

Lygus rugulipennis on chrysanthemum: Supplemental prey effects and an evaluation of trap plants

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Abstract

The European tarnished plant bug, *Lygus rugulipennis* Poppius, is considered a major pest in chrysanthemum nurseries in The Netherlands. Adults puncture plant's apical meristem, after which the growing point splits and growth is inhibited. Flower buds and flowers can also be severely damaged. Both types of damage result in economic losses for growers. Despite the importance of this pest for chrysanthemum nurseries, there is only very limited information about *L. rugulipennis* development on chrysanthemum plants, *Chrysanthemum* × *morifolium* Ramat., and whether *L. rugulipennis* can be controlled using trap plants is not known. We investigated whether: (1) *L. rugulipennis* could develop from egg to adult on the vegetative and flowering stages of chrysanthemum; (2) their performance was enhanced when a supplemental prey source (*Ephestia kuehniella* Zeller eggs) or another common pest (the green peach aphid, *Myzus persicae* Sulzer) was present; and (3) there were alternative plant species more attractive than chrysanthemum for use as trap plants for local pest control or monitoring of *L. rugulipennis*. *L. rugulipennis* developed on both vegetative and flowering chrysanthemum stages without any additional food source. Nonetheless, when chrysanthemum was supplemented with *E. kuehniella* eggs, *L. rugulipennis* achieved the best performance in terms of the number of adults developed and faster developmental time. Interestingly, *L. rugulipennis* developed faster on chrysanthemum infested with the aphid *M. persicae* compared to non-infested plants, however, there was no difference in the number of adults developed. In a trap plant experiment with 16 plant species in the vegetative stage, we found that white mustard, *Sinapis alba* L., was significantly more attractive than chrysanthemum to both adult and nymph *L. rugulipennis*. Further research is needed to evaluate the potential of *S. alba* as a trap plant for monitoring *L. rugulipennis* and how the presence of prey in the crop influences *L. rugulipennis*.

KEYWORDS

Ephestia kuehniella, European tarnished plant bug, horticultural pest, integrated pest management, *Myzus persicae*, *Sinapis alba*

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1 | INTRODUCTION

Some members of the genus *Lygus* Hanh (Heteroptera) are highly damaging pests to a range of crops (McBrien & Millar, 1999; Rämert et al., 2001). The most common phytophagous species of this genus in Europe is the European tarnished plant bug, *Lygus rugulipennis* Poppius (Heteroptera: Miridae) (Holopainen, 1986; Schwartz & Foottit, 1998; Varis, 1972). The damage caused by *L. rugulipennis* is via feeding punctures and saliva rich in degrading plant tissue enzymes, such as polygalacturonase (D'Ovidio et al., 2004; Frati et al., 2006; Strong, 1970; Varis, 1972). In addition, *L. rugulipennis* can inflict damage during oviposition because females use their ovipositors to lacerate host plant tissue to lay eggs causing deformations and growth losses in the affected host (Conti & Bin, 2001; Holopainen, 1986; Salerno et al., 2007; Varis, 1972). Furthermore, *L. rugulipennis* can also vector plant viruses (Turka, 1985).

Lygus rugulipennis has been recorded on 437 herbaceous and woody host plants from 57 different plant families (Holopainen, 1989; Holopainen & Varis, 1991; Schwartz & Foottit, 1998). Among those plants, oviposition or nymphal development has been observed in 59 plant species (Holopainen & Varis, 1991). Families with the most recorded host plants are Asteraceae, Brassicaceae, and Fabaceae (Holopainen & Varis, 1991). Several host plants are important commercial crops, such as alfalfa, *Medicago sativa* L. (Fabaceae), potato, *Solanum tuberosum* L. (Solanaceae), sugar beet, *Beta vulgaris* L. (Amaranthaceae), and strawberry, *Fragaria × ananassa* Duchesne (Rosaceae) (Easterbrook et al., 2003; Holopainen & Varis, 1991).

In Dutch chrysanthemum nurseries, *L. rugulipennis* is considered a pest insect that causes major crop losses (Hennekam et al., 2012). Damage can easily be observed as plants infested with *L. rugulipennis* are up to 15 cm shorter than non-infested plants of the same age. This damage is caused by one or a few *L. rugulipennis* adults puncturing the plant's apical meristem, after which the growing point splits and its growth is inhibited. *L. rugulipennis* also damages flowers, resulting in spots on petals, irregular growth, and indentations, making flowers non-marketable products. Despite the importance of this pest for chrysanthemum nurseries, nothing is known about the ability of *L. rugulipennis* to develop its full life cycle on chrysanthemum plants, *Chrysanthemum × morifolium* Ramat. (Asteraceae) (Hennekam et al., 2012; Holopainen & Varis, 1991).

Although *L. rugulipennis* is considered as a pest, it is also an omnivore feeding on both plants and prey, like many other mirids (Coll & Guershon, 2002). Mirids range from phytozoophagy (prey-feeding herbivores) to zoophytophagy (plant-feeding carnivores) (Coll & Guershon, 2002). For example, the predator *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) is released as a predator in tomato crops, but is also considered as a pest when densities are getting high (Moerkens et al., 2020). Within the genus *Lygus*, the phytozoophagous western tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae), is an important pest in cotton, but also a predator of whiteflies (Hagler & Naranjo, 2005). Prey feeding has

also been reported for *L. rugulipennis* on other pests such as aphids (Holopainen & Varis, 1991; Salerno et al., 2007). However, to what extent the presence of supplemental prey affects the performance of *L. rugulipennis* on chrysanthemum plants is not known. Besides *L. rugulipennis*, other pests can be present in Dutch chrysanthemum nurseries, for example, the cotton aphid, *Aphis gossypii* Glover, and the green peach aphid, *Myzus persicae* Sulzer (both Hemiptera: Aphididae), are regularly found on this crop and can increase population size rapidly (Vehrs et al., 1992). They damage by sucking on plant parts and producing honeydew which encourages black sooty mould (Vehrs et al., 1992). Concurrent damage by *Lygus* pests and aphids can thus be common in chrysanthemum nurseries, yet is not known how the performance of *L. rugulipennis* is affected when aphids are present on the same plant.

As part of biological pest control, supplemental food, like *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs is often supplied to released natural enemies as part of a 'standing army' approach (Messelink et al., 2014; Pijnakker et al., 2020). This enables populations of biological control agents to be sustained and ready to eliminate pest insects early after crop colonization. However, if the pest is an omnivore, some caution is needed, because the provided food sources may promote pest growth (Leman & Messelink, 2015; van Rijn et al., 2002). Hence, pests might take advantage of biological control strategies aimed to favour carnivore insects. It is not known if *L. rugulipennis* benefits from supplemental food in chrysanthemum crops.

A promising alternative to the use of chemical control for *L. rugulipennis* is the implementation of 'trap crops'. A trap crop represents a plant that is more attractive for a pest than the main crop itself (Shelton & Badenes-Perez, 2006). Such attractive plants can be placed either between or around the main crop and act as a sink for the pest, thus facilitating supplemental management practices aimed at pest removal or elimination (Shelton & Badenes-Perez, 2006). This method is successfully used in various crops (reviewed by Hokkanen, 1991; Shelton & Badenes-Perez, 2006). For *Lygus* spp., it has been shown that *M. sativa* can be successfully used as a trap crop (Sevacherian & Stern, 1974; Swezey et al., 2013). In organic strawberry fields in California, *L. hesperus* preferred the interplanted trap crop *M. sativa* over organic strawberries (Swezey et al., 2007). Bugs present in the trap crop were then regularly removed by vacuuming—bugvac (Swezey et al., 2007). It was subsequently shown that *L. hesperus* did not disperse from *M. sativa* into strawberry plants (Swezey et al., 2013). Whether trap crops can also be implemented in chrysanthemum nurseries to control *L. rugulipennis*, as *M. sativa* works for *L. hesperus*, has not been investigated yet.

In this study, we conducted a series of experiments to investigate the performance and development of *L. rugulipennis* on chrysanthemum plants, *C. × morifolium*, and whether trap plants can be used for monitoring *L. rugulipennis*. Specifically, we investigated whether: (1) *L. rugulipennis* can develop from egg to adult on chrysanthemum in the vegetative and flowering stages without additional food; (2) the presence of supplemental prey source (*E. kuehniella* eggs) as well as another common pest (the aphid *M. persicae*) can affect the

performance of *L. rugulipennis* on chrysanthemum in the vegetative stage; and (3) there are alternative plant species more attractive than chrysanthemum for *L. rugulipennis*. In total, 16 plant species in the vegetative stage were tested for their potential function as trap plants for *L. rugulipennis*.

2 | MATERIALS AND METHODS

2.1 | Insects

A colony of *L. rugulipennis* was established with individuals collected from potato fields in The Netherlands. *L. rugulipennis* nymphs and adults were reared separated inside transparent buckets (10.8 L, H 26.3 cm, Ø 27.0 cm; Houweling group, Bleiswijk, The Netherlands [NL]) closed with pantyhose to provide ventilation and prevent bugs from escaping. Bugs were reared with a diet consisting of pods of the flat bean, *Phaseolus vulgaris* L. (Fabaceae), and frozen eggs of *E. kuehniella* (Koppert B.V., Berkel & Rodenrijs, NL). Food was changed twice a week. The rearing was kept in a climate cabinet ($22 \pm 1^\circ\text{C}$, 70% relative humidity (RH) and 16:8 (L:D) photoperiod) at the Business Unit Greenhouse Horticulture, Wageningen University and Research (WUR), Bleiswijk, NL.

2.2 | Development of flowering and non-flowering chrysanthemum plants

A greenhouse trial was set up to test the performance of *L. rugulipennis* on chrysanthemum plants, *C. x morifolium*, either in the vegetative or in the flowering stage. Six *C. x morifolium* potted plants (pot Ø = 35 cm) of the cultivar 'Baltica' (Deliflor, Maasdijk, NL), grown without the use of crop protection products, were placed in a BugDorm cage (W 75 × D 75 × H 115 cm; MegaView Science Co. Ltd., Taichung, Taiwan). Fertigation was supplied to the plants by an automatic dripping system during the experiment. To prevent adults or nymphs from drowning in water, a plate with vermiculite was placed under the pot. The greenhouse was set up at standard chrysanthemum nursery settings ($20 \pm 1^\circ\text{C}$ daytime, $17 \pm 1^\circ\text{C}$ nighttime, 70% RH).

Per cage, five males and five females *L. rugulipennis* of 1-week-old, was released for 72 h and allowed to lay eggs. After 72 h all adults were removed. Three times a week, cages were checked for the presence of nymphs which were monitored until they reached the adult stage, after which they were removed. At the start of the experiment, vegetative chrysanthemum cuttings were about 10 cm and chrysanthemum with flower buds/flowers were about 70 cm tall. When flower buds emerged on vegetative plants, they were immediately removed. In both treatments, three replicates were performed and we scored the following fitness-related proxies: (1) the number of offspring developed until the adult stage; (2) the sex ratio of the developed adults, and (3) the developmental time from egg until the adult stage.

2.3 | Development in the presence of supplemental food or aphids

A greenhouse trial was set up to test the performance of *L. rugulipennis* on potted chrysanthemum plants in the vegetative stage. The following treatments were tested: (1) clean plants (control); (2) *E. kuehniella* eggs, provided once a week (ad libitum) on the chrysanthemum plants; and (3) *M. persicae* aphids. Aphids (approximately 100 mixed-stage instars; ad libitum) were introduced at the beginning of the experiment. Plants were weekly observed for the presence of aphids. In case there were less than 100 aphids present on a plant, more aphids were added till approximately 100 individuals were present. Aphids came from a culture of sweet pepper, *Capsicum annuum* L. (Solanaceae).

One *C. x morifolium* potted plant (pot Ø = 12 cm) of the cultivar 'Tempo Time' (SV.CO, De Lier, NL), grown without the use of crop protection products, was placed in a BugDorm cage (W60 × D60 × H60 cm). Fertigation was supplied to the plants by an automatic dripping system during the experiment. To prevent adults or nymphs from drowning in water, a plate with vermiculite was placed under the pot. The greenhouse was set up at standard chrysanthemum nursery settings ($20 \pm 1^\circ\text{C}$ daytime, $17 \pm 1^\circ\text{C}$ nighttime, 70% RH).

Per cage, five males and five females *L. rugulipennis* of 1-week-old, was released for 48 h and allowed to lay eggs. After 48 h all adults were removed and the treatments with supplemental prey were added. Three times a week, cages were checked for the presence of nymphs which were monitored until they reached the adult stage, after which they were removed. At the start of the experiment, all chrysanthemum plants had the same size (having several branches and about 20 cm tall). When flower buds emerged, they were immediately removed. For each treatment, three replicates were performed. Cages were placed in a block design inside a greenhouse. We scored the following fitness-related proxies: (1) the number of offspring developed until the adult stage; (2) the sex ratio of the developed adults, and (3) the developmental time from egg until the adult stage.

2.4 | Trap plant experiment

To screen for plant species more attractive for *L. rugulipennis* adults than chrysanthemum plants, a multiple-choice experiment with 16 different plant species (Table 1) was set up under greenhouse conditions. Plants were selected based on literature or on preliminary investigations. All 16 plant species were grown from seeds or young plant material (Table 1). To ensure plants were free of pesticides, biological seeds were used. Lavender, *Lavandula angustifolia* Mil. 'Hidcote' (Lamiaceae) and common nettle, *Urtica dioica* L. (Urticaceae) plants were bought/collected 2 months before the experiment started and placed per plant species in a quarantine cage and checked weekly on the presence of pest insects, which were removed when present. *L. angustifolia* was pruned after buying, so

TABLE 1 Plant species used for the trap plant experiment. Host plants for adults, nymphs, and oviposition as recorded by Holopainen and Varris (1991) are indicated with an X.

Plant family	Plant species	Common name	Recorded host plant	Recorded oviposition or nymphal growth	Collection
Amaryllidaceae	<i>Allium sativum</i> L.	Garlic			Bulbs, biological garden, Leerdam, The Netherlands
Amaranthaceae	<i>Amaranthus caudatus</i> L.	Love-lies-bleeding	X		Seeds, Intratuin, Zevenhuizen, The Netherlands
Asteraceae	<i>Artemisia vulgaris</i> L.	Common mugwort	X	X	Seeds, Cruydt-Hoeck, Nijeberkoop, The Netherlands
	<i>Chrysanthemum</i> L. 'Baltica'	Chrysanthemum 'Baltica'			Cuttings, Deliflor, Maasdijk, The Netherlands
	<i>Leucanthemum vulgare</i> Lam.	Oxeye daisy	X	X	Seeds, Cruydt-Hoeck, Nijeberkoop, The Netherlands
Brassicaceae	<i>Brassica nigra</i> L.	Black mustard	X		Seeds, Cruydt-Hoeck, Nijeberkoop, The Netherlands
	<i>Sinapis alba</i> L.	White mustard	X		Seeds, Cruydt-Hoeck, Nijeberkoop, The Netherlands
Cucurbitaceae	<i>Cucurbita pepo</i> L. 'Jack O'Lantern'	Pumpkin Jack O'Lantern 'amateur variety'			Seeds, Intratuin, Zevenhuizen, The Netherlands
Fabaceae	<i>Medicago sativa</i> L.	Alfalfa	X	X	Seeds, Cruydt-Hoeck, Nijeberkoop, The Netherlands
	<i>Phaseolus vulgaris</i> L.	Bean 'stambonen bruine noordhollandse'	X	X	Seeds, TuinPlus, Heerenveen, The Netherlands
	<i>Trifolium repens</i> L.	White clover			Seeds, Cruydt-Hoeck, Nijeberkoop, The Netherlands
Lamiaceae	<i>Lavandula angustifolia</i> Mill. 'Hidcote'	Lavender 'Hidcote'			Plants, Intratuin, Zevenhuizen, The Netherlands
Scrophulariaceae	<i>Ocimum basilicum</i> L.	Lettuce leaf basil			Seeds, Intratuin, Zevenhuizen, The Netherlands
	<i>Verbascum thapsus</i> L.	Great mullein	X		Seeds, Cruydt-Hoeck, Nijeberkoop, The Netherlands
Solanaceae	<i>Solanum tuberosum</i> L.	Potato	X	X	Potatoes, supermarket, Vlaardingen, The Netherlands
Urticaceae	<i>Urtica dioica</i> L.	Common nettle	X	X	Plants, nature area, Bleiswijk, The Netherlands

Note: All plants were kept in the vegetative stage during the experiment.

the newly produced shoots would be free of pesticides. *U. dioica* was grown for a month after which new shoots were potted in new pots and placed in new cages for a month before the start of the experiment. All 16 plant species were placed in the vegetative stage in a large cage with insect netting (W120 x D120 x H120 cm). Plants were about the same size and placed randomly on a table in the greenhouse arranged in rows of four by four plants, with equal distance between the plants (30cm, measured from the middle of the pot). Six replicates (cages) were performed using a randomized experimental design.

At the start of the experiment, 35 males and 35 females *L. rugulipennis* of 1-week-old, were released in the middle of the cage. One week later, another 15 males and 15 females *L. rugulipennis* of 1-week-old, were released per cage. To monitor the abundance of *L. rugulipennis* adults found on the plants as a proxy to assess 'attraction', three times a week during a period of 6 weeks plants were visually inspected and the number of *L. rugulipennis* adults were recorded per plant. At the end of the experiment, all plants were assessed and the number of *L. rugulipennis* nymphs and adults were recorded per plant. Adults were caught first from all plants in all replicates with an insect aspirator. The numbers of nymphs per plant were counted after cutting plants in pieces and shaking these plant parts above a large container. During the experiment all plants were kept in the vegetative stage, no additional food was added and fertigation was supplied by an automatic dripping system. The experiment was conducted at $20 \pm 1^\circ\text{C}$, 70% RH.

2.5 | Statistical analysis

Data from the development experiments were analysed with generalized linear mixed models (GLMMs). The number of *L. rugulipennis* recorded from test and control plants were analysed using GLMMs with Poisson error distribution and a log link function, modelling the treatment as a fixed effect and the replicate as a random effect. The development time of *L. rugulipennis* was analysed using GLMMs with

Gamma error distribution and a reciprocal link function, fitting the treatment as a fixed effect and the replicate as a random effect. The sex ratio of *L. rugulipennis* was analysed using GLMMs with binomial error distribution and a logit link function, modelling the treatment as a fixed effect and the replicate as a random effect. The significance of the fixed terms in the GLMMs was determined using Likelihood Ratio Tests comparing the full model with and without the factor in question (Crawley, 2007). Model fit was assessed with residual plots. Post-hoc tests for the developmental experiment with additional food or aphid treatment (*E. kuehniella* eggs, *M. persicae*, and no food as control) were carried out using the *glht* function in the *multcomp* package in R (Bretz et al., 2010). Trap plant data were analysed with chi-square tests with Bonferroni correction for multiple comparisons to investigate plant's differences in attraction to *L. rugulipennis* compared to chrysanthemum. Preferences for both adults and nymphs were compared using chi-square tests to determine whether the observed distribution of bugs on plants significantly diverged from a 50:50 distribution, which is expected if *L. rugulipennis* do not display any preferences. All statistical analyses were performed with the R software version 3.1.3 (R Core Team, 2013).

3 | RESULTS

3.1 | Development on flowering and non-flowering chrysanthemum plants

Lygus rugulipennis developed on chrysanthemum, *C. x morifolium*, plants in the vegetative and flowering stage. In both treatments, eggs laid by females successfully hatched and nymphs develop into adults (Figure 1a). A higher number of *L. rugulipennis* developed into an adult when fed on reproductive parts of chrysanthemum compared to vegetative parts (Poisson GLMM, $\chi^2 = 35.773$, $df = 1$, $p < 0.001$) (Figure 1a). However, no significant effects in development time (binomial GLMM, $\chi^2 = 0.299$, $df = 1$, $p = 0.585$) (Figure 1b) or sex ratio were found (gamma GLMM, $\chi^2 = 1.158$, $df = 1$, $p = 0.282$).

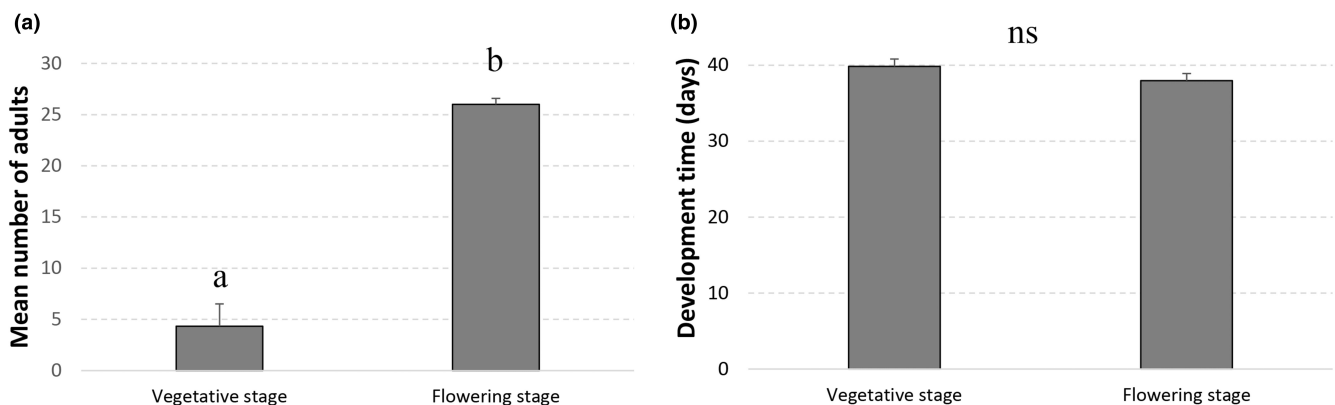


FIGURE 1 (a) Mean numbers per replicate of *Lygus rugulipennis* adults developed and (b) development time in days of *L. rugulipennis* from egg until adult stage on *Chrysanthemum x morifolium* 'Baltica' plants either in the vegetative or flowering stage. Different letters above the bars indicate significantly different means between treatments. NS indicate no significant differences between treatments (GLMM, $p < 0.05$).

3.2 | Development in the presence of supplemental food or aphids

The performance of *L. rugulipennis* on vegetative chrysanthemum plants was affected by the presence of additional food (Figure 2a). When *M. persicae* were present on the plants, a higher number of *L. rugulipennis* offspring developed into adults compared to the control (non-aphid infested plants), however, this difference was not significant (Tukey test, $z = 1.961$, $p = 0.120$). Significantly more offspring developed into adults when the additional food (*E. kuehniella* eggs) was present compared to the control ($z = 4.068$, $p < 0.001$), and more offspring developed into adults when *E. kuehniella* eggs were present compared to the presence of *M. persicae* aphids ($z = 2.487$, $p = 0.033$).

Aphid presence on chrysanthemum plants significantly reduced the development time (Figure 2b) of *L. rugulipennis* compared to control plants (Tukey test, $z = 4.073$, $p < 0.001$). The same effect was found when *E. kuehniella* eggs were present compared to the control ($z = 6.582$, $p < 0.001$). The presence of *E. kuehniella* eggs or *M. persicae* aphids on chrysanthemum plants did not affect the sex ratio of *L. rugulipennis* compared with the control (binomial GLMM, $\chi^2 = 4.553$, $df = 2$, $p = 0.103$).

3.3 | Trap plant experiment

Lygus rugulipennis adults were recorded on 14 of 16 plant species during the experiment (Figure 3). No adults were recorded on *L. angustifolia* 'Hidcote' and on white clover, *Trifolium repens* L. (Fabaceae). The highest percentage of adults were recorded on white mustard, *Sinapis alba* L. (Brassicaceae), love-lies-bleeding, *Amaranthus caudatus* L. (Amaranthaceae) and chrysanthemum 'Baltica' (Figure 3). *S. alba* was the only species that was significantly more attractive to *L. rugulipennis* adults than chrysanthemum ($\chi^2 = 10.376$, $df = 1$, $p < 0.001$) (Figure 3). *A. caudatus* ($\chi^2 = 2.456$, $df = 1$, $p = 0.117$), black mustard, *Brassica nigra* L. (Brassicaceae) ($\chi^2 = 0.03$, $df = 1$, $p = 0.859$) and *S. tuberosum* ($\chi^2 = 0.072$, $df = 1$, $p = 0.788$) were as equally

attractive as chrysanthemum. Chrysanthemum was significantly more attractive than the other 11 plant species (Figure 3).

Nymphs were recorded on all plant species, except on garlic, *Allium sativum* L. (Amaryllidaceae). A total of 1024 *L. rugulipennis* nymphs were recorded on the plants. The highest percentage of nymphs were recorded on *S. alba*, *A. caudatus*, and chrysanthemum 'Baltica' (Figure 4). *S. alba* was the only plant species on which significantly more *L. rugulipennis* nymphs were found than on chrysanthemum ($\chi^2 = 11.977$, $df = 1$, $p < 0.001$) (Figure 4). On *S. tuberosum* ($\chi^2 = 0.497$, $df = 1$, $p = 0.481$) and *U. dioica* ($\chi^2 = 4.445$, $df = 1$, $p = 0.350$) equally nymphs were found compared to chrysanthemum. On chrysanthemum significantly more nymphs were found than on 12 of the tested plant species (Figure 4).

4 | DISCUSSION

In this study, we showed that *L. rugulipennis* can develop from egg to adult on chrysanthemum plants without supplementary food. Our results indicated that more offspring develop into adults on plants at the flowering stage than on vegetative plants. Presumably, *L. rugulipennis* nymphs use pollen and nectar of chrysanthemum flowers which might be food sources of higher quality than leaves for nymphal survival, although we found no differences in terms of development time. Besides plant quality, also cannibalism among nymphs might have determined the number of nymphs developing to the adult stage since cannibalism has been observed by Hagler et al. (2020) for *L. hesperus* at low levels. Although *L. rugulipennis* develops better on chrysanthemum plants at the flowering stage, they are also able to reproduce successfully on small, vegetative chrysanthemum plants without any additional food source. This finding suggests that *L. rugulipennis* can establish a growing population inside a chrysanthemum nursery. Since most nymphs will probably develop in the flowering plants, growers should thus take precautions when harvesting them to avoid nymphs dispersing onto younger plants at the vegetative stage which they still can continue their development.

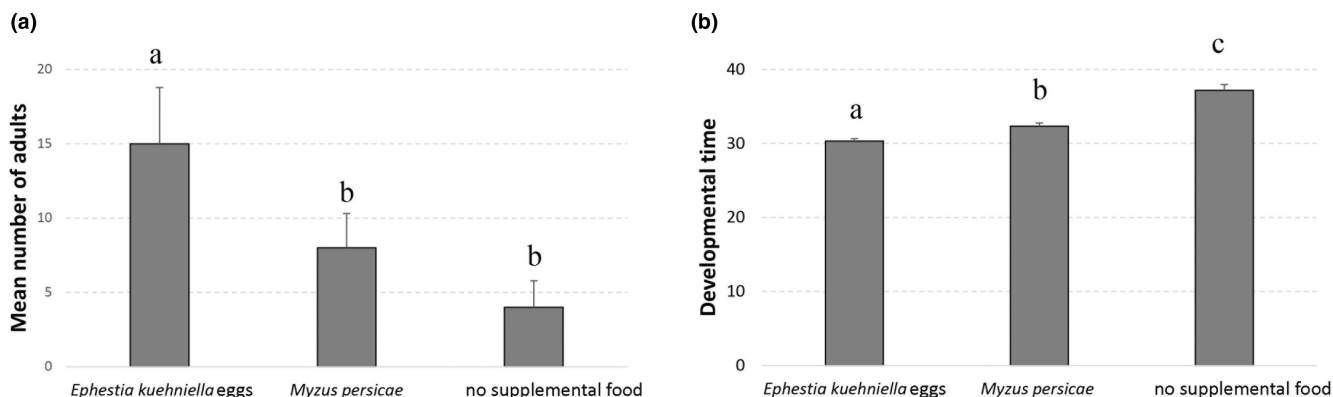


FIGURE 2 (a) Mean numbers per replicate of *Lygus rugulipennis* adults developed and (b) development time in days of *L. rugulipennis* from egg deposition until adult stage on *Chrysanthemum x morifolium* 'Tempo Time' in vegetative stage supplemented with *Ephestia kuehniella* eggs, the presence of the common pest *Myzus persicae* aphids and no food (clean plant; control). Different letters above bars indicate significantly different means among treatments (GLMM, $p < 0.05$).

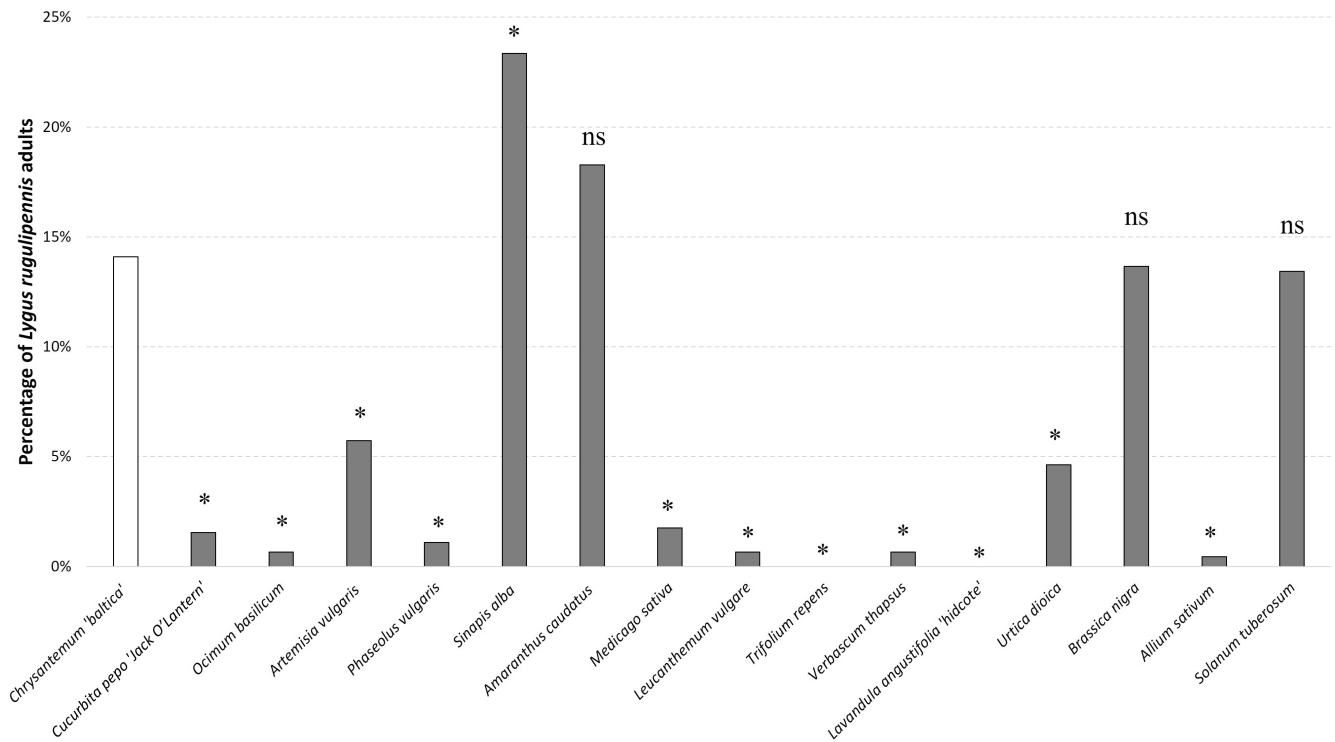


FIGURE 3 Percentage of *Lygus rugulipennis* adults recorded on 16 plant species during the trap plant experiment based on the sum of all observations. The asterisks above bars indicate significant differences between recorded *L. rugulipennis* adults on *Chrysanthemum × morifolium* 'Baltica' compared with the other plant species ($p < 0.05$ according to Chi-square test with Bonferroni correction). NS, no significant differences.

It is currently common practice to grow rows of different stages of chrysanthemum in the same nursery to provide a continuous supply of marketable plants. Because of this working method, it will be very difficult for growers to get rid of *L. rugulipennis* in their nurseries as they can develop on all stages of chrysanthemum as well *L. rugulipennis* will continuously damage plants which affects production.

Lygus rugulipennis developed faster when *M. persicae* were present on chrysanthemum plants, suggesting that infestations of *M. persicae* can promote *L. rugulipennis* in the crop. Growers should thus aim to control other pests in chrysanthemum nurseries to avoid faster development of *L. rugulipennis*. We hypothesized that there might be two possible mechanisms that could explain these findings. First, the most obvious reason for the better performance of plants with prey is the higher nutritional value of this mixed diet compared to a diet of only plant material (Coll & Guershon, 2002). The pest *L. rugulipennis* is not a strict herbivore, but rather an omnivore, which seems to be beneficial for this pest's performance and may explain its successful survival in many cropping systems. *Lygus rugulipennis* prefers to feed on apical meristems and flower buds of plants (Varis, 1972), but it can also predate on other insects (Holopainen & Varis, 1991). We have seen *L. rugulipennis* nymphs and adults feeding on *M. persicae*, as well Varis (1972) reported that *L. rugulipennis* fed on eggs of the mangold fly, *Pegomya betae* Curtis (Diptera: Anthomyiidae), and Salerno et al. (2007) reported feeding on the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), and on pupae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), providing evidence that *L. rugulipennis* has a diet consisting of both plants and

insects. Similarly, other *Lygus* species are reported to feed on a wide variety of prey including several lepidopteran and hemipteran species (reviewed by Wheeler, Wheeler, 1976, Wheeler, 2001). The finding of Salerno et al. (2007), together with ours, supports our hypothesis that *L. rugulipennis* can prey directly on aphids and thus the increased performance that we observed when *L. rugulipennis* feeds on plants infested with aphids might be due to direct trophic effects. The second possible reason is that plant-mediated interactions between the two herbivores play a role. Herbivores, such as aphids, activate salicylic acid (SA) defence signalling pathways in host plants which facilitate the subsequent attack of other herbivores inducing jasmonic acid (JA) defence signalling pathway (Ponzo et al., 2013; Stam et al., 2014). Future research should evaluate whether phytohormonal signalling between SA and JA occurs when *L. rugulipennis* feeds on chrysanthemum plants attacked by *M. persicae*.

We also showed that *L. rugulipennis* achieved the best performance (i.e., developed 6.8 days faster compared to the control) when *E. kuehniella* eggs are supplemented as a food source. As *E. kuehniella* eggs represent high-quality additional food sources for supporting predators such as *Orius laevigatus* Fieber and *Orius majusculus* Reuter (both Hemiptera: Anthocoridae) in chrysanthemum nurseries (Messelink et al., 2019; Pijnakker et al., 2020), a side effect might be that *L. rugulipennis* can benefit from the addition of supplementary food sources. We suggest that caution is needed in chrysanthemum nurseries to maximize the performance of biological control agents while minimizing the benefits for pests.

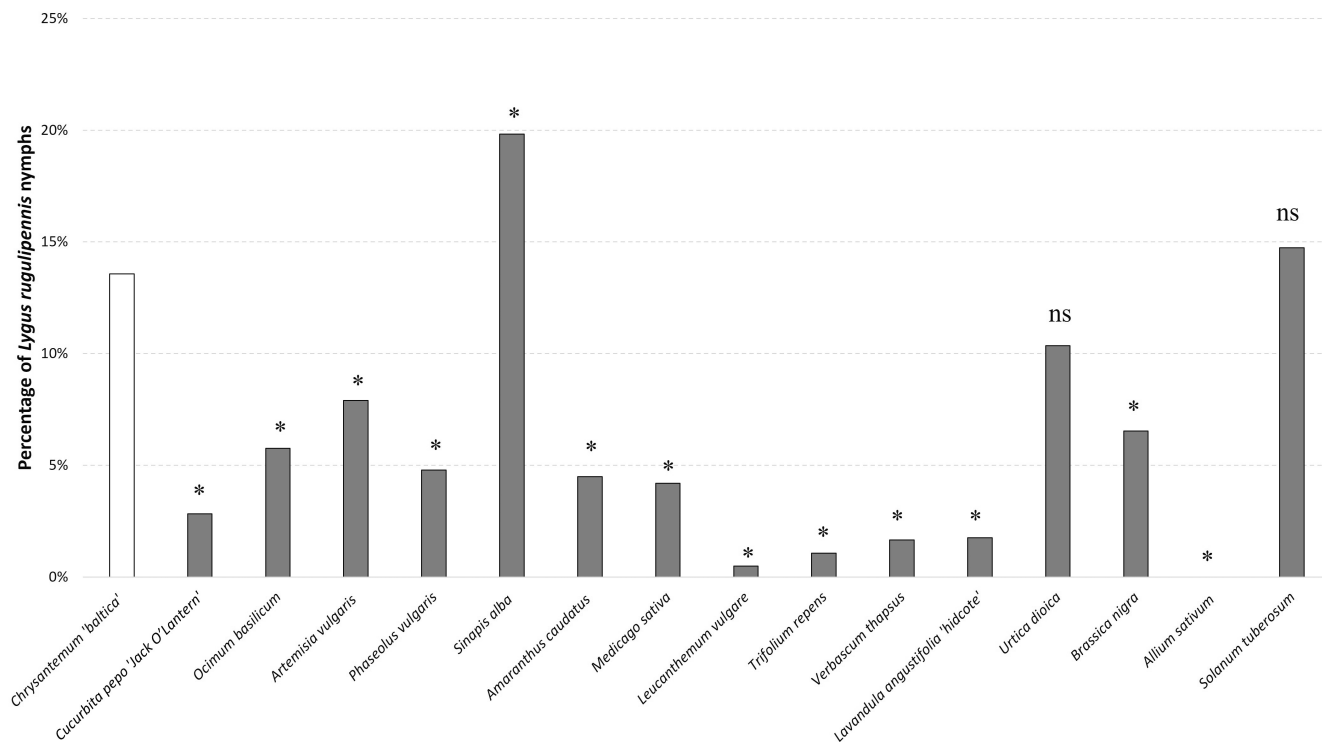


FIGURE 4 Percentage of all *Lygus rugulipennis* nymphs recorded on 16 plant species at the end of the trap plant experiment. The asterisks above bars indicate significant differences between recorded *L. rugulipennis* nymphs on *Chrysanthemum x morifolium* 'Baltica' compared with the other plant species ($p < 0.05$ according to Chi-square test with Bonferroni correction). NS, no significant differences.

Currently, there is a lack of effective biological control agents commercially available that can specifically control *L. rugulipennis* (Hennekam et al., 2012); in this perspective, the development of trap plants represents thus a promising alternative to chemical control. The result of our trap plant experiment shows that from the 15 selected plant species, *S. alba* was the most attractive plant in the vegetative stage for both nymphs and adults of *L. rugulipennis*, when compared with chrysanthemum, while *M. sativa* did not elicit the expected attraction. Some studies have indeed indicated that *M. sativa* can be used as a successful trap crop for *Lygus* spp. (Sevacherian & Stern, 1974; Swezey et al., 2013) and *M. sativa* is recorded as a host plant for *L. rugulipennis* (Holopainen & Varis, 1991). However, we observed in our trap plant experiment a low attractiveness of *L. rugulipennis* for *M. sativa*. In England, Easterbrook and Tooley (1999) found no consistent reduction of *L. rugulipennis* populations when using the trap plant scented mayweed, *Matricaria recutita* L. (Asteraceae) and *M. sativa* as a barrier strip around late-season strawberries. Only a delay was observed in the nymphal population build-up in strawberries surrounded by *M. recutita* compared to strawberries without a barrier strip. Accinelli et al. (2005) found that the use of *M. sativa* as a trap crop gave insufficient results for managing *L. rugulipennis* in lettuce in Italy. This indicated that *M. sativa* is not an adequate trap crop for *L. rugulipennis* in the combination of these tested crops, although positive results have been demonstrated for the closely related *L. hesperus* (Barman et al., 2010; Swezey et al., 2007). Taken together these studies highlight the challenges of finding an effective trap crop as results are often specific and context-dependent, in other

words, the stage (vegetative/flowering) of a trap crop compared to the stage (vegetative/flowering) of the crop is a very important factor for a pest to decide which plant is the most attractive. Although trap crops are often more attractive than the crop, they are unable to reduce the damage to the crop (e.g., Ondiaka et al., 2016), so other control methods have to be combined like vacuum devices (Swezey et al., 2007) or localized insecticide treatments (Dumont & Provost, 2019; Pansa & Tavella, 2009; Zhang et al., 2020).

Our study indicated that *S. alba* in the vegetative stage is a potential candidate trap plant as part of an integrated approach to monitor and control *L. rugulipennis* in chrysanthemum nurseries. *S. alba* can also be used as a trap crop for other pests (reviewed by Badenes-Pérez, 2019) including the agricultural pest *Nezara viridula* L. (Heteroptera: Pentatomidae) in sweet corn, *Zea mays* L. (Poaceae) (Rea et al., 2002). *S. alba* also attracts aphidophagous hoverflies and parasitoids of several pest insects like aphids and moths, during flowering (reviewed by Badenes-Pérez, 2019). Thus *S. alba* could be implemented in chrysanthemum nurseries for multiple benefits to the crop.

Although promising results have been found in our study, more research is needed with *S. alba* to conclusively demonstrate its value as a trap plant. Greenhouse trials with *S. alba* in chrysanthemum nurseries are needed to investigate whether (1) *L. rugulipennis* can move from chrysanthemum fields to *S. alba* and (2) trapping *L. rugulipennis* on *S. alba* is sufficient for releasing the pest pressure in chrysanthemum nurseries. Other crucial aspects that must be considered to maximize the 'trapping' effect require the identification of the optimal number and placement of *S. alba* plants in a chrysanthemum nursery.

We showed that flowering chrysanthemum plants were more suitable for *L. rugulipennis* development but we only tested chrysanthemum in the vegetative stage in our trap plant experiment. Further studies should determine the importance of flowering trap plants and flowering chrysanthemum, and whether *S. alba* can attract other pest species present in chrysanthemum. Another important aspect is that in chrysanthemum nurseries several chrysanthemum varieties are present. The attractiveness of these varieties has to be tested between each other and between *S. alba* as it might be that some varieties are more attractive to *L. rugulipennis*. As we also used two varieties in our study, there was a difference of almost 3 days in development time between both varieties (see Figures 1b and 2b), however, we cannot compare our findings in this study with each other, as both experiments were conducted in other greenhouses and another period of the year, which could have an influence on the development time. Finally, as we demonstrated that the presence of alternative prey is also beneficial for *L. rugulipennis*, this could make chrysanthemum even more attractive than a trap plant. More information is needed on the effects of supplementary feeding on biocontrols on pest populations. Recently, Fountain et al. (2021) demonstrated a push-pull system for *L. rugulipennis* management in UK commercial strawberry crops based on hexyl butyrate dispensers as a push element and all-green Unitraps baited with female *L. rugulipennis* sex pheromone as the pull element. This combination of synthetic semiochemicals showed a significant reduction in crop damage by *L. rugulipennis*. This new push-pull system could be tested in chrysanthemum in combination with trap plants to monitor and reduce *L. rugulipennis*.

AUTHOR CONTRIBUTIONS

JBW, MB, and GJM conceived the research and designed trials, JBW and MB conducted experiments, contributed material, and analysed data. AC analysed data and conducted statistical analyses. JBW wrote the manuscript and GJM secured funding. All authors read and approved the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from <https://zenodo.org/record/6839232#.YtFaLYTP1PY>. Data Citation: Jozef B. Woelke, Machteld Bouw, Antonino Cusumano, & Gerben J. Messelink. (2022). *Lygus rugulipennis* on chrysanthemum: supplementary prey effects and an evaluation of trap plants. <https://doi.org/10.5281/zenodo.6839232>.

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