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ORIGINAL ARTICLE

The effectiveness of *Chrysoperla carnea* (Neuroptera: Chrysopidae) and *Beauveria bassiana* (Ascomycota: Hypocreales) as control agents of *Neophilaenus campestris* (Hemiptera: Aphrophoridae) a vector of *Xylella fastidiosa*

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Key words. Predation, natural enemies, biological control, entomopathogenic fungus, bacterium, median lethal concentration, transmission, spittlebug foam

Abstract. The effectiveness of two biological control agents, *Chrysoperla carnea* (Neuroptera: Chrysopidae) and the entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Hypocreales) against nymphs and adults of *Neophilaenus campestris* (Hemiptera: Aphrophoridae) was determined under laboratory conditions. First, different nymphal stages of *N. campestris* were presented to different larval stages of *C. carnea*. Second, the effect of the foam produced by *N. campestris* nymphs on the larvae of *C. carnea* predation was evaluated. Finally, four concentrations of a wild strain of *B. bassiana*, BbGEp1, were sprayed on plants in order to determine their lethality for adults of *N. campestris*. Second and 3rd-instar larvae of green lacewing larvae are capable of capturing and killing 3rd and 5th-instar nymphs of *N. campestri*s. The percentage of 3rd-instar lacewing larvae that killed nymphs was significantly higher than that were killed by second-instar larvae. Second-instar larvae killed significantly more 3rd-instar nymphs than 5th-instar nymphs. Third instar lacewing larvae killed an average (± SEM) of 1.50 ± 0.31 5th-instar nymphs and 2nd-instar larvae killed very few nymphs. Spittlebug foam reduced, but did not prevent predation. The lethality of the entomopathogenic *B. bassiana* BbGEp1 used against adults of *N. campestris* was characterized by an LC_{50} value of 1.61×10^6 conidia/mL and LT_{50} of 3.63 days at 1×10^7 conidia/mL. The present study provides new and valuable data on the activity of two promising biological control agents of vectors of the bacterium *Xylella fastidiosa*. Further research is needed to confirm the results presented here and on the cost effectiveness of using these control agents as alternatives to synthetic insecticides for preventing the further spread of *X. fastidiosa* in Europe.

INTRODUCTION

Xylella fastidiosa Wells (Xanthomonadales: Xanthomonadaceae) is a gram-negative pathogenic bacterium from America. At present, it infects at least 690 species of plants belonging to 88 families (Gibin et al., 2023). In America, it causes Pierce's disease of grapevine and variegated chlorosis in citrus. This bacterium was detected in olive trees, oleander and almond in Italy in 2013 (Saponari et al., 2013). *X. fastidiosa* subsp. *pauca* resulted in "olive quick decline syndrome", which affected 10,000 ha of olive trees in the Lecce Region of Apulia (Italy) (Martelli et al., 2016). Subsequently this bacterium was recorded in France, Spain, Portugal and Germany (Bragard et al., 2019). This raised concerns about the health of crops in Europe. *X. fastidiosa* is transmitted by xylem-feeding Auchenorrhyncha (Hemiptera) of the families Cicadellidae, Aphrophoridae and Cercopidae (Frazier & Freitag, 1946; Redak et al., 2004). This bacterium is recorded in, but there is no evidence that it is transmitted by the phloem-feeding *Euscelis lineolatus* Brulle (Elbeaino et al., 2014). In America, most of the vectors of *X. fastidiosa* belong to the subfamily Cicadellinae [e.g. *Graphocephala atropunctata* (Signoret) and *Homalodisca vitripennis* (Germar) in North America; *Bucephalogonia xanthophis* (Berg) and *Dilobopterus costalimai* Young in South America]. In Europe, where leafhoppers are less abundant, the three confirmed vectors to date belong to the family Aphrophoridae: *Philaenus spumarius* L. (Saponari et al., 2014), *Neophilaenus campestris* Fallén and *Philaenus italosignus* Drosopolous and Remane (the last two confirmed in the laboratory; Cavalieri et al., 2019).

Given that currently there is no effective treatment for this disease, vector control is one of the main ways to prevent further spread of *X. fastidiosa*. Recently, researchers have strived to find effective methods of controlling the insect vectors, mainly *P. spumarius* in Europe, apart from

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interventions for removing ground vegetation (Morelli et al., 2021).

Regarding chemical control, results obtained mainly using olive trees, indicate pyrethroids and neonicotinoids are highly effective, with percentage mortality of nymphs and adults of *P. spumarius* ranging from 76.7% to 100% (Dongiovanni et al., 2018, 2020; Izquierdo & Sabaté, 2018; Dáder et al., 2019). However, insects can develop resistance to synthetic chemical pesticides, which also pollute the environment and are detrimental to beneficial arthropods and incompatible with organic management. Furthermore, some insecticides are prohibited in the EU, e.g. neonicotinoids (The European Commission, 2023). Thus, safer products and methods for reducing the abundance of the vectors of *X. fastidiosa* need to be developed. In this respect, biological control could potentially be an alternative to synthetic pesticides, which is also compatible with sustainable crop management. Currently, there is a dearth of data on the activity of natural enemies of *X. fastidiosa* vectors in Europe. Liccardo et al. (2020) and Lahbib et al. (2022) propose introducing the predatory hemipteran *Zelus renardii* Kolenati (Hemiptera: Reduviidae) as an inundation strategy for reducing the abundance of *P. spumarius*. These authors report the incidence of pathogens fell to below 10% using this hemipteran under experimental conditions. Benhadi-Marín et al. (2020) report predatory efficiency of the spiders *Araniella cucurbitina* Clerck and *Synaema globosum* Fabricius on *P. spumarius* under laboratory conditions, which is a type-II functional response for *A. cucurbitina* and type-I for *S. globosum*. Molinatto et al. (2020) report a maximum percentage parasitism of adults of *P. spumarius* of 17.5% in vineyards in the Piemonte region (Italy) by *Verrallia aucta* Fallen (Diptera: Pipunculidae). In contrast, percentage parasitism of the eggs of *P. spumarius* by *Ooctonus vulgatus* Haliday (Hymenoptera: Mymaridae) reaches 69% in Corsica, Italy (Mesmin et al., 2020). Regarding insect pathogens, only Vicente-Díez et al. (2021) report the effects of four entomopathogenic species of nematodes and their cell-free supernatants on *P. spumarius* nymphs. Under laboratory conditions, 78%–90% percentage mortality was attributed to nematodes and 64% to a specific cell-free supernatant.

Green lacewings (Neuroptera: Chrysopidae) are an important component of agroecosystems due to their role in pest control (Ridgway & Kinzer, 1974; Ridgway & Murphy, 1984). Lacewing larvae can prey on a wide range of soft-bodied and thin-cuticle arthropods such as caterpillars, aphids, whiteflies, cicadellids, psyllids, coccids, thrips, mealybugs and mites (Canard et al., 1984; Ridgway & Murphy, 1984; Senior & McEwen, 2001). In Southern Europe and the Mediterranean Basin, *Chrysoperla carnea* (Stephens) is abundant in crops susceptible to *X. fastidiosa*, such as olive groves and citrus orchards (Campos, 2001; Davila et al., 2003). There is, however, no data on how these predators affect vectors of *X. fastidiosa*. Entomopathogenic fungi are important natural enemies of arthropods. They are lethal for a wide range of taxa including lepidopterous larvae, aphids, thrips, flies and mites (Dauda et al., 2018), and are currently used in biological control programs (e.g. classical, augmentation or conservation; Shah & Pell, 2003). Furthermore, some species are used to control major pests in olive groves (e.g. Yousef et al., 2017), but there are no reports on how these natural enemies could be used to potentially to control vectors of *X. fastidiosa* in Europe.

The aim of this laboratory study is to determine the potential of the common green lacewing *C. carnea* and the entomopathogenic fungus *Beauveria bassiana* as biological control agents of different stages of *N. campestris*, the most abundant vector of *X. fastidiosa* in crops in southwestern Spain (Morente et al., 2018). The predatory potential of second and third-instar larvae of *C. carnea* in terms of the number of third and fifth-instars of *N. campestris* nymphs killed and the protection provided by the foam they produce were evaluated. Furthermore, the lethal effects of a wild strain of *B. bassiana* on *N. campestris* adults were analysed and the median lethal concentration (LC_{50}) calculated.

MATERIAL AND METHODS

Insects and entomopathogenic fungi

Since it is not possible to rear *N. campestris*, nymphs and adults collected from natural populations were used in the bioassays. *N. campestris* nymphs and adults were collected in vineyards in the surroundings of "Camino del Chaparral", located in Huelva province southwestern Spain (37°16′22″N; 6°35′18″W), at an altitude of roughly 80 m a.s.l. The surveys were conducted in April and May 2022, when nymphs and adults start emerging. Plants with signs of foam, mainly *Lolium rigidum* Gaudin, *Polypogon* sp., *Cynodon dactylon* (L.) Pers. and *Avena sterilis* L. (Poales: Poaceae), were placed in a plastic container and transported to the laboratory. Given the high sensitivity of the nymphs to environmental changes, they were maintained on the host plants that were collected on and kept in a chamber under standard conditions of 22 ± 2°C, 65% relative humidity and a photoperiod of 14L : 10D (hereafter, laboratory conditions) before starting the bioassays. Adults were collected using a battery powered field aspirator (Insecta-Zooka, BioQuip, Rancho Dominguez, CA) and once in the laboratory, they were transferred to 10-cm-diameter plastic pots planted with *Bromus hordeaceus* L. and placed in 29 × 29 × 29 cm plastic cages with three side panels of polyester netting for ventilation (BugDorm® 1, Bio-QuipProducts Inc., Rancho Rodríguez, CA, USA) and kept under laboratory conditions until required for bioassays.

Commercially available 2nd-instar larvae of the green lacewing *C. carnea* (Chrysocontrol 1000®, Agrobio SL, Almería, Spain) were used in the bioassays. To obtain 3rd instar larvae, which were also used in the bioassays, a batch of larvae were fed ad libitum with commercially-produced *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (Ephes control 10g® Agrobio SL, Almería, Spain). All *C. carnea* larvae were starved for 12 h and kept under laboratory conditions before use in the bioassays.

A wild strain of *Beauveria bassiana* (BbGEp1) isolated from larvae of *Xanthogaleruca luteola* Müll. (Coleoptera: Chrysomelidae), provided by Prof. Hani Aldebis (Agronomy Department, University of Córdoba), was used in the bioassays. This strain is known to be lethal to several insect taxa (e.g. Diptera: Coleoptera; unpubl. data). Conidial suspensions for bioassays were prepared from a culture on potato dextrose agar kept at 25°C in the dark. Conidia were scraped off the cultures and suspended in sterilised

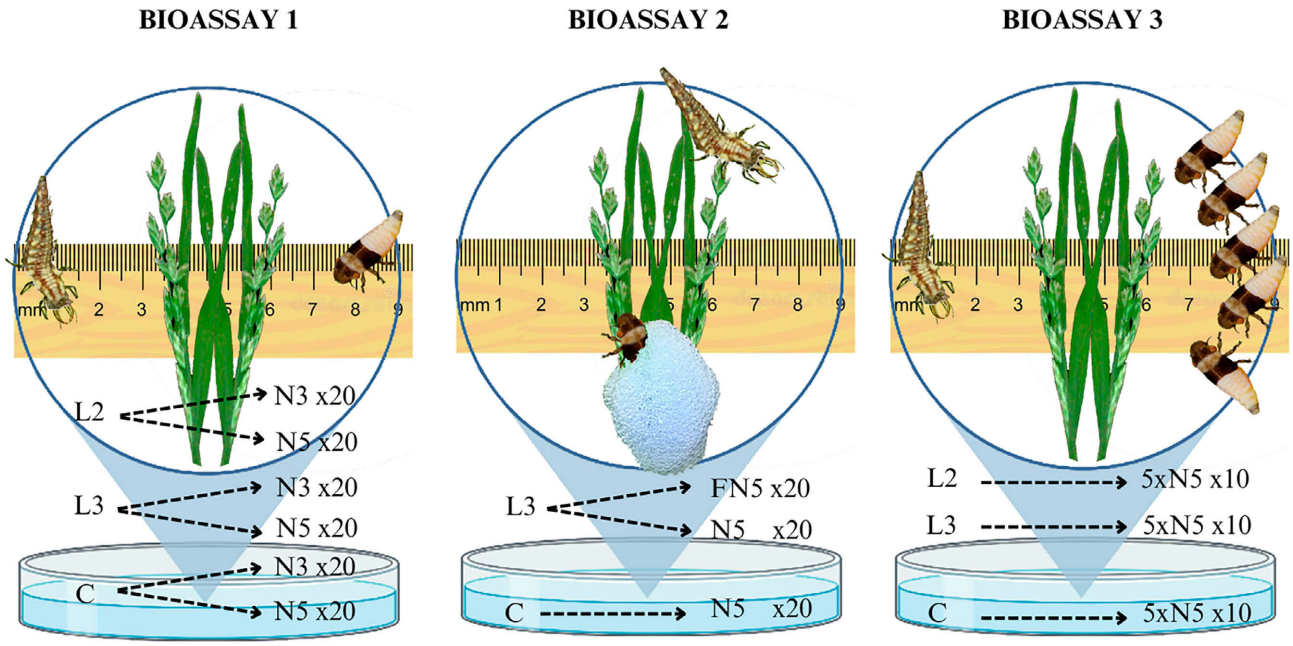

Fig. 1. Schematic diagrams of the three bioassays carried out to assess the number of nymphs of *Neophilaenus campestris* killed by larvae of *Chrysoperla carnea*, with the number of replicates per treatment. C indicates control treatment without a predator. L and N indicate the instars of the larvae and nymphs, respectively.

water (with 0.1% v/v of Tween 80) and then filtered through a double layer nylon mesh to remove hyphae. The conidia concentration was determined using a Neubauer haemocytometer. The resulting concentration was 3.36×10^7 conidia/mL.

All the bioassays were carried out in the morning between 8.00 and 8.30 a.m. The insects were illuminated using ca. 650 lx by Grolux fluorescent lamp (F36W/GRO T8, Feilo Sylvania, Shanghái, China) and placed on a white table. The lacewing larvae and *N. campestris* nymphs were carefully transferred to the dishes and cages using an entomological brush.

Number of nymphs of *N. campestris* killed by larvae of *C. carnea*

The first bioassay sought to determine if the second (CCL2) and third instar (CCL3) larvae of *C. carnea* were able catch and kill third (NCN3) and fifth instar nymphs (NCN5) of *N. campestris* (Fig. 1). Before testing, one 8 cm *Poa annua* L. plant was placed in the centre of a 9 cm Petri dish. The test started when one *C. carnea* larva and one *N. campestris* nymph were placed in the dish 5 cm from each other and 2 cm from the edge of the dish. Based on preliminary tests, an 8 h period ensured that predation occurred, which enabled comparison between different treatments. The numbers of prey captured in each dish, were recorded every hour for up to 8 h after the prey were offered. In all bioassays, predation was recorded when a nymph died and was consumed by a lacewing. Four combinations of larvae and nymphs were established: (1) CCL2 with NCN3; (2) CCL2with NCN5; (3) CCL3 with NCN3; and (4) CCL3 with NCN5. In addition, for each nymphal instar, a control treatment consisting of a dish containing one *P. annua* plant and one *N. campestris* nymph was used to assess the number of nymphs that died naturally. Groups of 20 replicates for each combination and control were established.

A second bioassay aimed to determine the effect of the foam produced by nymphs of *N. campestris* on the larvae of *C. carnea*. Before testing, NCN5 were transferred to a *P. annua* plant growing in a plastic pot for 24 h or until the nymph produced foam (Fig. 1). The plant with the fifth-instar nymph encased in foam was placed in the centre of a 9 cm Petri dish. The test started when one CCL3 was placed in the Petri dish 5 cm away from the plant. As in the first bioassay, the prey captured in each dish was recorded every hour for up to 8 h. In addition, a control treatment consisting one NCN5 encased in foam on a *P. annua* plant was used to assess the number of nymphs that died naturally. Groups of 20 replicates of foam encased nymphs and controls were established. Since few nymphs were available in the field, the third bioassay was carried out simultaneously with the first in order to compare it with the deaths recorded when the nymph had not produced foam (see above for the fourth combination in the first bioassay).

Finally, the third bioassay was used to compare and estimate the percentage deaths of nymphs recorded for CCL2 and CCL3 when a group of NCN5 nymphs was offered. Before testing, an 8-cm *P. annua* plant with five fifth instar nymphs was placed in the centre of a 9-cm Petri dish (Fig. 1). The test started when one *C. carnea* larva was placed in the dish 5 cm from the nymphs. Every hour the number of nymphs killed in each dish was recorded for up to 8 h with a final observation at 24 h. The control treatment, with one *P. annua* plant and five *N. campestris* nymphs was used to assess the number of nymphs that died naturally. Groups of 10 replicates for each larval instar and control were established. Since the third bioassay was carried out to determine how many nymphs were killed by one lacewing larva, we used the average number of nymphs consumed.

Lethality of *B. bassiana* for adults of *N. campestris*

To assess the lethality of the *B. bassiana* wild strain BbEp1 for adults of *N. campestris*, four concentrations were used: 1×10^7, 2×10^6, 4×10^5 and 8×10^4 conidia/mL. For each concentration and replicate, 10 mL of aqueous conidial suspension (with 0.1% v/v of Tween 80) was sprayed on to *B. hordeaceum* growing in 10-cm-diameter plastic pots. Later, 10 adults of *N. campestris* were transferred to the pots, placed in $29 \times 29 \times 29$ cm plastic cages (BugDorm® 1, Bio-QuipProducts Inc., Rancho Rodríguez, CA, USA) and kept under laboratory conditions. A control group was treated similarly with only 0.1% aqueous Tween 80, in order to assess the number of adults that died naturally. Groups of three

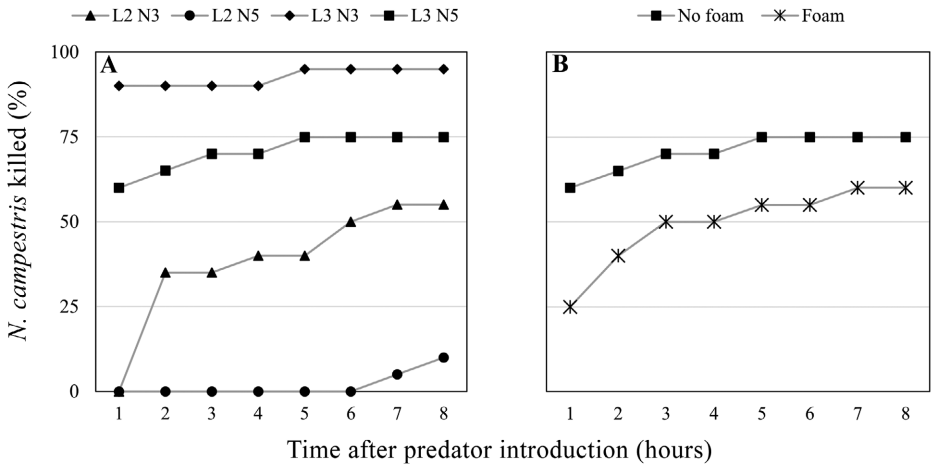

Fig. 2. A – Percentage of third (N3) and fifth (N5)-instar nymphs of *Neophilaenus campestris* killed by second and third-instar larvae of *Chrysoperla carnea*. B – Percentages of fifth-instar nymphs of *Neophilaenus campestris* nymphs covered in foam and without foam killed by third-instar larvae of *Chrysoperla carnea*. Each larva was offered one nymph (N = 20).

replicates per concentration and the control were established. Adult mortality was recorded daily up to 7 days after treatment. Dead adults were transferred to a Petri dish lined with moistened filter paper and kept under the same laboratory conditions. Fungal-induced mortality was confirmed by examining the dead adults using a stereomicroscopic. Only adults that were definitely killed by the fungus were included in the mortality-concentration analysis.

Statistical procedures

All analyses, except the mortality caused by *B. bassiana*, were done using R (R Core Team, 2022). The percentage of prey killed by larvae of *C. carnea* in the three bioassays was compared using generalized linear mixed models (GLMMs), including the number of nymphs killed and number that survived (using the cbind function) as the dependent variable fitted to a binomial distribution with a logit link function and predator and prey instars as the factors. The time after the introduction (hours) of the predator was included as a random factor. The models were checked for overdispersion and residual distribution using the DHARMa package (Hartig, 2022). In bioassay 1, pairwise comparisons of estimated marginal means for each group were carried out using the emmeans package (Lenth, 2023). Adult mortality caused by *B. bassiana* was subjected to a Probit analysis in order to produce a dose-mortality regression line (Finney, 1971) using the POLO Plus Program (LeOra Software Inc., Berkeley, CA, USA). The number of dead adults for each dose and replicate was pooled (n = 30) in order to estimate the LC_{50} values and 95% fiducial limits. Finally, the median lethal time (LT_{50}) when concentration exceeded 50% mortality was calculated following Biever & Hostteter (1971). The data and R codes supporting the findings of this study are available from https://doi.org/10.5281/zenodo.8282900.

RESULTS

Number of nymphs of *N. campestris* killed by larvae of *C. carnea*

Larvae of *C. carnea* killed nymphs of *N. campestris* in the three bioassays (Figs 2 and 3). In the first bioassay, both the ages of the larvae and the nymphs affected the result. CCL2 killed more *N. campestris* nymphs than CCL3 larvae (85% and 30%, respectively, $\chi^2 = 160.20$, df = 1, $p < 0.001$). In addition, a greater percentage of NCN3 was killed than of NCN5 (75% and 42.5%, respectively, $\chi^2 = 66.94$, df = 1, $p < 0.001$). At the end of the bioassay CCL3 killed 95% of NCN3 and 75% of NCN5, and CCL2 killed 55% of NCN3 and 10% of NCN5 (Fig. 2A) (p value < 0.0001, Tukey's adjustment method). No mortality was recorded in the control groups.

In bioassay 2, 75% of the nymphs without foam were killed compared with 55% of those with foam ($\chi^2 = 20.03$, df = 1, p < 0.001) (Fig. 2B). No mortality was recorded in the control.

Fig. 3 shows the results of bioassay 3 in which five NCN5 were provided for one second instar or third instar larva of *C. carnea*. No mortality was recorded in the control. The percentage of the NCN5 killed by CCL3 increased from 0.70 ± 0.21% at 1 h to 1.50 ± 0.31% at 24 h (Fig. 3). No NCN5, however, were killed by CCL2 after 8 h and it was only 0.1 ± 0.1% overall. The differences in the percentages of NCN5 killed by two larval instars of *C. carnea* is significant ($\chi^2 = 60.64$, df = 1, p < 0.001).

Lethality of *B. bassiana* for adults of *N. campestris*

The percentage of *N. campestris* killed *B. bassiana* wild strain BbGEp1 increased positively with the concentration.

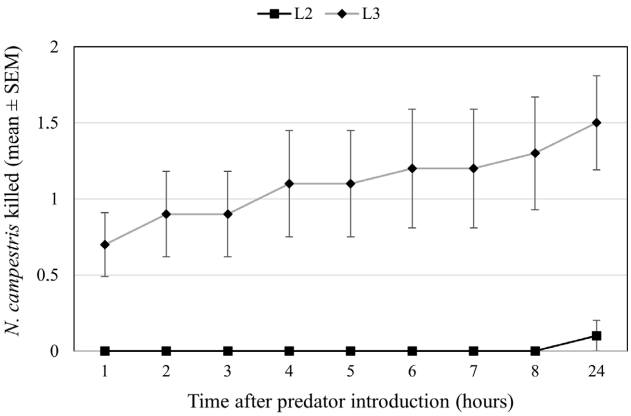

Fig. 3. Mean number of fifth-instar nymphs of *Neophilaenus campestris* killed by second (L2) and third (L3)-instar larvae of *Chrysoperla carnea*. Each larva was offered five nymphs (N = 10). Vertical lines are the standard errors.

Table 1. Percentage mortality recorded for each concentration seven days after application, median lethal time (LT_{50}), median lethal concentration (LC_{50}) and probit regression line parameters for *Beauveria bassiana* wild strain (BbGEp1) used against adults of *Neophilaenus campestris* (N = number of treated adults). LC_{50} was calculated from the probit regression line y = 0.94x – 5.86 (y = mortality; x = log concentration).

Concentration (conidia/ml)	N	Mortality (%)	LT_{50} (days)	LT_{50} (conidia/ml)	95% fiducial limits (conidia/ml)		χ^2 (df)	Slope ± SE	Intercept ± SE
					Lower	Upper			
0	30	0.00	–						
8×10^4	30	10.00	–						
4×10^5	30	33.33	–	1.61×10^6	8.73×10^5	3.33×10^6	1.08(2)	0.94 ± 0.18	–5.86 ± 1.07
2×10^6	30	46.67	–						
1×10^7	30	80.00	3.63						

The percentage mortality ranged between 10% and 80% seven days after application. The Probit analysis provided a good fit ($\chi^2 = 1.08$; df = 2) and the median lethal concentration (LC_{50}) had a value of 1.61×10^6 conidia/mL with 95% fiducial limits of 8.73×10^5 and 3.33×10^6 conidia/mL (Table 1). Mortality peaked between days 4 and 5 with a LT_{50} value of 3.63 days obtained when the highest concentration (1×10^7 conidia/mL; Table 1) was used.

DISCUSSION

Number of nymphs of *N. campestris* killed by *C. carnea*

The results indicate that larvae of *C. carnea* can kill nymphs of *N. campestris* under laboratory conditions. Although there is no data on the effectiveness of species of *Crysoperla* as controlling agents of Aphrophoridae under natural conditions, some authors report them attacking other Hemiptera and Auchenorrhyncha, mainly belonging to the family Cicadellidae, but also Membracidae and Ricaniidae, under laboratory, semi-field and field conditions (Daane et al., 1996; Weiser Erlandson & Obrycki, 2010; Wilson et al., 2015; Cuello et al., 2019; Kron & Sisterson, 2020; Prazaru et al., 2021; Mazza et al., 2021) or based on the use of molecular techniques (De León et al., 2006; Fournier et al., 2008). These authors report *C. carnea* attacking *Empoasca fabae* Harris, *Erythroneura variabilis* Beamer, *E. elegantula* Osborn, *Erasmoneura vulnerate* Fitch, *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae), *C. externa* (Hagen) attacking *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae) and *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae) and *C. rufilabris* (Burmeister) *Spissistilus festinus* Say (Hemiptera: Membracidae). Results presented indicate that *N. campestris* is a potential prey of *C. carnea*, adding for the first time the family Aphrophoridae to the list of Hemiptera susceptible to lacewing attack. It must be stressed, however, that a Petri dish is a very simple environment in which a predator does not have a choice of prey and the prey cannot hide or escape (Kron & Sisterson, 2020). Thus, the experiment recorded must be considered a best-case scenario for detecting for the first time the ability of *C. carnea* to feed on *N. campestris*. In addition, these findings should be supplemented by determining the presence and abundance of lacewings in herbaceous vegetation. In this regard, there are reports of adults and preimaginal stages of lacewings in the ground cover in Mediterranean woody crops (Alcalá-Herrera et al., 2019). Furthermore realistic and extensive field experiments are needed in order to confirm the effect of green lacewing larvae on vector populations in crops.

As expected, the percentage of 3rd instar larvae of the lacewing killed nymphs was almost three times that killed by 2nd instar larvae. In this context, 3rd instar larvae of *C. carnea* are more voracious than second or first-instar larvae (Sattar et al., 2007; Khan et al., 2012; Pacheco-Rueda et al., 2015). Similarly, and in support of our results, Cuello et al. (2019) report that the 3rd instar larva of *C. externa* consumes significantly more prey than its 2nd instar larva when attacking *G. brimblecombei* and *T. peregrinus* under similar conditions. In contrast, Mazza et al. (2021) report similar consumptions for 2nd and 3rd instar larvae of *C. carnea* feeding on *R. speculum*. In addition, prey size can have a strong effect on the number eaten (Rudolf, 2008). Our results indicate that the percentage of green lacewing larvae that killed 3rd instar nymphs was almost twice that of those that killed last-instar nymphs. Kron & Sisterson (2020) report higher percentage mortalities for 1st and 2nd instar nymphs of *S. festinus* than for 3rd, 4th and 5th instars when fed to larvae of *C. rufilabris*. Similarly, Pacheco-Rueda et al. (2015) report that *C. rufilabris* and *C. externa* larvae consume small nymphs of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). This preference for small prey may be because it is harder to capture larger prey and the handling time is greater (Milonas et al., 2011). In the present study, the results obtained for the 2nd- and 3rd-instar lacewing larvae are consistent with this notion: the percentage of larvae that killed small nymphs was over 5 times greater than the percentage that killed large nymphs. This should be taken into account when considering their use as biological control agents. Currently commercially-produced lacewings are used for mass release in field crops as an alternative to using insecticides. Therefore, it is important to know to what extent the different instars are effective and the size range of their prey. In this regard, Daane et al. (1996) tested how effective the inundative release of *C. carnea* was in suppressing the leafhoppers *E. variabilis* and *E. elegantula* in vineyards. These authors report that releases of *C. carnea* (9.6–29.5%) result in a greater reduction in leafhopper nymphs when lacewings are released as larvae than as eggs. In the case of *N. campestris*, further research is required in order to evaluate the potential of using *C. carnea* releases to control vectors in crops susceptible to *X. fastidiosa*.

There is little information on percentage predation under similar conditions and with a similar sized prey. Data for 3rd-instar larvae of *C. externa* feeding on *G. brimblecombei* and *T. peregrinus* nymphs in Petri dishes indicate greater than 20 nymphs per 24 h (Cuello et al., 2019). In contrast, Weisser Earlandson & Obrycki (2010) report that the 3rd-instar larvae of *C. carnea* kill 1.2 *E. fabae* nymphs every 24 h. It must be stressed that the last-instar nymphs of *N. campestris* are much larger than the prey mentioned above. In any event, the percentage predation recorded for the last-instar of *N. campestris* seem to be considerably lower than that reported for other common prey of *C. carnea*. For instance, Pérez-Guerrero et al. (2014), under similar conditions, report that in only 90 min, the 3rd instar larvae of *C. carnea* consumed more than four 2nd-instar larvae of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). Percentage mortality of nymphs of different instars should be analysed in future studies.

The presence of foam reduced, but did not prevent predation. Little is known about how foam affects natural enemies. Del Campo et al. (2011) describe the deterrent effects of *Aphrophora cribrate* Walker (Hemiptera: Aphrophoridae) foam and its metabolites on the ant *Formica excestoides* (Hymenoptera: Formicidae), in which the rate feeding of this ant decreased significantly after 5 min. In contrast, Vicente-Díez et al. (2021) report no effects on the virulence of entomopathogenic nematodes and cell-free supernatants from its symbiotic bacteria when exposed to *P. spumarius* (Hemiptera: Aphrophoridae) foam. In addition, the foam from other aphrophorids has antibacterial and antifungal activity (Chang et al., 2019; Sahayaraj et al., 2021). In the present study, feeding by lacewing larvae was delayed and reduced, which indicates a deterrent effect, which is similar to the results of Del Campo et al. (2011). In any event, it is clear that foam did not prevent larvae from eating nymphs. Further research is needed in order to determine whether lacewing larvae would attack nymphs of *N. campestris* if other prey is available, as in field conditions.

Lethality of *B. bassiana* for adults of *N. campestris*

Solutions containing the wild strain of *B. bassiana* BbGEp1 sprayed on plants killed adults of *N. campestris*. Percentage mortality ranged from 10% to 80% when concentrations ranged between 8×10^4 and 10^7 conidia/mL. There is no data on the extent to which the three confirmed vectors of *X. fastidiosa* in Europe (*P. spumarius*, *N. campestris* and *P. italosignus*) are susceptible to entomopathogenic fungi. There is some information on the lethality of entomopathogenic for the main vectors of *X. fastidiosa* in North America (e.g. *H. vitiprennis* and *H. coagulate*; Dara et al., 2007, 2008; Cabanillas & Jones, 2013). In general, Auchenorhyncha are susceptible to entomopathogenic fungi as natural infections are reported for several families: Cicadellidae (Toledo et al., 2006; Choudhary et al., 2012; Thangam et al., 2013), Cercopidae (Choudhary et al., 2012; Foieri et al., 2017) and Fulgoridae (Clifton et al., 2019). Little information, however, is available on the lethality of entomopathogenic fungi for Aphrophoridae. Yan et al. (2019) report that seven days after the application of solutions of *Pestalotiopsis* spp. many *Aphrophora flavipes* Uhler were dead. These authors report a percentage mortality of 84.7% when a concentration of 2.37×10^8 conidia/mL was used. This level of mortality is similar to that recorded for *N. campestris* (80%), but using a lower concentration of *B. bassiana* (1×10^7 conidia/mL). The lethality *B. bassiana* for *H. coagulate*, the main vector of *X. fastidiosa*, using the same concentration as used in the current study, resulted in a percentage mortality of 53.8% seven days after application (Dara et al., 2008). Kanga et al. (2004) report that *Pseudogibellula formicarum* caused 66% mortality of *H. coagulata* seven days after application using a concentration of 2×10^8conidia/mL. The method used by Cabanillas & Jones (2013) to estimate LC_{50} for the effect of *Isaria poprawskii* on *H. vitripennis* (expressed as conidia/mm^2) does not allow a direct comparison with results of the current study. However, the LT_{50} they cite for adults of *H. vitripennis* for a high concentration (2×10^8 conidia/mL) is 5.3 days. This is greater than time recorded for adults of *N. campestris* using a lower concentration (3.63 days at 1×10^7 conidia/mL). Thus, the lethality of *B. bassiana* BbGEp1 for adults of *N. campestris* is greater than that reported for other vectors of *X. fastidiosa*. Further field research is needed in order to confirm that this strain of entomopathogenic fungi is an efficient biological control agent against *N. campestris* infesting crops susceptible to *X. fastidiosa*. In this respect, it would be interesting to analyse to what extent this and other strains are active against vectors of *X. fastidiosa*. The purpose of this would be to ascertain the potential for spraying crops susceptible to *X. fastidiosa* when entomopathogenic fungi are currently used to control other major pests (e.g. olive; Yousef et al., 2017).

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