GIUSEPPE MAZZA^{a,*} - FRANCESCO BINAZZI^{a,*} - DANIELE MARRACCINI^b - LUCA BONCOMPAGNI^c -
GIUSEPPINO SABBATINI PEVERIERI^a - PIO FEDERICO ROVERSI^a - ELISABETTA GARGANI^a

EVALUATION OF *CHRYSOPERLA CARNEA* COMPLEX AND COCCINELLID PREDATORS AS BIOCONTROL AGENTS OF *RICANIA SPECULUM* (WALKER, 1851) (HEMIPTERA RICANIIDAE)

^a CREA – Research Centre for Plant Protection and Certification. Florence, Italy

^b Department of Agriculture, Food and Environment, University of Pisa. Pisa, Italy

^c Department of Biology, University of Florence. Florence, Italy

*These authors equally contributed to this paper

Corresponding Author: Giuseppe Mazza, giuseppe.mazza@crea.gov.it

Mazza G., Binazzi F., Marraccini D., Boncompagni L., Sabbatini Peverieri G., Roversi P.F., Gargani E. - Evaluation of *Chrysoperla carnea* complex and coccinellid predators as biocontrol agents of *Ricania speculum* (Walker, 1851) (Hemiptera Ricaniidae).

Ricania speculum is an alien insect feeding on several host plants belonging to several families, including important vegetables, crops, ornamental plants, wild plants as well as trees. For the first time, recently, *Chrysoperla* sp. and coccinellid beetles have been observed preying on this species in the field on *Citrus* sp. and *Laurus nobilis* plants. The main purposes of this work were to evaluate the possible predation capability of these groups of insects and determine their prey preference in laboratory trials.

In the choice trials, *R. speculum* nymphs were paired in a confined space and exposed for 24h to single predators: *Ch. carnea* complex and to the coccinellids *Cryptolaemus montrouzieri*, *Adalia bipunctata*, *Coccinella septempunctata*, and *Propylea quatuordecimpunctata*. Specimens of *Aphis rumicis*, an aphid frequently found on herbaceous plants, were used as a control prey. In the cage trials, a single *Ch. carnea* complex second-instar larva was placed into a jar containing a tomato plant with ten *R. speculum* nymphs.

In the choice trials, a high mortality percentage of both preys was observed with the *Ch. carnea* complex, while in those with Coccinellids only high *A. rumicis* mortality was reported. In the cage trials, 13% mortality of *R. speculum* was recorded after 24h while after 72h and 168h prey mortalities reached 18% and 25%, respectively. The results of the study showed that larvae of the *Ch. carnea* complex could be potential predators of *R. speculum*, while coccinellids do not seem promising predators of this alien species.

KEY WORDS: alien species; biological control; Chrysopidae; Fulgoromorpha; lacewings; natural enemies

INTRODUCTION

Ricania speculum (Walker, 1851) (Hemiptera: Fulgoromorpha: Ricaniidae), the black planthopper, is an alien species reported for the first time in Europe in 2009 (MAZZA *et al.*, 2014). Distributed in several countries of Southeast Asia (BOURGOIN, 2021), in a relatively short time, the species has spread to several Italian regions such as Liguria, Toscana, Veneto, Piemonte, and Lazio (MAZZA *et al.*, 2014; ROSSI & LUCCHI, 2015; SILVESTRI, 2017; MAZZA *et al.*, 2018). More recently, in 2019, it was also found in Friuli-Venezia Giulia and Emilia-Romagna (Marraccini D., pers. obs.).

Ricania speculum is a univoltine species, characterized by a wide polyphagia, which allowed a rapid expansion of this species in the invaded countries (e.g. MAZZA *et al.*, 2014; ROSSI & LUCCHI, 2015; MAZZA *et al.*, 2018). Females insert their eggs into the woody twigs of many host plants, including important vegetables, crops, ornamental plants as well as wild plants and trees, on which nymphs feed and develop. Many host plants, including important crops such as *Olea europaea* L. and *Vitis* spp., are reported to be susceptible, even though the potential economic impact of this pest has never been fully evaluated. Damages mainly include sap suction, wounds, and with-

ering of young shoots or branches where the eggs are laid (ROSSI *et al.*, 2015; MAZZA *et al.*, 2018). So far, *R. speculum* has not represented an actual problem, but its increasing populations spreading in new areas are raising some concern, not only for its direct damage but also for the indirect ones. In fact, this pest has also been reported to acquire grapevine Flavescence dorée phytoplasma, though it seems unable to transmit it (GALETTO *et al.*, 2019). No management strategies have been developed for this alien species even though green chromotropic traps have proved effective as a monitoring device (MAZZA *et al.*, 2019). No pesticides have been registered to control this insect and only a few natural enemies have been reported for the entire family Ricanidae; for example, ÇETIN & SEZEN (2016) investigated the bacterial flora of *Ricania simulans* (Walker, 1851) and tested with efficacy some bacterial isolates against this pest. GÜÇLÜ *et al.* (2010) studied the pathogenicity of *Lecanicillium muscarium* against *R. simulans* and discovered that nymphs were more susceptible than adults to this fungus. Regarding *R. speculum*, LAUDONIA *et al.* (2017) reported the first record of native parasitoids, which emerged from eggs collected in Liguria (Northern Italy). However, despite the high number of collected samples, only a few parasitoids emerged. Thus, no reports concerning the ef-

ficacy of natural enemies for controlling *R. speculum* are currently available.

In 2018 during monitoring and research activities on *R. speculum* distribution and host plants (MAZZA *et al.*, 2018), the following observations were made in the field for the first time, particularly on *Citrus* sp. and *Laurus nobilis* L. plants: green lacewings of the genus *Chrysoperla* Steinmann, 1964 (Neuroptera: Chrysopidae) on more than one occasion, and adults of the coccinellid *Coccinella septempunctata* Linnaeus, 1758 (Coleoptera: Coccinellidae), more rarely, attacked and fed on *R. speculum* nymphs. On the contrary, the same predators have never been reported to attack *R. speculum* adults (Marracini D., pers. obs.).

In most cases, chrysopid larvae are polyphagous predators feeding on several pests of economic importance (SENIOR & MCEWEN, 2001). A potential obstacle to the successful use of chrysopids in biological control is represented by the taxonomic complexity of the genus *Chrysoperla* that is currently under revision (TAUBER *et al.*, 2000; HENRY & WELLS, 2007). Indeed, *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae) appears to be a complex of many cryptic, sibling species, the *Chrysoperla carnea* complex or *carnea*-group (THIERRY *et al.*, 1992, 1998; HENRY *et al.*, 2001, 2002; MONSERRAT, 2016). They are particularly attracted to eggs and young larvae of Lepidoptera, aphids, spider mites, scales, psyllids, mealybugs, whiteflies, thrips, leafhoppers, and other soft-bodied preys (PRINCIPI & CANARD, 1984).

Lacewing larvae are highly predacious and cannibalistic, while adults feed mostly on nectar, pollen, and honeydew, even though some of them can be predators (PAPAS *et al.*, 2011).

The *Chrysoperla carnea* complex is not only relatively easy to rear and widely used in biological control but are also commercially available for greenhouse and field application (ATLIHAN *et al.*, 2004; CARILLO *et al.*, 2009; HUANG & ENKEGAARD, 2010). Unfortunately, the taxon referred to '*Chrysoperla carnea*' by the industry may include several taxa from different geographic regions. This represents a risk factor such as the introduction of alien species and the ecological and behavioural differences among the species of the complex (HENRY & WELLS, 2007). Thus, the clear identification of the best taxa suitable for correct biological control programs represents a continuous challenge (HENRY *et al.*, 2002; LOURENÇO *et al.*, 2006).

Immature and adult coccinellid beetles (Coleoptera: Coccinellidae) are also common and voracious predators feeding mainly on aphids or specific preys (MICHAUD, 2012). DIXON (2000) emphasized three attributes, which make members of the Coccinellidae family successful biological control agents: specificity, voracity, and a high rate of population increase. Coccinellids exhibit, in most cases, a narrow range of prey specificity, however, dominant species tend to be generalists. In fact, it is known that, after a consistent population increase due to aphid outbreaks, some of them are able to switch to alternative prey species contributing to the biological control of

pests, which serve only as supplementary food (MICHAUD, 2012).

Thus, the aims of the present work are to evaluate the potential role of *Ch. carnea* complex larvae and coccinellid adult beetles as predators of *R. speculum* nymphs, to verify their prey preference in choice trials and to evaluate the amount of prey that predators are able to kill in cage trials.

MATERIALS AND METHODS

Ricania speculum nymphs (first-third instars according to WILSON *et al.*, 2016) were collected in May-July 2018 and 2019 in Tuscany (Central Italy). A field survey was conducted in suburban areas in the municipality of Viareggio, province of Lucca (43°50'40''N; 10°16'15''E) in order to collect the species frequently observed here (MAZZA *et al.*, 2018; 2019).

In the same period, adult winged morphs of *Aphys rumicis* Linnaeus, 1758 and adults of *Co. septempunctata* were respectively collected on *Rumex* sp. and *Spartium junceum* L., during insect collection in countryside areas located close to Florence (43°43'54''N; 11°15'16''E).

The adult coccinellids *Cryptolaemus montrouzieri* Mulsant, 1853, *Adalia bipunctata* (Linnaeus, 1758), and *Propylea quatuordecimpunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) as well as eggs and larvae of the *Ch. carnea* complex (CrisoPAK®) were obtained from BIOPLANET® (Cesena, Italy). The bio-factories do not provide a taxonomic certification of the biocontrol agents and thus, specimens belonging to sibling species of the *Ch. carnea* complex, as previously explained into the introduction, could be potentially present within the same package.

In the laboratory, first instar larvae of *Ch. carnea* complex were kept in Petri dishes (9 cm diameter) and daily fed *ad libitum* with *Ap. rumicis* to obtain second and third instar larvae suitable for the tests. All stages of the *Ch. carnea* complex larvae were identified according to PANTALEONI (1983). Each single larva was kept individually in a Petri dish to avoid cannibalism.

Colonies of all the predator species (larvae of the *Ch. carnea* complex and adults of coccinellids) were maintained in Petri dishes (9 cm diameter) and they were daily provided with *Ap. rumicis* as food *ad libitum*, during the brief pre-conditioning step up and until the start of the tests.

Similarly, *R. speculum* colonies (nymphs) were reared in the same Petri dishes described above and fed with *L. nobilis* leaves until the beginning of the experiment; the leaves were replenished every two days.

All the insects were reared and maintained in a climatic chamber at + 26 °C ± 1.75, RH 65 ± 5%, and 16:8 L:D (HPP750, Memmert GmbH + Co. KG, Schwabach, Germany) and they were all starved for 24 h before the beginning of the trial according to HUANG & ENKEGAARD (2010). Individuals were separately placed into glass tubes (15 cm long and 2 cm diameter) closed at both ends by a 250-µm-mesh plastic net. The tubes with the speci-

Fig. I - Cage trials: Potted plants of tomato *Lycopersicon esculentum* Mill. cv. Rio with *Ricania speculum* nymphs and a second-instar larva of *Chrysoperla carnea* complex.

mens were then maintained in a climatic chamber at the same conditions described above.

CHOICE TRIALS

All the experiments were performed in Petri dishes (9 cm diameter) lined with moistened filter paper and a *L. nobilis* leaf. One single *Ap. rumicis* (used as positive control) and one *R. speculum* were placed with a fine brush at the opposite extremes of the leaf. Five minutes after the release of the two preys, a single predator from the colonies (*Ch. carnea* complex, $n = 23$ first instar larvae, $n = 23$ second instar larvae, $n = 23$ third instar larvae; *Cr. montrouzieri*, $n = 25$; *Ad. bipunctata*, $n = 23$; *Co. septempunctata*, $n = 17$; *P. quatuordecimpunctata*, $n = 18$) was placed at the center of the leaf in each dish. Insects were maintained in climatic chambers as described above and after 24 h, prey choice (i.e. predated insects) was assessed under a stereomicroscope following HUANG & ENKEGAARD (2010).

CAGE TRIALS

Potted tomato plants (*Lycopersicon esculentum* Mill. cv. Rio Grande of 10 cm height) bought from nursery, were placed into glass jars of 25 cm height and 15 cm diameter closed by a 250- μ m mesh plastic net (Fig. I). Plants were grown in a greenhouse under natural light and temperature conditions after being covered during their development with a fine mesh netting as protection from undesired attacks of other insects. Ten *R. speculum* nymphs were inserted into each jar ($n = 10$) containing a potted tomato plant. Two hours after the release of *R. speculum* nymphs, a single second-instar larva of the *Ch. carnea* complex was released into each jar. The second-instar larvae of this predator were selected for further

cage trials as they had recorded the maximum peak of predation (83%; see below the results section) and were relatively easy to handle in laboratory conditions. This time interval ensured that preys had dispersed throughout the plant. The number of consumed preys was counted after 24 h, 72h, and 168h for each of the 10 jars. All the jars were maintained at 25 ± 2 °C and $60 \pm 5\%$ RH in a controlled rearing room. The peculiar experimental conditions designed in the cage trials were aimed at investigating the searching ability and prey consumption rates of *Ch. carnea* complex second-instar larvae.

STATISTICAL ANALYSIS

In the choice trials, an exact McNemar's test was performed to compare mortalities of paired *Ap. rumicis* and *R. speculum* exposed to each of the investigated predators. A Chi-square test was carried out to compare *R. speculum* mortalities recorded in the three trials involving exposure to different *Ch. carnea* development stages (MACDONALD & GARDNER, 2000; McDONALD, 2014; ZAR, 2010).

In the cage trials, mortalities of *R. speculum* exposed to second-instar *Ch. carnea* complex for various time intervals, were expressed as the number of consumed preys over the total number of preys and calculated after 24 h, 72h, and 168h for each of the 10 jars. Death rates at different time intervals were then compared by the non-parametric Friedman test, as data were not normally distributed and failed transformations. Pairwise comparisons were performed as Post hoc analysis using Wilcoxon signed-rank tests with Bonferroni correction (ZAR, 2010). Statistical analysis was carried out by the software SPSS 20.0.

Fig. II - Prey preferences of *Chrysoperla carnea* complex first-third instar larvae ($n = 23$ for each larval instar) on *Aphis rumicis* adults and *Ricania speculum* nymphs in laboratory choice tests. Asterisks indicate significant differences in the number of consumed preys (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

RESULTS

CHOICE TRIALS

In the first set of choice tests involving either *Ap. rumicis* adults or *R. speculum* nymphs exposed to the predator *Ch. carnea* complex, high mortality percentages of both species were observed (Fig. II). Specifically, in the first trial with the first instar larvae of *C. carnea* complex, 96% and 78% mortality of *Ap. rumicis* and *R. speculum* were respectively recorded, without a significant difference between the mortalities of the two species ($P = 0.125$). A similar result was obtained with the second instar larvae of *Ch. carnea* complex that caused high mortality of both *Ap. rumicis* and *R. speculum* (100% and

83% respectively; $P = 0.125$). Only in the third trial a significant difference between the mortalities of the two species was observed (100% for *Ap. rumicis* and 70% for *R. speculum*; $P = 0.016$).

Fig. III - Prey preferences of four Coccinellid adult predators (*Cryptolaemus montrouzieri*, $n = 25$; *Adalia bipunctata*, $n = 23$; *Coccinella septempunctata*, $n = 17$; *Propylea quatuordecimpunctata*, $n = 18$) on *Aphis rumicis* adults and *Ricania speculum* nymphs in laboratory choice tests. Asterisks indicate significant differences in the number of consumed preys (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Fig. IV - The percentage of *Ricania speculum* nymphs ($n = 100$) preyed upon by *Chrysoperla carnea* complex second-instar larvae ($n = 10$) after 24h, 72h, and 168h in the cage trials.

Ricania speculum mortality percentages, recorded in the three *C. carnea* trials, were quite similar and always high, ranging from 70% to 83%. The Chi-Square test detected no significant differences in the proportions of dead specimens ($\chi^2 = 1.139$; $df = 2$; $n = 69$; $P = 0.566$).

In the second set of choice-tests involving coccinellid predators released against *Ap. rumicis* and *R. speculum*, high aphid mortalities were observed (Fig. III). When adults of the predator *Cr. montrouzieri* were tested against the two preys, 92% mortality of *Ap. rumicis* was recorded while only 20% mortality *R. speculum* was observed ($P = 0.00004$). Similarly, the adults of *Ad. bipunctata* caused 96% mortality of *Ap. rumicis* and only 13% mortality of *R. speculum* ($P = 0.00004$). When *Co. septempunctata* was tested, full mortality rate was recorded for *Ap. rumicis* but not for *R. speculum* (100% and 53% respectively; $P = 0.008$). Also, with the predator *P. quatuordecimpunctata*, 100% mortality was recorded for *Ap. rumicis* while only 17% mortality was observed for *R. speculum* ($P = 0.00006$).

CAGE TRIALS

In the cage trials, significant differences were recorded in the mortality of *R. speculum* preyed upon by the second-instar larvae of the *Ch. carnea* complex after 24h, 72h, and 168h ($\chi^2 = 11.00$; $df = 2$; $n = 10$; $P = 0.004$). After 24h, 13% *R. speculum* mortality was recorded, whereas after 72h and 168h, 18% and 25% mortalities were observed, respectively (Fig. IV). Despite a 5% increase in mortality, no significant difference was observed from 24h to 72h ($Z = -1.890$; $P = 0.059$), and between 72h to 168h, with a 7% increase in mortality ($Z = -1.841$, $P = 0.066$). However, an overall significant increase in mortality was observed from 24h to 168h ($Z = -2.414$, $P = 0.015$).

DISCUSSION AND CONCLUSIONS

The results of our study showed that in laboratory choice trials, the *Chrysoperla carnea* complex preyed upon *Ricania speculum* nymphs, with quite similar and

very high mortality percentages. These values ranged between 70% to 83%, depending on the development stage of predator larvae. Several species of green lacewings have been reported to preferentially feed on aphids (e.g. CANARD, 2001), with a consumption of up to 160 aphids per day reported in ATLIHAN *et al.* (2004). Our results not only confirmed the voracious nature of the green common lacewing and its generalist behaviour, but also evidenced, for the first time, its ability to prey on *R. speculum*. In these trials, the *Ch. carnea* complex larvae were maintained on *Aphis rumicis* as rearing *pabulum*. This rearing period was, nonetheless, extremely brief and did not affect the predation ability of the chrysopid larvae, which were equally attracted to the two different preys, as highlighted by the results.

These results are noteworthy because *R. speculum* nymphs are more difficult to predate when compared to aphids. In fact, the antipredator strategies of *R. speculum* nymphs may comprise dodging around to the opposite side of a leaf or branch as well as jumping when a predator approaches it (DIETRICH, 2009). Moreover, *Ricania* nymphs have not only their body covered by enormous quantities of wax secretions but are also provided with a sort of flexible “wax tail”, which can be moved in a parasol-like fashion when nymphs are disturbed (HAMILTON, 2011; WILSON *et al.*, 2016). Thus, this wax can prevent parasites and predators from grasping the nymphs, allowing them to leap away in case of danger (DIETRICH, 2009). However, *Chrysoperla carnea* complex larvae may overcome such *R. speculum* defense strategies, as observed by our results.

In our trials, only third-instar larvae of the *Ch. carnea* complex were found to prefer *Ap. rumicis*, but no significant differences were detected. Moreover, no significant differences were observed in the efficacy of three tested *C. carnea* development stages. Nonetheless, second-instar larvae of this predator were selected for further cage trials as reported above.

In the cage trials, single larvae showed interesting results with 13%, 18%, and 25% mortality of *R. speculum* nymphs at the three tested time intervals. Notwithstanding the relatively low percentages, this experiment on

prey consumption is promising since single larvae of *Ch. carnea* complex and their preys were not confined to small Petri dishes but in a more complex experimental set-up involving potted tomato plants. Further semi-field/field trials should be performed using different experimental conditions including other host plants and different predator/prey densities. Indeed, as already reported in ATLIHAN *et al.* (2004), prey density had a considerable effect on consumption rate, development, and fecundity of this predator. In particular, prey consumption of *Ch. carnea* complex larvae rose with increasing prey density. In our trial, only a single larva of the *Ch. carnea* complex was used in each jar, while hundreds of these predators could be potentially released in the field. Interestingly, even though predators are known to respond to plant volatiles (SHARMA *et al.*, 2019), REDDY (2002) found that when using the olfactometer, *Ch. carnea* adults did not respond to odors emanating from tomato plants. However, the latter author used the non-predatory *Ch. carnea* adults and thus no comparisons can be made with our experiment. Further trials involving *Ch. carnea* complex larvae could be performed with other plant hosts since *R. speculum* showed a wide polyphagia (MAZZA *et al.*, 2018).

All the experiments performed with the four different species of coccinellid predators, clearly showed that these beetles preferentially preyed on aphids. It is worth noting that no promising results were obtained with adults, even though they theoretically should be the most suitable life stage for field release, as they are more voracious than larvae. Our results indicate that probably they are not effective biological control agents against *R. speculum*. A higher mortality rate of preys (53%) was only recorded in laboratory tests with *Co. septempunctata* (the coccinellid beetle observed in the field during the predation events on *R. speculum*), but a strong preference for aphids was evident since this species is quite specialist. According to MICHAUD (2012), coccinellids are characterized by prey specificity and most of them are aphidophagous or coccidophagous.

In conclusion, continuous field observations (Marracini D., pers. obs.) followed by laboratory trials, evidenced that the *Ch. carnea* complex could be more effective than coccinellid beetles as potential biocontrol agents of *R. speculum*. Nonetheless, additional aspects related to control efficiency require further evaluation in semi-field and field conditions. The accurate identification and use of the cryptic species of the *Ch. carnea* group could influence the effectiveness of this control agent leading to a more adequate control of this pest being mindful of ethical environmental stewardship, as highlighted by HENRY & WELLS (2007). Moreover, as already highlighted, the *Ch. carnea* complex is still not taxonomically resolved and thus the results could be affected since the tests could be performed with multiple species with different ecology.

Due to the aforementioned problems, the use of *Ch. carnea* complex as biocontrol agents must be evaluated with critical attention. Neither the composition nor the origin of the individuals used for massive breeding and sold in these commercial packages are known, thus posing a potential threat to the natural ecosystems.

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