Responses of adult Hydrellia lagarosiphon to a revised diet: Implications for life cycle studies and laboratory culturing…

Article *in* Entomologia Experimentalis et Applicata · September 2015

DOI: 10.1111/eea.12350

CITATIONS
0

READS
66

3 authors:

Rosie Mangan
TEAGASC - The Agriculture and Food Developm…
8 PUBLICATIONS **11** CITATIONS
SEE PROFILE

Tara Dirilgen
University College Dublin
6 PUBLICATIONS **18** CITATIONS
SEE PROFILE

Jan-Robert Baars
University College Dublin
56 PUBLICATIONS **368** CITATIONS
SEE PROFILE

E E A	12350	WILEY	Dispatch: 25.8.15	CE: Sumithra
Journal Code	Manuscript No.		No. of pages: 6	PE: Rajasekaran S

DOI: 10.1111/eea.12350

Responses of adult *Hydrellia lagarosiphon* to a revised diet: implications for life cycle studies and laboratory culturing techniques

Rosie Mangan*, Tara Dirilgen & Jan-Robert Baars

BioControl Research Unit, School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland

Accepted: 3 July 2015

Key words: biological control, Ephydridae, female fecundity, nutrition, reproduction, Hydrocharitaceae, Diptera, weed control, entomophagous

Abstract

Preservation of an insect culture under laboratory conditions is essential for its study. Numerous diets have been developed for entomophagous insects undergoing screening as biological control agents in attempts to improve the nutritional quality of food provided in laboratory settings. However, less emphasis has been given to developing a more effective laboratory diet for phytophagous insects with the adult life stage not dependant on the target species. The larvae of *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae) mine the leaves of *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae). This species is currently under consideration as a biological control agent of this aquatic invasive. Rearing techniques for the adult stage of other *Hydrellia* species have been developed but current diets are not ideal as they result in relatively low reproduction rates. We compared alternative nutritional regimes and quantified their impact on life history attributes of *H. lagarosiphon*. The diets included the previously developed yeast-sugar diet, a newly developed insect-derived diet, and a diet that combined the two. Total fecundity was significantly higher for females on an insect-derived diet compared with the traditional carbohydrate diet and the net reproductive rate (R_o) was also higher. Population doubling time (T_d) was lower, decreasing by 30% compared to the traditional laboratory diet developed for *Hydrellia* species. Adult females fed the combination diet, including both insect and non-insect foods, laid 30% fewer eggs than those reared on an insect diet alone. Consequently, insect derived nutritional regimes could improve culturing techniques significantly and if permission to release the agent is granted, this diet may benefit mass rearing efforts potentially saving time and reducing associated costs.

Introduction

The ability to rear insects under laboratory conditions is an essential component to facilitate research in biological control programs. Research on insects can be facilitated if the insect species undergoing evaluation can be colonized and mass produced in the quantities required for both pre-release screening and later for release (Glenister & Hoffmann, 1998; Nordlund, 1998; Smith & Nordlund, 1999, 2000; Riddick, 2009). *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), a leaf-mining fly, is currently being investigated as a biological control agent of the submerged invasive macrophyte *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae) in Ireland (Baars et al., 2010; Mangan & Baars, 2013) and New Zealand (Paynter, 2013).

Rearing techniques have been developed for *Hydrellia* flies under laboratory conditions, dating back to the late 1980s following the importation of *Hydrellia balciunasi* Bock and *Hydrellia pakistanae* Deonier into quarantine as part of a control effort on *Hydrilla verticillata* (L.f.) Royle in the USA (Buckingham et al., 1989). The laboratory diet described for *H. balciunasi* and *H. pakistanae* consists of a sugar–yeast hydrolyzate mixture (4 g yeast hydrolyzate, 7 g sucrose, 10 ml water) and a sugar–water mixture

*Correspondence and present address: Rosie Mangan, Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown 6140, South Africa. E-mail: manganrosemary@gmail.com

(1:1), and this has largely been the culturing method employed for the majority of *Hydrellia* species undergoing evaluation as biological control agents to date (Grodowitz et al., 1993, 1994, 1997; Balciunas & Burrows, 1996; Martin et al., 2010; Cabrera Walsh et al., 2012; Mangan & Baars, 2013; Sonal Varia, pers. comm.). This approach has been tested on *H. lagarosiphon* (Mangan & Baars, 2013), and although it is not unusual that fecundity varies between congeneric species, the egg production was almost 70% lower than that of adult female *H. pakistanae* fed on the same artificial diet under laboratory conditions (Buckingham et al., 1989; Buckingham & Okrah, 1993).

Oogenesis is a nutrient-limited process and nutritional supply can significantly impact egg production (Wheeler & Center, 1996). The comparatively low oviposition rates for *H. lagaroisphon* fed on the classical artificial diet suggest it is inadequate and does not promote female oviposition to its full potential. Numerous diets have been developed for entomophagous insects undergoing screening as biological control agents in an attempt to improve the nutritional quality of the diet under laboratory conditions (Abdel-Salam & Abdel-Baky, 2001; Arijs & De Clercq, 2001; Specty et al., 2003; De Clercq et al., 2005; Hamasaki & Matsui, 2006). This is primarily driven by the need to produce large numbers of natural enemies of high quality for experimental purposes during the screening process and also mass rearing of the population prior to release (Glenister & Hoffmann, 1998; Nordlund, 1998; Smith & Nordlund, 1999, 2000; Riddick, 2009).

An extensive body of literature exists on artificial diets for insect predators (Thompson, 1999; Riddick, 2009); however, little consideration has been given to developing a more effective laboratory diet for phytophagous insects where adult stages require an alternative food supply other than the plant. In the field, adult *Hydrellia* flies prey on very small insects or insects trapped in the surface film. These include amongst others the adult and pupal stages of aquatic macroinvertebrates like mayflies (Ephemeroptera), non-biting midges (Chironomidae), or terrestrially derived insects including other species of *Hydrellia* (Denoir, 1970). Other components of their diet include yeasts, fungi, Cyanophyta, Chrysophyta, nectar, and leaf epidermis. In the laboratory, the adults of *H. lagarosiphon* are restricted to the artificial diet provided; however, they also scavenge and feed on dead adult *H. lagarosiphon*. Although the current laboratory diet contains key dietary components capable of supporting adult reproduction (Mangan & Baars, 2013), it is important that the breadth of nutritional requirements of *H. lagarosiphon* be satisfied, as this ensures the continuous production of high-quality progeny in insects (Cohen, 2004). The absence of the insect component in the laboratory diet, usually available in the field, probably fails to maximize the rates of oviposition in *H. lagarosiphon* adults. Insect-derived materials supplied in the diet of insects appear to be generally beneficial, improving their growth, development, and fecundity (Nettles, 1990; Grenier et al., 1994). In this study, we attempted to improve the artificial diet used to rear *H. lagarosiphon* in the laboratory by including insect-derived materials. The consequence of different diets on the life table and demographic life parameters of *H. lagarosiphon* were determined.

Materials and methods

Insect and plant cultures

The insect cultures and experiments were conducted in the quarantine facility at University College Dublin (Ireland) in controlled environment rooms. The laboratory culture of *H. lagarosiphon* originates from a field collection made in July 2009 from the Eastern Cape, South Africa (32°33′52.01″S, 22°29′22.42″E). Laboratory cultures were reared in transparent plastic boxes (30 × 15 × 15 cm) fitted with a nylon mesh window maintained at 21.7 ± 0.1 °C under fluorescent lighting in a long-day cycle (L16:D8 h). Cultures were initiated with up to 40 adults, placed into the boxes containing about 30 excised shoot tips of *L. major* in ca. 4–5 cm of dechlorinated water. Flies were fed with a yeast hydrolysate-sugar solution [4 g Bacto™ TC yeastolate (BD Diagnostics, Oxford, UK), 7 g sugar, 10 ml H_2O; Buckingham et al. (1991)] and honey provided on floating feeding stations. The yeastolate contains an indeterminate mixture of amino acids, peptides, polysaccharides, vitamins, and minerals. After 3–4 days exposure the adults were removed and the container was filled with water and additional plant material. The plant material was field collected throughout the year from Lough Corrib (County Galway, Ireland) and maintained in 600-l circulation tanks kept in a polytunnel. The current study was initiated after populations were reared for at least 15 generations, and specimens used in trials all originated from the same generation.

Drosophila melanogaster cultures

A laboratory culture of *D. melanogaster* Meigen was obtained from Blades Biological Ltd. (Kent, UK). The initial culture consisted of ca. 50 individuals from each life stage: adults, pupae, larvae, and eggs. *D. melanogaster* adults were transferred and reared in transparent plastic cylindrical containers (13.5 × 13.2 cm), fitted with a nylon mesh window on the lid. An approximately 1-cm layer of Blades' *Drosophila* quick mix medium™ was added to each container, which was mixed with 100 ml water to provide the larvae with nutrition. Approximately 5 g of

Bacto TC yeastolate was added to the culture to provide the adults with nutrition. After a 7-day exposure the adults were removed and transferred to a new container.

Preparation of diets

Female fecundity was assessed under each of four dietary conditions. (1) Traditional diet: 4 g Bacto TC yeastolate, 7 g sugar and 10 ml H_2O solution was prepared and absorbed using a strip of tissue (0.5 × 1.5 cm). This was placed in a Petri dish alongside a gauze-covered drop of honey; (2) Insect diet: Adult *D. melanogaster* were placed in a freezer for 2 min, removed, and the abdomen was crushed with a sterile forceps to expose the abdominal cavity. Ten adult *D. melanogaster* were placed in ddH_2O and presented on a small square of gauze in a Petri dish; (3) Combination diet: a combination of diets (1) and (2) was provided in roughly equal proportions; (4) Starvation: no food was provided. Each replicate was replaced with fresh food every 2nd day to prevent contamination.

Fecundity experiment

To determine the fecundity of *H. lagarosiphon*, newly emerged flies (<12 h old) were collected and ca. 160 mating pairs were isolated (ca. 40 per dietary condition). The adult mating pairs were placed in 0.5-l transparent plastic containers and exposed to five fresh *L. major* shoot tips in 6–7 cm of dechlorinated water. A Petri dish (3.5 × 1.1 cm) provided a surface to place the food source. The containers were covered with a nylon mesh. Fecundity trials were run for 5, 10, and 15 days (15 days represents 80% of the average adults life stage at 20 °C: 90% of all eggs are laid within this time period, after which egg viability declines to 25%; Mangan & Baars, 2013). Every 5 days, female fecundity was recorded for each replicate and mating pairs were transferred to a new container. At the end of each trial period a sub-set of replicates (ca. 12–15 replicates from each dietary condition) was terminated. The mean number of eggs laid every 5 days and the total number of eggs laid during a 15-day period was recorded.

Egg viability

All eggs laid during the 5-, 10-, and 15-day trial periods were monitored to assess egg viability. All eggs were counted and then monitored daily for a 14-day period for egg hatch. Each container and its contents were thoroughly investigated under a microscope for split eggs, indicating emergence, and larvae present, indicating survival to the next life stage. Survival was recorded as successful development from hatched eggs to the larval stage.

Life table and demographic growth parameters

A life table for *H. lagarosiphon* was constructed for each dietary condition using the development times, survivorship rates of all the life stages, and sex ratios of the offspring, as calculated for *H. lagarosiphon* by Mangan & Baars (2013), in combination with female fecundity assessed under the four dietary conditions in this study. The following demographic parameters were calculated from the life tables: net reproductive rates (R_o), mean generation time (T_c), intrinsic rate of natural increase (r_m), and population doubling time (T_d). Mean demographic parameter estimates with standard errors were then generated using bootstrap pseudo-replication of the net female maternity life table data using methods proposed by Pilkington & Hoddle (2006). This method created 1 000 pseudo-replicates for each demographic parameter.

Statistical analysis

Mean female fecundity and egg viability, and the pseudo-values for R_o, r_m, T_c, and T_d were analysed with ANOVA followed by Tukey's honestly significant difference (HSD) tests ($\alpha = 0.05$) using STATISTICA 7 (StatSoft, Tulsa, OK, USA).

Results

Reproduction, development, and life table parameters

The diet with which the fly was reared significantly affected the fecundity of female *H. lagarosiphon* (ANOVA: $F_{3,31} = 21\ 369.72$, $P<0.01$). The mean number of eggs laid during a 15-day period was lowest when adults were starved, with females laying on average seven eggs. This increased to around 84 eggs when adults were supplied with an insect diet (Table 1). Egg viability was not significantly different among food treatments. However, when adults were starved egg viability decreased significantly, particularly when compared to when adults were reared on an insect diet ($F_{3,31} = 2\ 016.74$, $P<0.01$).

Differences in development and reproductive characteristics of adult females among diets were also reflected in the life table statistics (Table 1). The net reproductive rate (R_o) of *H. lagarosiphon* fed on an insect diet was higher than on the traditional laboratory diet ($F_{3,3996} = 212\ 921.2$, $P<0.01$). Similarly, the population doubling time (T_d) ($F_{3,3996} = 19\ 109.3$, $P<0.01$) was different among the dietary conditions and was lowest when adults were reared on an insect diet. Mean generation time (T_c) was also different between fed and starved treatments ($F_{3,3996} = 692\ 337.1$, $P<0.01$) and females that were not provided with food did not survive for more than 5 days, resulting in a reduced mean generation time of 53 days. Finally, the intrinsic rate of

Table 1 Mean (± SE) fecundity, subsequent egg viability, and various life table parameters of female *Hydrellia lagarosiphon* monitored for a period of 15 days on each of four diets

Parameter	Traditional diet	Insect diet	Combination diet	Starvation
Total no. eggs (/female)	34.5 ± 2.2a	84.2 ± 10.6b	61.3 ± 9.2ab	7.0 ± 1.0a
Egg viability (%)	72.8 ± 2.6ab	83.5 ± 2.2b	74.9 ± 2.3ab	52.1 ± 2.2a
Net reproductive rate R_o (no. females/female)	11.5 ± 0.1a	33.1 ± 0.4c	18.2 ± 0.4b	0.8 ± 0.0a
Intrinsic rate of increase r_m (no. females/female/day)	0.04 ± 0.0b	0.06 ± 0.0d	0.05 ± 0.0c	−0.01 ± 0.0a
Mean generation time T_c (days)	57.2 ± 0.2b	57.2 ± 0.2b	57.7 ± 0.1b	53.0 ± 0.0a
Population doubling time T_d (days)	16.2 ± 0.5d	11.4 ± 0.0b	13.9 ± 0.1c	−106.2 ± 12.4a

Means within a row followed by different letters are significantly different (Tukey HSD: P<0.05).

increase (r_m) of the flies fed on the traditional laboratory diet was lower than adults reared on both the combination and insect diet ($F_{3,3996}$ = 14 568.0, P<0.01).

Dietary conditions also affected the egg laying patterns of *H. lagarosiphon* (Figure 1). Adult females that were starved only laid eggs in the first 5 days, as both males and females failed to survive beyond this time, presumably due to malnutrition. Adults fed the traditional laboratory diet laid the majority of their viable eggs (74%) during the first 10 days of egg production with a notable decrease in the last 5 days of the experiment. Viable egg production among adults reared on an insect diet was consistently higher throughout the trial period compared to adults fed the traditional laboratory diet.

Discussion

The main objective of this study was to develop a more suitable diet for *H. lagarosiphon* and investigate the impact it has on the reproductive and developmental attributes of the fly. Previous studies have demonstrated how the use of insects and insect materials in artificial laboratory diets can enhance the survival and oviposition of entomophagous insects. Under field conditions, *Hydrellia* feed on a broad range of dead insects (Denoir, 1970); however, the original laboratory diet developed for these flies is devoid of any insect or insect-derived components (Buckingham et al., 1989). Our study indicates that the inclusion of insects in the diet of *H. lagarosiphon* is extremely beneficial. The insect diet appears to be highly nutritious and adults reproduced with more success compared to adults fed the traditional diet of sugar–yeast hydrolyzate mixture developed by Buckingham et al. (1989) and used since (Grodowitz et al., 1993, 1994, 1997; Balciunas & Burrows, 1996; Cabrera Walsh et al., 2012; Mangan & Baars, 2013; Sonal Varia, pers. comm.).

Knowledge of the biology and rearing techniques of *Hydrellia* is essential in using these natural enemies in a biological control program. Biology and development

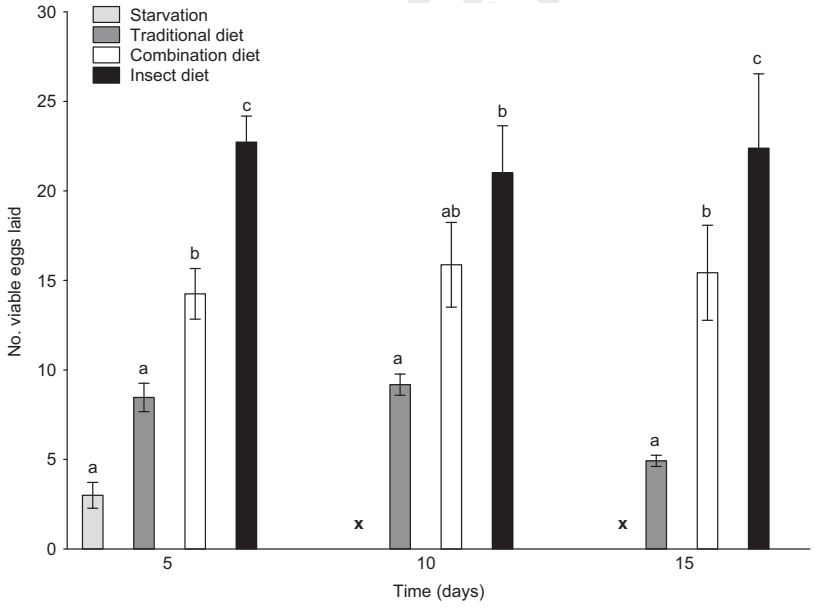

Figure 1 Mean (± SE) number of viable eggs laid by adult female *Hydrellia lagarosiphon* supplied with four dietary conditions: starvation, traditional diet, combination diet, or insect diet, after 5, 10, and 15 days. No females on a starvation diet survived to day 10. Bars within time periods headed by a different letter are significantly different (Tukey HSD: P<0.05).

work revealed the potential for colonization success of *H. lagarosiphon* in Europe by calculating the number of annual generations from a degree-day model for this species (Mangan & Baars, 2013). The results indicated that *H. lagarosiphon* would be capable of completing between one and eight generations per year across Western Europe. However, population growth was calculated based on adult reproductive performance when adult mating pairs were supplied with the traditional sugar–yeast hydrolyzate diet. The demographic growth estimates in the current study now show that the published estimates (Mangan & Baars, 2013) are conservative. This study reveals that a diet more closely resembling that utilized under field conditions results in higher population growth as indicated by the demographic growth parameters. The inclusion of insects in the diet enhances rates of egg laying, increasing the net reproductive rate (R_o) by 200% and decreasing the population doubling time (T_d) by 30% compared to the traditional laboratory diet developed by Buckingham et al. (1989).

These results demonstrate the usefulness of employing a nutritional regime that more closely resembles the field diet and how it can drastically increase reproduction in *H. lagarosiphon* and potentially the impact this agent will have on target weed populations. The study also demonstrates the usefulness of investigating food sources that more closely resemble those in the wild and that can support and increase the development and reproductive rates of natural enemies under laboratory conditions. Successful production of large numbers of natural enemies is essential when they are required for experimental purposes during the screening process and later for mass rearing prior to release.

Acknowledgements

We gratefully acknowledge the ongoing help we have received from the staff of Inland Fisheries Ireland and the assistance provided by colleagues in the Department of Zoology and Entomology, Rhodes University, South Africa, and also the Irish Research Council, and the CAISIE Project EU Life+ Program for the provision of funding. We would like to thank Steve Compton for his editorial assistance in the preparation of the manuscript.

References

Abdel-Salam AH & Abdel-Baky NF (2001) Life table and biological studies of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) reared on the grain moth eggs of *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae). Journal of Applied Entomology 125: 455–462.

Arijs Y & De Clercq P (2001) Development of an oligidic diet for *Orius laevigatus* nymphs using a deletion-addition approach. Mededelingen van de Fakulteit der Landbouwkundige en Toegepaste Biologische Wetenschappen van de Rijksuniversiteit Gent 66: 315–320.

Baars J-R, Coetzee J, Martin G, Hill MP & Caffrey JM (2010) Natural enemies from South Africa improve the prospects for biological control of *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae) in Europe. Hydrobiologia 656: 149–158.

Balciunas JK & Burrows DW (1996) Distribution, abundance and field host-range of *Hydrellia balciunasi* Bock (Diptera: Ephydridae) a biological control agent for the aquatic weed *Hydrilla verticillata* (L.f.) Royle. Australian Journal of Entomology 35: 125–130.

Buckingham GR & Okrah EA (1993) Biological and Host Range Studies with Two Species of Hydrellia (Diptera: Ephydridae) that Feed on Hydrilla. Technical Report A-93-7. US Army Engineer Waterways Experiment Station, Vicksburg, MS, USA.

Buckingham GR, Okrah E & Thomas MC (1989) Laboratory host range tests with *Hydrellia pakistanae* (Diptera: Ephydridae), an agent for biological control of *Hydrilla verticillata* (Hydrocharitaceae). Environmental Entomology 18: 164–171.

Buckingham GR, Okrah EA & Christian-Meier M (1991) Laboratory biology and host range of *Hydrellia balciunasi* (Diptera: Ephydridae). Entomophaga 36: 575–586.

Cabrera Walsh G, Magalí Dalto Y, Mattioli FM, Carruthers RI & Anderson LW (2012) Biology and ecology of Brazilian elodea (*Egeria densa*) and its specific herbivore, *Hydrellia* sp., in Argentina. BioControl 57: 1–15.

Cohen AC (2004) Insect Diets. CRC Press, Boca Raton, FL, USA.

De Clercq P, Bonte M, Van Speybroek K & Bolkmans K (2005) Development and reproduction of *Adalia bipunctata* (Coleoptera: Coccinellidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Phycitidae) and pollen. Pest Management Science 61: 1129–1132.

Denoir DL (1970) A systematic and ecological study of Nearctic *Hydrellia* (Diptera: Ephydridae). Smithsonian Contributions to Zoology 68: 1–147.

Glenister CS & Hoffmann MP (1998) Mass-reared natural enemies: scientific technological, and informational needs and considerations. Mass-Reared Natural Enemies: Application, Regulation, and Needs (ed. by R Ridgway, MP Hoffmann, MN Inscoe & CS Glenister), pp. 242–247. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD, USA.

Grenier S, Greany PD & Cohen AC (1994) Potential for mass release of insect parasitoids and predators through development of artificial culture techniques. Pest Management in the Subtropics: Biological Control—A Florida Perspective (ed. by D Rosen, FD Bennett & JL Capinera), pp. 181–205. Intercept Publishers, Andover, UK.

Grodowitz MJ, Center TD, Snoddy E & Rives E (1993) Status of the Release and Establishment of Insect Biocontrol Agents of Hydrilla. Proceedings of the 27th Annual Meeting of the Aquatic Plant Control Research Program, Bellevue, WA, USA, pp. 93–98.

Grodowitz MJ, Center TD, Snoddy E & Dray FAJr (1994) Release and Establishment of Insect Biocontrol Agents for the Management of Hydrilla. Proceedings of the 28th Annual Meeting of the Aquatic Plant Control Research Program, Baltimore, MD, USA, pp. 181–201.

Grodowitz MJ, Center TD, Cofrancesco AF & Freedman JE (1997) Release and establishment of *Hydrellia balciunasi* (Diptera: Ephydridae) for the biological control of the submersed aquatic plant *Hydrilla verticillata* (Hydrocharitaceae) in the United States. Biological Control 9: 15–23.

Hamasaki K & Matsui M (2006) Development and reproduction of an aphidophagous coccinellid, *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae), reared on an alternative diet, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. Applied Entomological Zoology 41: 233–237.

Mangan R & Baars J-R (2013) Use of life table statistics and degree day values to predict the invasion success of *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), a leaf mining fly of *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae), in Ireland and the rest of Europe. Biological Control 64: 143–151.

Martin GD, Coetzee JA & Baars J-R (2010) *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae), a potential biological control agent for the submerged aquatic weed *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae). African Entomology 21: 151–160.

Nettles WC (1990) In vitro rearing of parasitoids: role of host factors in nutrition. Archives of Insect Biochemistry and Physiology 13: 167–175.

Nordlund DA (1998) Capacity and quality: keys to success in the mass rearing of biological control agents. Natural Enemies of Insects 20: 169–179.

Paynter Q (2013) Feasibility of Biocontrol of *Lagaorsiphon major* in New Zealand. Landcare Research Contract Report LC 1234. Landcare Research, Auckland, New Zealand.

Pilkington LJ & Hoddle MS (2006) Use of life table statistics and degree-day values to predict the invasion success of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae), an egg parasitoid of *Homalodisca coagulata* (Hemiptera: Cicadellidae), in California. Biological Control 37: 276–283.

Riddick EW (2009) Benefits and limitations of factitious prey and artificial diets on life parameters of predatory beetles, bugs, and lacewings: a mini-review. BioControl 54: 325–339.

Smith RA & Nordlund DA (1999) Automation of insect rearing – a key to the development of competitive augmentative biological control. Natural Enemies of Insects 21: 70–81.

Smith RA & Nordlund DA (2000) Mass rearing technology for biological control agents of *Lygus* spp. Southwest Entomologist 23: 121–127.

Specty O, Febvay G, Grenier S, Delobel B, Piotte C et al. (2003) Nutritional plasticity of the predatory ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae): comparison between natural and substitution prey. Archives of Insect Biochemistry and Physiology 52: 81–91.

Thompson SN (1999) Nutrition and culture of entomophagous insects. Annual Review of Entomology 44: 561–592.

Wheeler GS & Center TD (1996) The influence of hydrilla leaf quality on larval growth and development of the biological control agent *Hydrellia pakistanae* (Diptera: Ephydridae). Biological Control 7: 1–9.