

***Eupeodes americanus* and *Leucopis annulipes* as potential biocontrol agents of the foxglove aphid at low temperatures**

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Abstract: The foxglove aphid, *Aulacorthum solani* Kaltenbach (Hemiptera: Aphididae), has become a significant pest in ornamental horticulture as it can build high populations at low temperatures from 10-18 °C. Currently, only chemical control is used, as no commercially available biological control agent is effective at these temperatures. Two new potential biocontrol agents were evaluated in the laboratory at low temperatures: the silverfly, *Leucopis annulipes* Zetterstedt (Diptera: Chamaemyiidae) and the American hoverfly, *Eupeodes (Metasyrphus) americanus* (Wiedemann) (Diptera: Syrphidae). Active flight, oviposition and larval voracity were evaluated at 12 °C, 15 °C and 18 °C. The number of eggs laid by females was calculated after 7 days (18 °C, 15 °C, and 12 °C) and 14 days (12 °C). The number of aphids consumed by larvae was determined after 8 days and 8 hrs (12 °C), 6 days and 6 hrs (15 °C) and 5 days (18 °C). Results indicated that hoverflies laid a significantly higher number of eggs than silverflies at all temperatures. When given an additional 7 days at 12 °C, oviposition increased for both species. Hoverfly larvae consumed 2 times more aphids than silverfly larvae at 12 °C, 15 °C and 18 °C. Globally, the present study demonstrates a clear superiority of the hoverfly over the silverfly at low temperatures, and identifies it as a potential biocontrol agent of the foxglove aphid.

Key words: Syrphidae, Chamaemyiidae, biological control, *Aulacorthum solani*, greenhouses

Introduction

The foxglove aphid, *Aulacorthum solani* Kaltenbach (Hemiptera: Aphididae) is a growing problem in horticulture (Jandricic *et al.*, 2014). Agricultural production in North-eastern America starts in January and February. The temperature in greenhouses is kept low in order to save on heating costs (University of Minnesota's Center for Urban and Regional Affairs and Center for Sustainable Building Research, 2013). Contrary to other species of aphids, *A. solani* has a high fecundity at 5 °C, 10 °C, and 20 °C, where few natural enemies are effective (Jandricic *et al.*, 2010). There is no current biological control agent commercially available for use at low temperatures (10 °C to 20 °C) against the foxglove aphid (Schüder *et al.*, 2004; Katsarou *et al.*, 2005; Alotaibi, 2008; Lee *et al.*, 2008; Jalali *et al.*, 2010; Jandricic, 2013). Insecticides currently used against aphids in Canada on ornamental crops include Intercept™ 60 WP (imidacloprid) and Endeavor® 50 WG (pymetrozine) (Ministry of Agriculture, Food and Rural Affairs, 2014).

The Nearctic *Eupeodes (Metasyrphus) americanus* (Wiedemann) (Diptera: Syrphidae) is a generalist aphid predators (Rojo *et al.*, 2003). The biology of *E. americanus* is poorly known, however other syrphids that prey upon aphids are active early in the spring and late in

the fall; when temperatures are low (Dixon *et al.*, 2005; Tamaki *et al.*, 1967). This is consistent with the thermal development threshold of other syrphid species, which is between 5 and 7 °C (Hart *et al.*, 1997; Honěk & Kocourek, 1988). The second potential biocontrol agent, the silverfly, *Leucopis annulipes* Zetterstedt (Diptera: Chamaemyiidae) consumes many species of aphids (Satar *et al.*, 2015) and live within the colony without eliciting defensive responses from their prey (Fréchette *et al.*, 2008).

The aim of the present study was to assess the potential of two new biological control agents, the American hoverfly and the silverfly for the repression of *A. solani*. To achieve this, active flight, female oviposition and larval voracity for both predators were evaluated and compared at low temperatures.

Material and methods

Insect rearing

All insect rearings were carried out at the Université du Québec à Montréal (UQÀM) in the Laboratoire de Lutte Biologique. Foxglove aphids (*Aulacorthum solani*) were reared on green pepper plants, *Capsicum annuum* L. (Solanaceae) (18 °C, 16 L:8 D photoperiod and 60% R.H). Hoverfly (*Eupeodes americanus*) rearings were based on the Frazer method (Frazer, 1972). Adults were reared in controlled conditions (22 °C during the day, 19 °C at night and 60% R.H.). Eggs were collected and kept in Petri dishes at 4 °C, 60% R.H. Larvae were reared on green peach aphids, *Myzus persicae* Sulzer (Hemiptera: Aphididae). Silverfly (*Leucopis annulipes* Zetterstedt) were reared on potato plants inoculated with *M. persicae* following Gaimari & Turner (1996) methodology and kept in the same growth chamber as hoverflies.

Active flight capacity experiment

Active flight capacity for the American Hoverfly and Silverfly was studied at 12, 15, and 18 °C. A minimum of 16 males and 16 females of each species aged of 18 to 36 hours at the beginning of the test were individually placed in the center of a Plexiglas[®] box which was placed in a Conviron[™] growth chamber at 700 LUX and 60% R.H. Insects were given a 30 minutes acclimation period before starting the tests and each individual was observed for a period of 10 minutes. The presence or absence of active flight, as well as the delay from introduction of the insect in the box to the observation of active flight, was noted.

Egg-laying experiment

The number of eggs laid by American hoverfly and silverfly females was measured at 12 °C, 15 °C, and 18 °C. Twenty females and twenty males from each species, aged less than 24 hrs were collected from the colony established in the laboratory. One male and one female were introduced in a transparent plastic cylinder containing a green pepper plant with 6 to 8 leaves inoculated with 200 foxglove aphids of different stages. Cylinders were placed in a growth chamber for 7 days at 12, 15, and 18 °C and 14 days at 12 °C, 16 L:8 D, 50% R.H. Tests were repeated at 12 °C for 14 days to account for prolongation of egg maturation. The number of eggs laid as well as the number of larvae on the plant was counted at the end of each trial.

Larval voracity experiment

The number of aphids consumed by American hoverfly and silverfly larvae was calculated at 12, 15, and 18 °C, 16 L:8 D, 50% R.H. Green pepper plants were placed in the same type of cylinders described earlier for the egg-laying tests. Thirty 2nd instar foxglove aphids were

placed on each green pepper plant. The treatment consisted of the introduction of a stage I American hoverfly larva or silverfly larva. A control was carried out without predators. Cylinders were put inside a Conviron™ growth chamber for 5 days at 18 °C, 6 days and 6 hours at 15 °C, and 8 days, 8 hours at 12 °C. At the end of each test, the number of living aphids was calculated as such: initial density (30 aphids) – number of living aphids. If a larva was feeding on an aphid at the moment of data collection, it was considered as dead. Daily consumption was also calculated: total consumption/ duration of the test according to the temperature tested for.

Data analysis

Statistical analyses were performed with the statistical package JMP® 12 (JMP, 2015). All experiment variables were analyzed by a two-way ANOVA and a Tukey's HSD test was used to identify treatments that were significantly different from each other.

Results and discussion

Delays before active flight (in seconds) varied according to all 3 temperatures for *E. americanus* ($F_{2, 141} = 15.6$, $P < 0.0001$; $\mu_{18} = 59.1$; $\mu_{15} = 110.1$; $\mu_{12} = 210.6$) and *L. annulipes* ($F_{2, 64} = 18.8$, $P < 0.0001$; $\mu_{18} = 60.6$; $\mu_{15} = 81.69$; $\mu_{12} = 387.3$). No significant difference was observed between the two species at 12 °C ($F_{2, 201} = 11.5$, $P = 0.5673$), 15°C ($F_{2, 201} = 11.5$, $P = 0.6306$), and 18 °C ($F_{2, 201} = 11.5$, $P = 0.9999$). However, all *Eupeodes americanus* demonstrated active flight at 18 °C, 88.7% could fly at 15 °C, while only 14.5% could fly at 12 °C. For *L. annulipes*, 88.7% demonstrated active flight at 18 °C, 24.1% at 15 °C, and 7.8% at 12 °C.

In the egg-laying trial, the average number of eggs laid by *E. americanus* ($\mu_{18} = 113$; $\mu_{15} = 46.9$; $\mu_{12} = 11.6$) was significantly greater than *L. annulipes* ($\mu_{18} = 2.09$; $\mu_{15} = 1.18$; $\mu_{12} = 0$) after 7 days ($F_{2, 223} = 84.73$, $P < 0.0001$) and 14 days at 12 °C ($F_{2, 51} = 53.12$, $P < 0.0001$; $\mu = 101$ for the hoverfly and $\mu = 1.33$ for the silverfly). When given an additional 7 days (14 days in total), the average number of eggs laid at 12 °C went from 0 to 1.33 eggs for the silverfly and from 11.6 eggs to 101 eggs for the hoverfly. This is the first study that examines female oviposition performance at low temperatures for *E. americanus*.

Average daily aphid consumption was at least 2 times greater for the hoverfly larvae ($\mu_{18} = 4.54$; $\mu_{15} = 3.65$; $\mu_{12} = 2.80$) when compared to the silverfly ($\mu_{18} = 1.27$; $\mu_{15} = 1.05$; $\mu_{12} = 0.88$) ($F_{2, 235} = 175.55$, $P < 0.0001$). Daily aphid consumption was significantly greater for the hoverfly when compared to the silverfly at 18 °C, and 12 °C ($F_{2, 235} = 235.05$, $P < 0.0001$). Total aphid consumption was significantly greater for the hoverfly ($\mu_{18} = 22.7$; $\mu_{15} = 22.8$; $\mu_{12} = 23.3$) when compared to the silverfly ($\mu_{18} = 6.35$; $\mu_{15} = 6.59$; $\mu_{12} = 7.29$) at 18 °C, 15 °C, and 12 °C ($F_{2, 235} = 222.38$, $P < 0.0001$).

According to what occurs in most invertebrates, performances of both *E. americanus* and *L. annulipes* were negatively affected at low temperatures. However, while *L. annulipes* is clearly not an adequate biological control agent against the foxglove aphid at low temperatures, the American hoverfly demonstrated a high potential against the target aphid and may be considered for commercialization. This is the first study to look at the efficiency of the American hoverfly against the foxglove aphid in terms of active flight, oviposition, and voracity at low temperatures. Finally, this study may contribute to the commercialization of a new biological control agent and the reduction of pesticide use (Frank, 2010).

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References

- Alotaibi, S. 2008: Mass production and utilization of the predatory midge, *Aphidoletes aphidimyza* Rondani for controlling aphids. *Global J. Biotech. Biochem.* 3: 1-7.
- Dixon, A. F. G., Jarošík, V. & Honěk, A. 2005: Thermal requirements for development and resource partitioning in aphidophagous guilds. *Eur. J. Entomol.* 102: 407-411.
- Frank, S. D. 2010: Biological control of arthropod pests using banker plant systems: Past progress and future directions. *Biol. Control.* 52: 8-16.
- Frazer, B. D. 1972: A simple and Efficient Method of Rearing Aphidophagous Hoverflies (Diptera: Syrphidae). *J. Entomol. Soc. B. C.* 69: 23-24.
- Fréchette, B., Larouche, F. & Lucas, E. 2008: *Leucopis annulipes* larvae (Diptera: Chamaemyiidae) use a furtive predation strategy within aphid colonies. *Eur. J. Entomol.* 105: 399-403.
- Gaimari, S. D. & Turner, W. J. 1996: Methods for Rearing Aphidophagous *Leucopis* spp. (Diptera: Chamaemyiidae). *J. Kans. Entomol. Soc.* 69: 363-369.
- Hart, A. J., Bale, J. S. & Fenlon, J. S. 1997: Developmental threshold, day-degree requirements and voltinism of the aphid predator *Episyrphus balteatus* (Diptera: Syrphidae). *Ann. Appl. Biol.* 130: 427-437.
- Honěk, A. & Kocourek, F. 1988: Thermal requirements for development of aphidophagous Coccinellidae (Coleoptera), Chrysopidae, Hemerebiidae (Neuroptera), and Syrphidae (Diptera): some general trends. *Oecologia* 76: 455-460.
- Jalali, M. A., Tirry, L. & De Clercq, P. 2010: Effect of temperature on the functional response of *Adalia bipunctata* to *Myzus persicae*. *BioControl* 55: 261-269.
- Jandricic, S. E., Wraight, S. P., Bennett, K. C. & Sanderson, J. P. 2010: Developmental Times and Life Table Statistics of *Aulacorthum solani* (Hemiptera: Aphididae) at Six Constant Temperatures, With Recommendations on the Application of Temperature-Dependent Development Models. *Environ. Entomol.* 39: 1631-1642.
- Jandricic, S. E. 2013: Investigations of the biology of the pest aphid *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae) and of biological control agents for control of multi-species aphid outbreaks in greenhouse floriculture crops (Doctoral Dissertation). Cornell University, 260 pp.
- Jandricic, S. E., Mattson, N. S., Wraight, S. P. & Sanderson, J. P. 2014: Within-Plant Distribution of *Aulacorthum solani* (Hemiptera: Aphididae), on Various Greenhouse Plants with Implications for Control. *J. Econ. Entomol.* 107: 697-707.
- JMP 2015: SAS Institute Inc. (Version 12.1). Cary, NC, USA.
- Katsarou, I., Margaritopoulos, J. T., Tsitsipis, J. A., Perdakis, D. Ch. & Zarpas, K. D. 2005: Effect of temperature on development, growth, and feeding of *Coccinella septempunctata* and *Hippodamia convergens* reared on the tobacco aphid, *Myzus persicae nicotianae*. *BioControl* 50: 565-588.

- Lee, S. G., Kim, H. H., Park, G. J., Kim, K. H. & Kim, J. S. 2008: Development model of foxglove aphid, *Aulacorthum solani* (Kaltenbach). Korean J. Appl. Entomol. 47: 359-364.
- Ministry of Agriculture, Food and Rural Affairs 2014: Guide to Greenhouse Floriculture Production. Publication 370, Toronto, Canada.
- Rojas, S., Gilbert, F., Marcos-García, M. A., Nieto, J. M. & Mier Durante, M. P. 2003: A world review of predatory hoverflies (Diptera, Syrphidae: Syrphinae) and their prey. Centro Iberoamericano de la Biodiversidad (CIBIO) Ediciones, Universidad de Alicante, Alicante, Spain.
- Satar, S., Raspi, A., Özdemir, I., Tusun, A., Karacaoğlu, M. & Benelli, G. 2015: Seasonal habits of predation and prey range in aphidophagous silver flies (Diptera Chamaemyiidae), an overlooked family of biological control agents. B. Insectol. 68: 173-180.
- Schüder, I., Hommes, M. & Larink, O. 2004: The influence of temperature and food supply on the development of *Adalia bipunctata* (Coleoptera: Coccinellidae). Eur. J. Entomol. 101: 379-384.
- Tamaki, G., Landis, B. J. & Weeks, R. E. 1967: Autumn populations of green peach aphid on peach trees and the role of syrphid flies in their control. J. Econ. Entomol. 60: 433-336.
- University of Minnesota Center for Urban and Regional Affairs and Center for Sustainable Building Research 2013: Cold Climate Greenhouse Resource: A guidebook for designing and building a cold-climate greenhouse. The Regents of the University of Minnesota, USA.