Article

Studies Aimed at Evaluating the Impact of Generalist Predators on the Leafhopper *Erasmoneura vulnerata* Populations

Stefan Cristian Prazaru [1, *], Giulia Zanettin [1], Alberto Pozzebon [1*], Paola Tirello [1], Francesco Toffoletto [1], Davide Scaccini [1], and Carlo Duso [1, *]

[1]Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, viale dell'Università 16, 35020 Legnaro, Italy; stefancristian.prazaru@phd.unipd.it, 0000-0002-0458-8994 (S.C.P.); giuliazanettin22@gmail.com, (G.Z.); paola.tirello@unipd.it, (P.T.); francesco.toffoletto@studenti.unipd.it (F.T.); davide.scaccini@phd.unipd.it , 0000-0002-6850-7911 (D.S.)
*Correspondence: carlo.duso@unipd.it, 0000-0002-2600-2536 (C.D.); alberto.pozzebon@unipd.it, 0000-0002-2445-7211 (A.P.).
Tel.: +39-0498272805 (C.D.); Tel.: +39-0498272806 (A.P.).

Simple Summary: *Erasmoneura vulnerata*, a grapevine leafhopper native to North America, was detected in Europe in the early 2000s. Although it is considered a minor pest in native areas, outbreaks of this species are reported in North-eastern Italy. In this study, we investigated the potential of two generalist predators, i.e., *Chrysoperla carnea* and *Orius majusculus*, in controlling *E. vulnerata* through laboratory and semi-field experiments. Both species showed to be promising, and thus field trials were planned. Predator releases in vineyards reduced *E. vulnerata* abundance by about 30%. Since naturally occurring *E. vulnerata* antagonists exert a moderate impact and the effectiveness of natural insecticides is limited, the augmentative release of generalist predators can be considered a complementary tool in controlling *E. vulnerata* populations in vineyards.

Abstract: Outbreaks of the Nearctic leafhopper *Erasmoneura vulnerata* represent a recent issue for winegrowers in Southern Europe, in particular in North-eastern Italy. Problems are frequent in organic vineyards because insecticides labeled for organic viticulture show limited effectiveness towards leafhoppers. On the other hand, the response by naturally occurring predators and parasitoids to *E. vulnerata* populations in vineyards is often unable to keep leafhopper densities under levels that are acceptable for winegrowers. Here we evaluated the potential of two generalist predators, i.e., *Chrysoperla carnea* and *Orius majusculus*, in controlling *E. vulnerata* populations. Laboratory and semi-field experiments were carried out to evaluate both species' predation capacity on *E. vulnerata* nymphs. Then, predators were released in vineyards colonized by large *E. vulnerata* populations. Both predator species exhibited a remarkable voracity in the laboratory and significantly reduced leafhopper densities in semi-field experiments. Predator releases in vineyards reduced leafhopper densities by about 30%. Results obtained in laboratory, semi-field, and field experiments are discussed to implement these biocontrol agents' use in pest control tactics.

Keywords: *Erasmoneura vulnerata*; augmentative biological control; invasive pests; grapevine; *Chrysoperla carnea*; *Orius majusculus*; generalist predators

1. Introduction

Erasmoneura vulnerata (Fitch) (Hemiptera: Cicadellidae) is native to North America where it is reported as a minor pest of grapevines compared to other leafhopper species [1]. This pest was detected in Europe (North-eastern Italy) in 2004 [2] but did not cause outbreaks in the newly invaded areas until 2016 [3]. Since then, issues due to *E. vulnerata* infestation are increasing in the Veneto region. *Erasmoneura vulnerata* moved to Slovenia [4], is spreading in Northern Italy, and recently has been detected in Switzerland [5]. Therefore, an increase in problems caused by this new pest is expected. Recent studies

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carried out in North-eastern Italy showed that *E. vulnerata* could complete three generations per year, and overwintered adults colonize vineyards showing a remarkable edge effect [6]. Sometimes, adults damage shoots at the bud-break, but the second generation is usually the most harmful.

In most cases, the response by naturally occurring antagonists to *E. vulnerata* outbreaks seems unable to keep population densities at acceptable levels for winegrowers. It should be mentioned that adults cause nuisance to pickers during harvest time. The use of insecticides can achieve leafhopper control in conventional viticulture, but natural insecticides (to be used in organic farms) are less effective [6]. The side effects of insecticides are a matter of concern, and reduction of their use in Europe are the focus of the Directive 2009/128/EU [7] and, more recently, of Farm to Fork Strategy [8]. Therefore, the identification of feasible alternatives to insecticides is a priority in Europe. Augmentative biocontrol strategies through the release of predators and parasitoids could be useful to control *E. vulnerata* populations. In California vineyards, green lacewings (Neuroptera: Chrysopidae) were released to control leafhoppers, i.e., *Erythroneura variabilis* Beamer and *Erythroneura elegantula* Osborn, with positive results [9,10]. In the present study, the impact of two generalist predators, i.e., *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and *Orius majusculus* (Reuter) (Hemiptera: Anthocoridae) on *E. vulnerata* populations was tested in the laboratory, semi-field, and field conditions. Both species are well-known generalist predators of various hemipterans and have been frequently detected in Italian vineyards [11–14].

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2. Materials and Methods

2.1. Laboratory experiments

Laboratory experiments were carried out to assess the capacity of *C. carnea* and *O. majusculus* to prey upon *E. vulnerata* nymphs. Predators were supplied by Bioplanet (Cesena, FC, Italy). Laboratory-reared 3rd instar nymphs of *E. vulnerata* were transferred onto grapevine leaf disks inside plastic Petri dishes (90 mm of diameter, 15 mm of height) used as experimental arenas. Grapevine leaves were collected in the University of Padua (Italy) experimental farm and washed with water plus Tween (0.15% w/w) before the experiment. Three prey densities (5, 10 and 20 leafhopper nymphs per Petri dish) were considered as prey offer to predators. Three treatments were set for each prey density: *C. carnea* (3rd instar larvae), *O. majusculus* (adults), Control (no predators). A single *C. carnea* larva or *O. majusculus* adult were transferred onto an experimental arena immediately after placing *E. vulnerata* nymphs. The number of living and dead leafhopper nymphs, and predators was recorded 24 hours from the beginning of the experiment. Insect remains were considered as dead individuals. Experimental arenas were maintained in a climatic chamber at 23 ± 2 °C and 70-80% RH with a photoperiod of 16L:8D.

2.2 Semi-field experiments

Two semi-field trials were carried out using single potted vines that were confined inside insect-proof cages (BugDorm-4S2260, MegaView Science Education Services Co., Ltd., Taiwan). Cages were placed in outdoor conditions under the shade and protected from the rain. Experimental units were set up in the University of Padua's experimental farm, and the experiments were carried out from May to August 2019. *Erasmoneura vulnerata* infestation was set differently in the two experiments. Each vine was infested by 60 (2nd–4th instar) *E. vulnerata* nymphs in the first experiment. In the second experiment, three *E. vulnerata* adults (two females and one male) were confined onto a vine and allowed to reproduce during June and July; then, adults were removed, and the number of living nymphs was estimated by using a portable magnifying lens before predator releases. Each treatment was replicated five times. In both experiments, *Chrysoperla carnea* larvae and *O. majusculus* adults were released on vines in a ratio of 1 predator/10 nymphs (*C. carnea*) or 1 predator/30 nymphs (*O. majusculus*). *Erasmoneura vulnerata* individuals and predators were transferred into cages using a pencil brush. In the second experiment, infestation density was assessed prior to predator releases. Predator-prey ratios reflected producer instructions (Bioplanet) for the control of other homopteran pests. Nymph density was assessed two weeks after predator release by counting the number of living *E. vulnerata* inside each cage. For both experiments and all cages, we calculated the population growth rate of *E. vulnerata* during the experiment using the following formula:

$$rt = \mathrm{Ln}\,(Nt \;/\; Nt\text{-}\,\tau\,) / \tau.$$

Nt - τ represents the nymph density in each cage before predator releases, Nt is the nymph density observed in each cage at the end of the experiment, and τ is the time in days after predator release (i.e., 15 in both experiments). With $rt > 0$, the population increases, with $rt < 0$ means that the population decreases, while $rt = 0$ means that the population is stable.

2.3. Field experiments

The impact of *C. carnea* and *O. majusculus* on *E. vulnerata* populations was assessed in field conditions. Predators were released in two infested vineyards. The first trial was carried out in a vineyard located at Conegliano (North-eastern Italy, 45°52’53.05’’N, 12°17’00.26’’E, 77 m a.s.l.) in 2018. This vineyard comprised the cultivar Merlot and was trained with the Guyot system. The second trial was carried out in a vineyard located at

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Ponte di Piave (North-eastern Italy, 45°72'78.39''N, 12°46'82.44''E, 11 m a.s.l.) in 2019. It comprised the cultivar Cabernet Sauvignon, trained with the Bellussi system. No insecticides were applied in plots considered in this study. Predators were provided by Bioplanet.

In the first experiment (2018), three treatments were compared: (1) *C. carnea* release; (2) *O. majusculus* release; (3) Control. Each treatment comprised four replicates, each having five vines (approximately 20 m^2 of vine canopy). About 30 *C. carnea* larvae or 4 *O. majusculus* adults were released per m^2 of canopy in the respective treatment. These figures reflected producer instructions (Biooplanet) for the control of other homopteran pests. Predators were released on 21st July. They were manually distributed on the permanent cordon and the canopy. Sampling was carried out to evaluate leafhopper and predator densities before (on 20th July) and after releases (on 28th July, 4th and 11th August). In each sampling date, 100 leaves per treatment were randomly collected and transferred to the laboratory, where they were observed under a Wild M3 stereomicroscope to assess the abundance of *E. vulnerata* and the released predators.

In the second experiment (2019), the same treatments considered in the previous experiment were compared. They comprised four replicates of three vines (approximately 24 m^2 of canopy). As in the first experiment, predator releases were done, but two releases were performed on 9th and 21st August. Sampling was carried out to evaluate leafhopper and predator densities before and after releases. In each sampling date, 100 leaves per treatment were transferred to the laboratory to assess the abundance of *E. vulnerata* and the released predators using previous procedures.

2.4. Statistical analysis

Data from laboratory and semi-field experiments were analyzed using logistic regression with the GLIMMIX procedure of SAS, ver. 9.4 [15], considering the binomial distribution of the data. In this analysis, the number of surviving nymphs at the end of the experiment over the initial number of insects was considered as dependent variable. An F test (P = 0.05) was used to assess the effect of independent variables, i.e., predator species, prey density and their interactions. A Tukey's test (P = 0.05) was used as post-hoc to evaluate differences among predator treatments and in each of the three levels of prey density considered. Data were arcsin $\sqrt{n}$ transformed prior the analysis.

Data from field trials was analyzed using a Repeated Measures Linear Mixed Model with the MIXED procedure of SAS [15]. The model was used to test the effect of predator species, time, and their interactions on the number of *E. vulnerata* motile forms observed during the experiment. The effect of independent variables was tested using the F test (P = 0.05). Degrees of freedom were estimated with the Kenward and Roger method. A Tukey's test (P = 0.05) to the least-square means was applied to evaluate the differences among treatments. The models' assumptions were evaluated by inspecting diagnostic plots of model residuals, and data on leafhoppers density were log (n + 1) transformed before the analysis.

3. Results

3.1. Laboratory experiment

All predators survived during the laboratory experiment. *Chrysoperla carnea* and *O. majusculus* actively preyed upon *E. vulnerata* nymphs and significantly affected their survival rates compared to the control ($F = 45.00$; d.f. = 2, 46; $P < 0.0001$). No differences emerged between the two predators (5 nymphs: $F = 0.22$; d.f. = 1, 46; $P = 0.824$; 10 nymphs: $F = 1.36$; d.f. = 1, 46; $P = 0.905$; 20 nymphs: $F = 1.59$; d.f. = 1, 46; $P = 0.806$; Figure 1).

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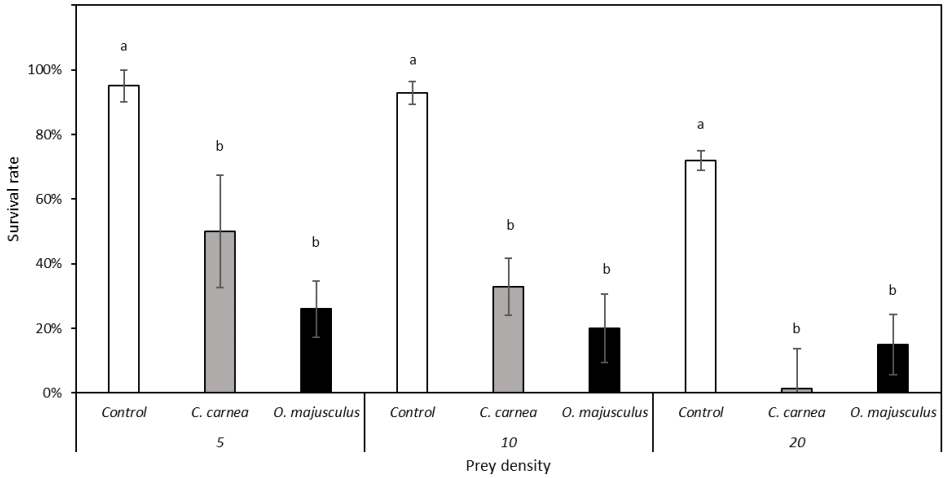

Figure 1. Survival rates (mean ± SE) of *Erasmoneura vulnerata* nymphs offered as prey to *C. carnea* or *O. majusculus* in the laboratory experiment. Different letters indicate significant differences at Tukey's test (P = 0.05).

3.2. Semi-field experiments

In the first experiment, predator releases significantly reduced leafhopper densities (F = 39.94; d.f. = 2, 12; P < 0.0001; Figure 2A). Different outcomes between the two predatory species were recorded, as *E. vulnerata* population decrease was higher in *C. carnea* compared to *O. majusculus* releases (F = 4.09; d.f. = 1, 12; P = 0.0040; Figure 2A).

In the second experiment, the number of *E. vulnerata* nymphs detected prior to the predator releases was similar among the cages (70 ± 5.04 mean ± std. err.; F = 0.67; d.f. = 2, 12; P = 0.5279). Leafhopper densities were significantly reduced by the two predators (F = 37.88; d.f. = 2, 12; P < 0.0001; Figure 2B), and no differences between them were observed (F = 0.8531; d.f. = 1, 12; P = 0.9805; Figure 2B).

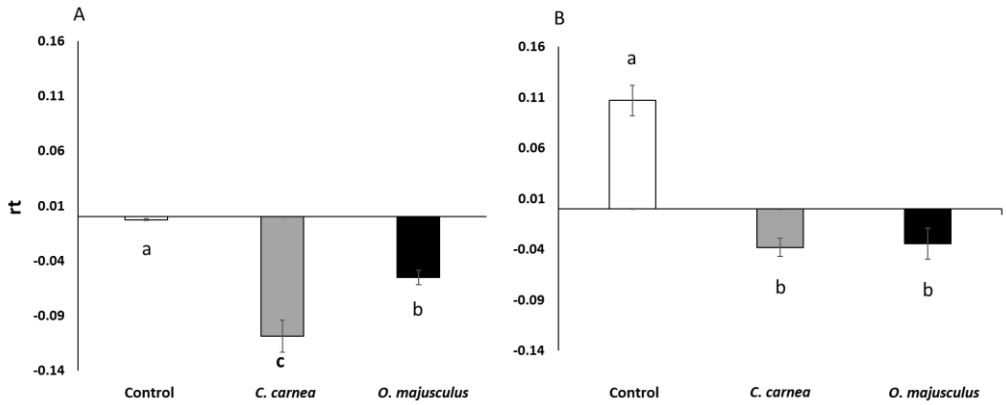

Figure 2. Population growth rate (mean ± SE) of *Erasmoneura vulnerata* observed in semi-field experiments in the three treatments (*C. carnea, O. majusculus*, and Control) and two weeks after predator releases. In the first experiment (A), 60 nymphs per cage were introduced, while in the second experiment (B), the population of nymphs was generated by the inoculation of three adults. In

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both experiments predators were introduced in a ratio of 1/10 nymphs (*C. carnea*) or 1/30 nymphs (*O. majusculus*). Data are expressed as growth rate comparing the population level before and after the predator introduction. Different letters indicate significant differences at Tukey's test (P = 0.05).

3.3. Field experiments

In the first experiment (2018), *E. vulnerata* densities increased after releases and then declined in all treatments. Leafhopper populations significantly fluctuated over sampling dates (F = 36.29; d.f. = 3, 27; P < 0.0001). Predator releases significantly affected leafhopper population densities (F = 4.03; d.f. = 16.6; P = 0.037; Figure 3) with different outcomes between the two predators. Leafhopper in *O. majusculus* release plots were lower as compared to the control plots (F = 2.82; d.f. = 16.6; P = 0.036) while no differences emerged between *C. carnea* releases plots and Control (F = 1.66; d.f. = 2, 16.6; P = 0.348). No significant differences in leafhopper densities between *O. majusculus* and *C. carnea* were found (F = 1.17; d.f. = 1, 16.6; P = 0.78).

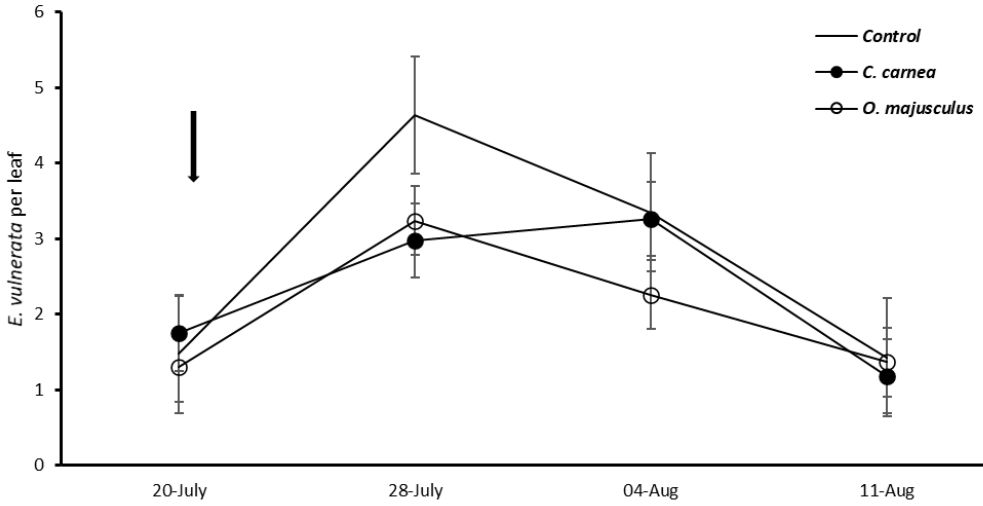

Figure 3. *Erasmoneura vulnerata* infestation (mean ± SE) in vineyard plots characterized or not by predator releases (experiment 1, 2018). The arrow indicates the predator release event.

In the second experiment (2019), *E. vulnerata* densities slightly increased in the second sampling date and then declined. Predator releases significantly affected leafhopper population densities compared to the control (F = 136.9; d.f. = 2, 71; P < 0.0001; Figure 4). The effect of time and the interaction "treatment*time" were significant (F = 453.81; d.f. = 7, 71; P < 0.0001; F = 10.04; d.f. = 14, 71; P < 0.0001, respectively). There were no differences between the leafhopper densities observed in the two predator release treatments (F = 0.69; d.f. = 1, 71; P = 0.494) that were lower compared to the control (*C. carnea:* F = 14.03; d.f. = 1, 71; P < 0.0001; *O. majusculus:* F = 14.71; d.f. = 1, 71; P < 0.0001; Figure 4).

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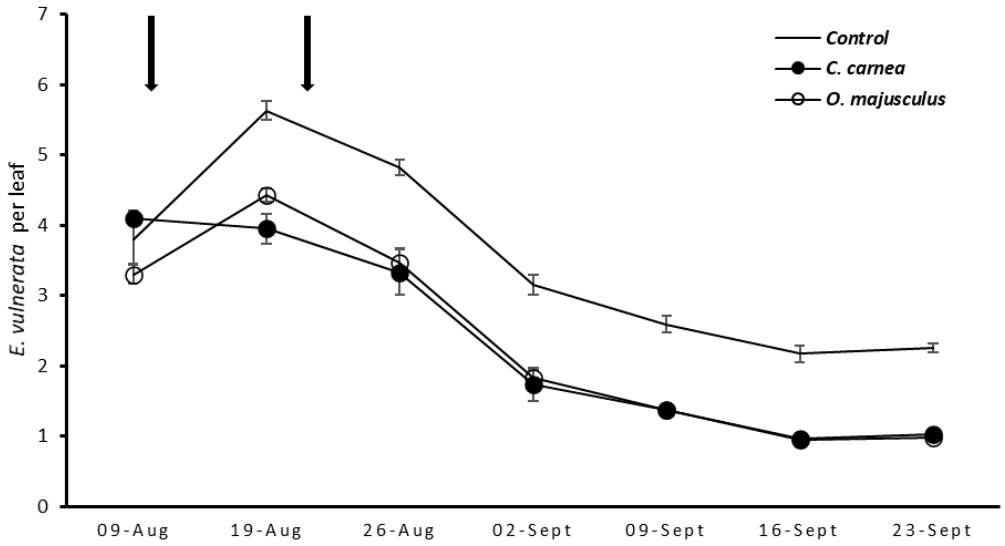

Figure 4. *Erasmoneura vulnerata* numbers (mean ± SE) in vineyard plots characterized or not by predator releases (experiment 2, 2019). Each arrow indicates predator release events.

4. Discussion

Both predators preyed actively upon *E. vulnerata* nymphs and significantly reduced their densities in semi-field and field conditions.

Orius majusculus preys upon a variety of arthropod species, such as thrips, leafhoppers, aphids, lepidopterans, and spider mites [16–18]. It has been commonly detected in various crops (included grapevines) in North-eastern Italy, preying upon homopterans and spider mites [11,12]. Despite predation upon grape leafhoppers was observed in the latter studies, *ad hoc* experiments were not planned. On the other hand, interactions between *O. majusculus* and leafhoppers were investigated in Spain. Ardanuy [19] observed early-season increases of *Orius* spp. in maize fields potentially related to the occurrence of leafhoppers, in particular *Zyginidia scutellaris* (Herrich-Schäffer). They examined the innate and learned preferences of *O. majusculus* toward volatiles emitted from plants infested by *Z. scutellaris*, *Spodoptera littoralis* Boisduval (Lepidopteran: Noctuidae), and *Dalbulus maidis* Delong & Wolcott (Hemiptera: Cicadellidae). Predators were markedly attracted by volatiles emitted from maize plants infested with *Z. scutellaris* or *S. littoralis*. Feeding by *Z. scutellaris* induces the emission of maize's HIPVs (herbivore induced plant volatiles) that attract anthocorids into maize fields. In our field experiments, the impact of *O. majusculus* on leafhopper populations was similar or slightly better compared to that of *C. carnea*.

Chrysoperla carnea is a generalist predator that may prey upon more than 70 species belonging to five orders, but Homopterans represent the preferred targets [20]. It has been widely used in augmentation biological control tactics against aphids and lepidopterans [21,22]. Few studies have examined the predation by *C. carnea* upon leafhoppers. Erlandson and Obrycki [23] compared the predation activity of *C. carnea* on the leafhopper *Empoasca fabae* Harris with that exhibited by the anthocorid *Orius insidiosus* (Say) and the coccinellid *Coleomegilla maculata* (De Geer). *Chrysoperla carnea* was the most voracious among tested predators, especially in the high-prey density trials [23]. The impact of lacewings on grape leafhoppers has been evaluated in California, where Daane et al. [9] released *C. carnea* in vineyards infested by *E. variabilis* and *E. elegantula*. In a first trial, *C. carnea* larvae were released into cages and leafhopper densities were reduced by 23.5–30.3%. Furthermore, the release of *C. carnea* larvae in vineyards obtained a significant

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reduction in leafhopper density (33.6 and 31.4% in the first and second generations, respectively), with about 20,000 larvae released per hectare [9]. However, unsatisfactory results were obtained in other trials using the same approach [9]. In additional field experiments, *C. carnea* eggs were released obtaining a significant leafhopper reduction (9.6% as a mean) in about a half of tested vineyards. Differences in release methods and prey densities were claimed as possible factors affecting these contrasting results. Interestingly, leafhopper density reduction was more relevant when lacewing larvae rather than eggs were released. Furthermore, prey densities had a significant role in the outcome of *C. carnea* releases, as predators could not reduce leafhopper densities below the economic injury thresholds in high pest pressure conditions [9]. Aspects related to augmentative releases of green lacewings (including *C. carnea*) were further evaluated in California vineyards [10]. A mixture of lacewing eggs and corn grit placed in paper cups was distributed to every 5th vine in every other row; this system was associated with low egg hatching and larvae dispersal. Egg hatching increased when they were dropped onto the vines from a moving flatbed trailer. In other trials, the effect of increasing release rates (from 6,175 to 1,235,000 eggs or larvae per hectare) was compared, but prey numbers were not correlated with release densities. Releases were more effective when nymphs were at the beginning of the generation (before peak). Larval releases are confirmed to be more effective than egg releases.

Results obtained releasing *C. carnea* against *E. vulnerata* were similar to those observed in California against phylogenetically close leafhopper species [9]. However, the results of our field experiments (for both predator species) were less convincing compared to those reported in the laboratory and semi-field studies. These discrepancies could be due to many factors, and among them, release techniques, the occurrence of alternative prey, and climatic conditions could be the most important. In grapevine training systems we considered, the permanent cordon grows at 1-2 m from the ground level. Therefore, a number of released predators can fall and disperse after releases. The use of cups located on the cordon could reduce the dispersion of predators and the potential cannibalism, but it requires high handling time. In one of the experimental vineyards, spider mites occurred at a moderate level, and this factor could represent a distraction towards the leafhopper prey. The alternate of heavy rains and drought typical of summertime in the study area could have reduce release success. Further studies are needed to elucidate the role of factors that may influence these generalist predators' effectiveness in controlling *E. vulnerata* populations in realistic conditions.

5. Conclusions

Promising results obtained with *O. majusculus* and *C. carnea* suggest implementing release techniques and optimize their release density to improve the impact of anthocorids and lacewings on *E. vulnerata* populations. These results have to be considered within Integrated Pest Management strategies in vineyards where *E. vulnerata* can be a severe threat for grapevine production.

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Informed Consent Statement: Not applicable.

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