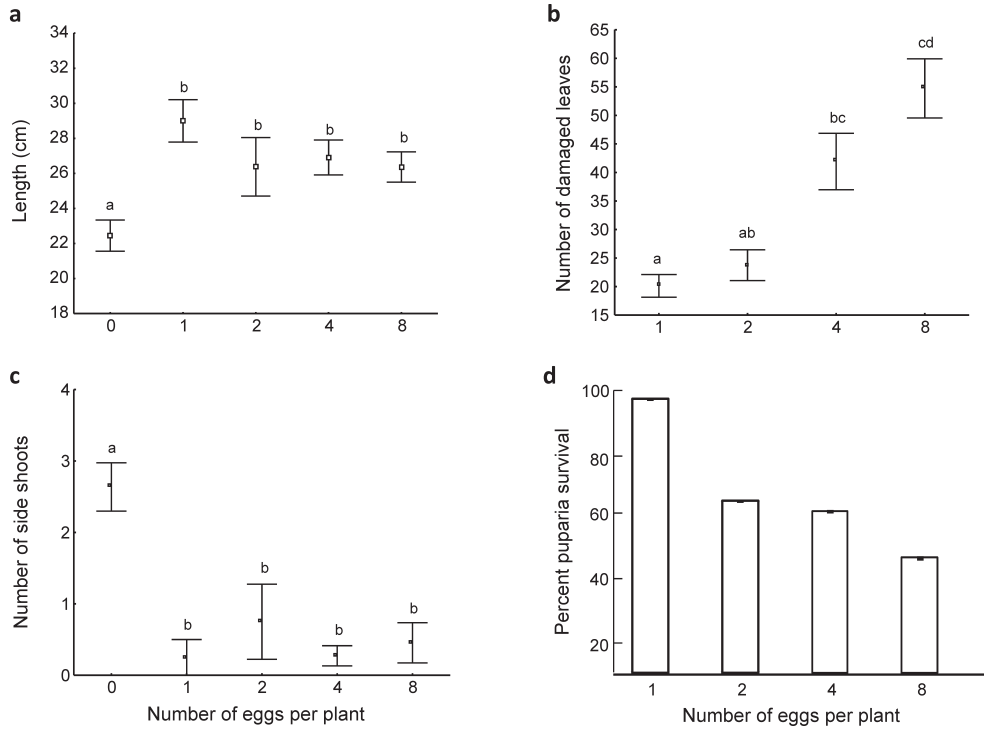

Fig. 2. Impact of *Hydrellia lagarosiphon* on *Lagarosiphon major* in relation to the number of *H. lagarosiphon* eggs placed on the shoots. **a**, Differences in *L. major* shoot length (cm) ($F_{(4,44)} = 4.72$, $P > 0.05$). **b**, Number of damaged *L. major* leaves per shoot ($H_{4,\ n=49} = 39.6$, $P > 0.05$). **c**, Number of *L. major* branches produced per shoot ($H_{4,\ n=49} = 24.3$, $P > 0.05$). **d**, Number of *H. lagarosiphon* puparia per shoot of *L. major* ($H_{4,\ n=49} = 40$, $P > 0.05$). Error bars represent S.E.; means followed by the same letter are not significantly different.

of roots produced by the shoots as there was no significant difference between the control and the different egg densities (0,1,2,4 and 8 eggs per shoot) ($H_{(4,49)} = 39.59$, $P < 0.05$). On average, *L. major* produced 17 ± 0.1 (S.E.) roots per plant.

DISCUSSION

Hydrellia lagarosiphon has a similar biology to other species of *Hydrellia* that are specific to other Hydrocharitaceae species, namely the Australian species, *H. balciunasi*, and the Asian species, *H. pakistanae,* which attack *H. verticillata* (Wheeler & Center 2001), and *Hydrellia* sp. n. which feeds on *E. densa* (Cabrera Walsh *et al*. 2012). These species and *H. lagarosiphon* lay eggs predominantly on protruding vegetation and shoot tips, and the neonate larvae prefer to move to the crown of the plant to feed initially on the softer younger shoots, and then move down the plant in search of new leaves. The larvae feed between the upper and lower leaf epidermal tissue layers, effectively reducing the plant's ability to photosynthesize and giving the leaf the appearance of being mined (Wheeler & Center 2001). Pupariation occurs between the epidermal layers of the leaf and adults live on the surface of the water and water body edge, moving *via* walking and short hopping flights.

Experiments in tanks investigating the relationship between the invasive *H. verticillata* and *H. pakistanae* showed that larval feeding damage to 10–30 % of leaves on a stem reduced the maximum rate of light-saturated photosynthesis of the plant by almost 40 %, and when leaf damage reached 70 % of leaves on a stem, photosynthetic rates were reduced by up to 60 % (Doyle *et al.* 2002). In order for the plant's daily respiratory requirements to no longer be met, leaf damage would have to reach densities of 70–90 % (Doyle *et al.* 2002). However, Wheeler & Center (2001) indicated that it would take approximately 4000 fly larvae/m^2 during a single generation to damage 60–70 % of the whorls of the *H. verticilliata* plant. *Hydrellia*

pakistanae was released in the U.S.A. in 1990 and recent surveys have shown that numbers never reach such densities, even under optimal conditions. The highest field densities recorded for *H. pakistanae* translated to approximately 15 % of the whorls damaged (Wheeler & Center 2001). In the indigenous range of *H. lagarosiphon,* on average 58 % of stems contained *H. lagarosiphon*, while sites with 100 % of stems infected were not uncommon. These comparatively higher densities suggest that field populations of the fly could significantly reduce photosynthesis of *L. major*, but this needs to be tested. The newest plant growth and growth tips were generally the most susceptible to larval damage. Similar damage has been recorded with other *Hydrellia* spp. and their host plants (Deonier 1971). In these laboratory experiments with *H. lagarosiphon*, only high larval densities (8 larvae per shoot) seemed to have a significant impact on the plant, although shoots were only exposed to a single generation of flies. Accumulative damage over several generations at low fly densities may cause significant levels of damage.

Hydrocharitaceae species such as *L. major* are known to form dense mats and canopies, limiting competition from other species (Howard-Williams & Davies 1988; Van *et al*. 1998; James *et al*.1999; Caffrey *et al*. 2007, 2010). *Hydrellia lagarosiphon* was shown to reduce branching by *L. major,* thus potentially restricting the plant's ability to form a dense canopy. Laboratory competition studies in the U.S.A. revealed that *H. verticillata* is a much stronger competitor than *Vallisneria americana* Michx at high nutrient levels, forming a dense surface canopy that competitively excludes *V. americana* (Van *et al.* 1998). When *H. pakistanae* was introduced, larval damage reduced the *H. verticillata* canopy in the top 30 cm of the water column. As a result, there was a significant shift in the competitive balance between *H. verticillata* and *V. americana* because the presence of *H. pakistanae* reduced competition in favour of *V. americana* (Van *et al.* 1998). The impact of a herbivore may be subtle, leading to a gradual reduction in the host plant's health, thereby increasing its susceptibility to competition from other vegetation (Pantone *et al.* 1989; Wheeler & Center 2001; Coetzee *et al*. 2005). Thus, even at densities lower than recorded in its indigenous range, *H. lagarosiphon* may be able to reduce the biomass of *L. major,* thereby opening areas for native plant re-growth, similar to the reduction recorded in *H. verticillata* when grown under the influence of herbivory and in competition with native plant species (Grodowitz *et al*. 2007).

Hydrellia lagarosiphon shows promise as a biological control agent, but abiotic factors could influence its success or failure. Possible mitigating abiotic factors may be similar to those experienced by *H. pakistanae* on *H. verticillata* in the U.S.A., namely cold winter temperatures which reduce both the fly's activity and the presence of its host plant (Wheeler & Center 2001), while wind, wave action, temperature and humidity tolerance restrict oviposition to sheltered habitats (Deonier 1971). However, from field observations, low temperatures do not appear to limit the distribution of *H. lagarosiphon* in its indigenous range. All large infestations of *L. major* were restricted to areas of South Africa that experience winter temperatures between –2 and +4 °C. Thus *H. lagarosiphon* should be able to survive in the more temperate climates of Europe and New Zealand where *L. major* has become a considerable problem in different freshwater habitats (Rattray *et al*. 1994; Caffrey *et al*. 2011). Research in invaded countries also raised concerns that *L. major* may not form surface-reaching weed beds that are typical of the indigenous range, thereby denying the fly the opportunity to oviposit on protruding plant parts (J. Clayton, NIWA, 2010 pers. comm.). However, our field observations in South Africa indicate that surfacing plants are not essential to ensure persistence of the fly because adults will lay eggs on the surface of the water and possibly on adjacent plants that are at the surface.

While the third trophic level is seldom considered to be important in weed biological control programmes (Harvey *et al.* 2010), parasitoids of *Hydrellia* spp. have been recorded from around the world (Deonier 1971; Hesler 1995; Wheeler & Center 2001; Diaz *et al.* 2009; Kula 2009). Thus, parasitism of *H. lagarosiphon* was expected in its native range and may possibly occur in its introduced range. The effect of parasitism on biological control agent populations is a relatively unstudied field, and warrants more investigation. However, Hill & Hulley (1995) argued that, based on investigations in South Africa, native parasitoids rarely affect the success of biological control agents and that no candidate agent should be rejected solely on its susceptibility to attack by native parasitoids. In contrast, Paynter *et al.* (2010) argued that para-

sitism is significantly associated with the failure of agents to suppress weed populations and that one should select agents that have species-poor parasitoid faunas in their country of origin. However, at field sites where parasitism levels were relatively high, *H. lagarosiphon* numbers remained high.

The genus *Hydrellia* is highly specialized and predominantly specific to aquatic plant species (Deonier 1971). Of 46 *Hydrellia* spp. found in the Holarctic region, where the genus has received most attention, 32 have host plants in the Hydrocharitaceae, Alismataceae or Potamogetonaceae (Buckingham *et al.* 1989). Twenty-one of the *Hydrellia* species listed by Deonier (1971) were reported to have only one host plant, while 10 species were recorded from one plant genus, one species from one plant family, 11 species from two plant families, and two species from three plant families. Only *H. griseola* (Fallkn) was reported from more than three plant families (Buckingham *et al*. 1989). No-choice tests conducted on *Hydrellia* sp. n. associated with *E. densa* revealed larval feeding on only two other Hydrocharitaceae species, but very few of the larvae that fed survived to adulthood on the non-target hosts (G. Cabrera Walsh, USDA-ARS 2011, pers. comm.). Both Buckingham & Okrah (1993) and Deonier (1971) reported that late instar larvae of *Hydrellia* spp. may move to adjacent non-host plants for pupariation, but this does not cause significant damage to the new host.

The contribution of *H. pakistanae* to the management of *H. verticilliata* in certain areas of the U.S.A. using *Hydrellia* spp. provides evidence that *Hydrellia* spp. are suitable for release as biological control agents on Hydrocharitaceae (Baars *et al.* 2010). *Hydrellia lagarosiphon* is the most ubiquitous and common herbivore species associated with *L. major* within its native range. Its biology is well understood and it has proved easy to rear under laboratory conditions, thereby showing great potential as a candidate agent for *L. major*. However further damage assessments and host-specificity testing would be required by any country intending to use the fly as a biological control agent.

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