

ORIGINAL ARTICLE

“Alien versus predator”: predatory effect of coccinellid *Exochomus quadripustulatus* on the scale insect *Toumeyella parvicornis*. An open-field experimentation on the *Pinus pinea* of Rome

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Abstract The tortoise scale insect (*Toumeyella parvicornis*) is rapidly spreading in stone pine (*Pinus pinea*) forests and urban parks of Mediterranean Europe. Its current distribution and potential spread is concerning as, so far, it already includes three different European countries. Pest management strategies based on endotherapeutic treatments have a limited time coverage and are unfeasible on large scales. Biological control can be a valuable alternative to contain the spread of *T. parvicornis*, as highlighted by some recent studies conducted with predators under controlled conditions. Although promising outcomes, open-field efficacy is still poorly explored. This study aimed to fill this gap in knowledge through an open-field assessment of the predation impact of *Exochomus quadripustulatus* and its capability of reducing *T. parvicornis* infestation level. Adult ladybug females were released on stone pine groups divided as follows: (i) plants treated only with ladybugs, (ii) plants pre-treated with bio-insecticide prior the release of the ladybugs, and (iii) an untreated control. The stone pine groups that received the ladybugs application, showed a lower infestation level (in terms of *T. parvicornis* adult females) than the untreated control, for most of the evaluation period. Results showed, for the first time, evidence of *E. quadripustulatus* efficacy in open-field applications, confirming the previously positive outcomes observed under laboratory and semi-field conditions. The outcomes of this study, accordingly, open the door to future biological control programs.

Key words biological control; ladybugs; pest management; stone pine; tortoise scale insect

Introduction

The recent European expansion of the pine tortoise scale insect, *Toumeyella parvicornis* (Cockerell, 1897) (Cockerell & Quaintance, 1897) (Hemiptera: Coccidae) is in-

creasing the demand for effective and low-impact control strategies which, however, are further complicated by the typical environment where *Pinus pinea* L., the host plant, is usually growing. Stone pines are either present in urban/suburban parks of many Mediterranean areas or in forests devoted to pine nuts production. These environments are favorable for the spread of this pest, increasing the complexity of management.

The reduced dimensions of the insect (0.2–2 mm), the size and the umbrella-like crown shape of the host plant,

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and the infestation localized on plant canopies is the main limitation for an early detection of *T. parvicornis*. As the usual height of adult plants is around 15 m, inspections on the canopies and/or sample collection require cranes and qualified personnel. The costs of monitoring activities are further increased by the identification of the specimens, which requires a stereomicroscope to clearly observe either the typical bilocular pores on the body of females or the microscopic characters of the preimaginal stages (Garonna et al., 2018). The recent publication of the DNA barcoding sequences (Di Sora et al., 2023c) could open the door to further molecular-based tools that might support the morphological identification of this species, even if it is not going to solve the problem of sample collection.

The ontogenic cycle of *T. parvicornis* is well known and is similar in all newly introduced areas, except for the number of generations. After egg hatching, crawlers (the first preimaginal stage) colonize healthy plants mainly carried by the wind. From the second preimaginal stage, instead, adult females lose the legs and remain anchored to the twigs through the piercing-sucking mouthparts. Adult males, instead, remain mobile for the overall duration of the life cycle (Hamon & Williams, 1984).

The infestation can be accordingly divided in two phases. During the first stage of the infestation, plants are apparently healthy and the detection can be carried out only by inspecting the canopy of the tree. During the advanced stage, instead, a pool of secondary fungal hosts develop on the honeydew produced by *T. parvicornis*. Fungal growth may bring to the typical black color of the canopy, a sign of molds' presence, and part of the plants to death (Chomnunti et al., 2014).

The pine tortoise scale insect is quickly adapting to the Mediterranean climatic conditions. The environmental suitability predicted by Di Sora et al. (2023a) has been recently confirmed by the first detections in France and Albania (EPPO, 2021; Di Sora et al., 2024b). In Italy, instead, the almost continuous stone pine belt along the coastal areas is speeding up the spread (EPPO, 2023), despite the intensive control actions that have been carried out, in the past years, using agrochemicals (Di Sora et al., 2022). To date, endotherapeutic abamectin is the only agrochemical-based treatment allowed on *T. parvicornis* to contain and prevent the spread in urban environments. However, although its persistence was considered sufficient for the containment of this pest (Bertin et al., 2022), more in-depth researches (Di Sora et al., 2022; 2023b) showed that endotherapeutic abamectin is strongly limited by two main factors: (i) the cost is high, and (ii) the coverage time of the treatments is shorter than expected. For this reason, there is the need for alternative solutions, pos-

sibly based on natural enemies. Biological alternatives that can be applied externally and directly to the foliage have not been studied for this pest, although some commercial products may have potential use. For example, the organic product known as Agricolle®, based on natural gelling agents containing polysaccharides that attach firmly to the bodies of certain insects (Biogard, 2025). Its insecticidal mechanism is therefore exclusively physical, like a glue. It may be particularly effective on small life stages, such as nymphs as already tested on other species (Nikolova, 2021).

The use of predatory insects for pest management is a valid alternative, especially when compared to more traditional chemical approaches (Furlong & Zalucki, 2010). Natural enemies are, in fact, a crucial factor for the natural balance inside an ecosystem (Mandal et al., 2019).

In North America, native area of *T. parvicornis*, the main predators belong to Coccinellidae (Rabkin & Lejeune, 1954); The main species are *Hyperaspis binotata* (Say, 1826) (Rabkin, 1939; Bradley, 1973), *Hyperaspis signata* (Olivier, 1808), *Chilocorus stigma* (Say, 1835), *Scymnus lacustris* LeConte, 1850 and *Coccinella transversoguttata* Faldermann, 1835 (Wilson, 1971). Predators belonging to Coccinellidae are widely distributed in Europe, including Italy, and some of them, such as *Cryptolaemus montrouzieri* Mulsant, 1850, are currently involved in biological control programs in agriculture and forest environments (Burgio et al., 2025).

Adaptations of local natural enemies (predators/parasitoids) to invasive species are common phenomena, even if this process is not immediate (Carlsson et al., 2009; Pintor & Byers, 2015). Conversely, the introduction of natural enemies from their native areas results, *de facto*, in the release of a second alien species (Van Lenteren, 2000; Symondson, 2002): this labor-intensive process should follow well-defined steps to ensure that there will not be deleterious consequences for the receiving ecosystem (Jeschke et al., 2014). For this reason, a preliminary assessment of the potential efficacy of local predators/parasitoids has been conducted.

Di Sora et al. (2024a) evaluated the predation efficiency of *Exochomus quadripustulatus* (Linnaeus, 1758) and *C. montrouzieri*, two common scale insect predators with stable populations in European pinewood forests, obtaining promising results under controlled and semi-controlled environments. Then, selecting between the two species, the only one that is native, proved to be effective in the preliminary phase and widely available commercially, the natural further step of this research would be an open field evaluation of the efficacy of the predator. Accordingly, this work aimed to fill this gap in knowledge by considering stone pine plants growing in the

“Parco Regionale dell’Appia Antica” (Rome, Italy). During the spring-autumn season 2024, groups of infested stone pines were selected to test two different combinations of treatments: (i) the release of gravid *E. quadripustulatus* females on the plants’ canopy, and (ii) the canopy treatment with an organic insecticide prior to predators’ release. We believe that this study can provide further information to the scientific community on the potential control of the local natural enemies on *T. parvicornis*, as a first step toward providing a rational solution to the problem of this invasive scale insect affecting European pines.

Materials and methods

Origin of the ladybugs and experimental field

Gravid specimens of *E. quadripustulatus* were provided by CBC Bioplanet Società Agricola SRL (Cesena, Italy). Same-age gravid females reared under the same environmental conditions were used for uniformity of initial conditions. Although specific details of the ladybugs’ rearing process are protected by industrial patents, the specimens developed, from egg to adult, under a constant temperature of 25 °C, a relative humidity of 70%, and a photoperiod of 16 : 8 h (light : dark).

The experimental field was located inside the Parco Regionale dell’Appia Antica (41°49′49.8″N 12°32′42.1″E, Rome, Italy), more specifically within Santa Maria Nova and Villa dei Quintili site, which are under the jurisdiction and management of Parco Archeologico dell’Appia Antica. The area, which has an extension of approximately 11 hectares, is an archaeological site featured by the typical Mediterranean vegetation, mainly stone pines (*Pinus pinea*) and holm oaks (*Quercus ilex* L.). Three different groups of stone pines were randomly selected, and the infestation level by *T. parvicornis* was assessed before the beginning of the experimentation (through statistical analysis following the same procedure as Section *Statistical analysis*, without treatments variable in the calculations). The plant groups were, at least 100 m far from each other, separated by buildings and/or by uncultivated fields with no vegetational continuity in the middle.

Plants were 70–80 years old, had a height of 20 meters, a mean diameter at breast height (DBH) of 60 cm, approximately, and they were equally infested (namely, no significant differences were assessed among plants in terms of *T. parvicornis* abundance, through canopy sampling, $P > 0.05$). Infestation level was assessed on April 22, 2024, at the beginning of the study, ensuring the same initial conditions for all the plants involved in the exper-

imentation. Additionally, it is worth mentioning that the stone pines under investigation had received endotherapeutic treatments with abamectin in previous years. However, no such treatments were performed in 2024, and multi-residual analyses conducted according to Di Sora *et al.* (2022; 2023b) confirmed the absence of abamectin in plant tissues. Two extra twigs per plant, collected during the first sampling date, have been analyzed using the liquid chromatography technique according to the methodology of Mulligan *et al.* (1996) and Madadlou *et al.* (2011). Abamectin concentration was calculated as the sum of Avermectin B1a, Avermectin B1b and delta-8,9 isomer of Avermectin B1a, expressed as Avermectin B1a. The values were associated with the respective extended uncertainty (confidence interval of 95%, coverage factor $k = 2$). The machine had a limit of quantification of 0.005 and a limit of detection of 0.003.

A datalogger (Elitech RC-51H) was placed in the field to record temperature and humidity over the months. During the trial, the tool recorded a mean temperature of 21 °C, a minimum and maximum temperature of 6 °C and 39 °C, respectively, and a mean relative humidity of 69% RH.

Treatments assessment

Plants were divided in three groups (Group I, Group II, Group III), corresponding to the different treatments considered in this study. Treatments were carried out between the second half of May and the first half of June, when the first peak of crawlers occurs (identified as the most susceptible stage) (Garonna *et al.*, 2018).

Group I was composed of six stone pines, located in the Santa Maria Nova site. On 27 May, plants were treated with water and Agricolle®, an organic polysaccharides-based insecticide that was high-pressure (15 bar) sprayed on the canopies through a Caffini Performer 3P motorized-pump. The overall solution was composed of 400 L of water and 1.2 L of Agricolle®; each plant received 66 L of solution, approximately, delivered using a basket crane. On 10 June, almost two weeks after the canopy spraying, 50 gravid females of *E. quadripustulatus* (50 gravid females for each tree for a total of 300 predators) were released on the main branches of the trees using a basket crane to reach the plants’ canopy. The original cup containing the ladybugs, provided by the seller, was opened and the individuals observed up to their spread.

Group II was composed of five stone pines, located in the Villa dei Quintili site. Unlike Group I, this treatment provided only for the release of ladybugs (same day

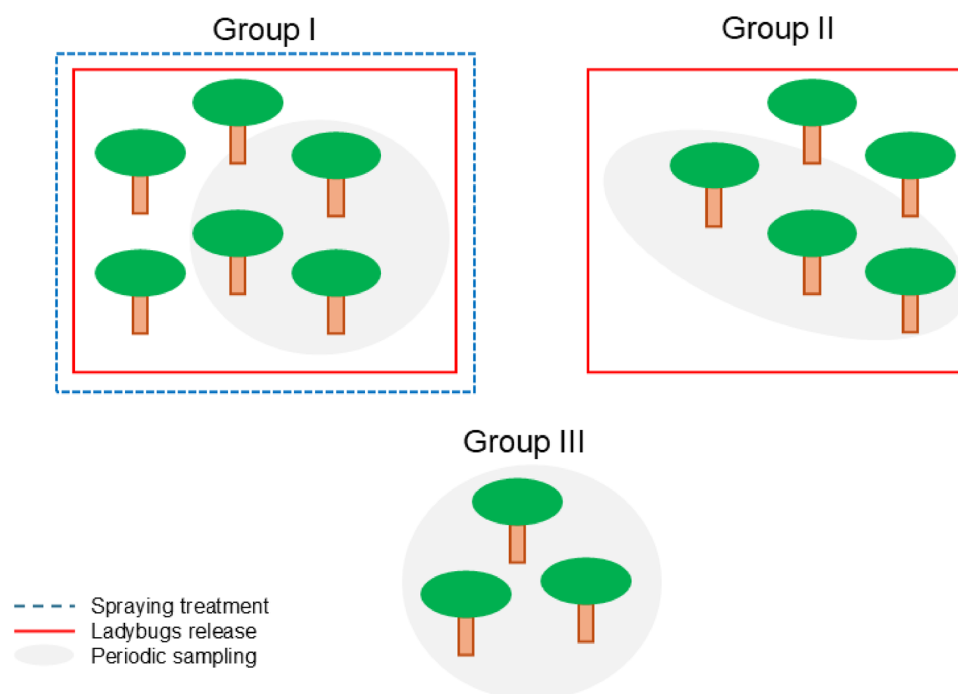


Fig. 1 Sampling and treatments design. The blue dotted-line square includes all the stone pines that received the spraying treatment. The square marked in red includes the stone pines where ladybugs were released. The gray circles indicate sub-groups of stone pines involved in the periodic samples collection.

and conditions of Group I), without spraying the plant canopies (50 gravid females for each tree for a total of 250 predators).

Group III was composed of 3 stone pines, located in the Villa dei Quintili site. This group did not receive any treatment, acting as an untreated control.

*Samples collection and assessment of *T. parvicornis* infestation level*

The field trial covered the period April 22–November 25, 2024. Every month, six twigs per plant, 20 cm-long, were randomly collected to assess the infestation level over time. Regular inspections were carried out, for operational reasons, only on three plants per group, even if treatments were carried out on a higher number, as detailed in Section *Treatments assessment*. Evaluations were focused on a smaller subgroup to ensure data collection where treatments were supposed to be more homogeneous. A graphical representation of the treated and sampled plants is shown in Fig. 1.

Plant canopies were first reached with a basket crane and then the six random twigs per plant were cut using pruning scissors. Samples were promptly stored in plas-

tic bags, labeled, and sealed for further laboratory identification and analysis.

Stone pine twigs were singularly inspected using a stereomicroscope (Nikon C-Leds SMZ 745 T with Digital C-Mount Camera TP 8000, 8.0 Mp color CMOS), counting the number of adult females standing on the bark, according to Di Sora *et al.* (2023b).

Statistical analysis

The dataset was analyzed in two steps that respectively aimed to: (i) assess the differences among the treatments over the season, and (ii) compare the three treatments at each sampling date, to identify when the differences occurred over time. An analogous approach was followed by Di Sora *et al.* (2022) to assess the efficacy of abamectin treatments, indicating either if a treatment is effective or after how long differences occur.

Either in the case (i) or in the case (ii), data were analyzed through a Generalized Linear Model with mixed-effect (GLMM), negative binomial distribution, and the Tukey's adjusted as a *post hoc* test ($\alpha = 0.05$). Treatment was considered as an independent variable, the number of *T. parvicornis* adult females as a response variable (esti-

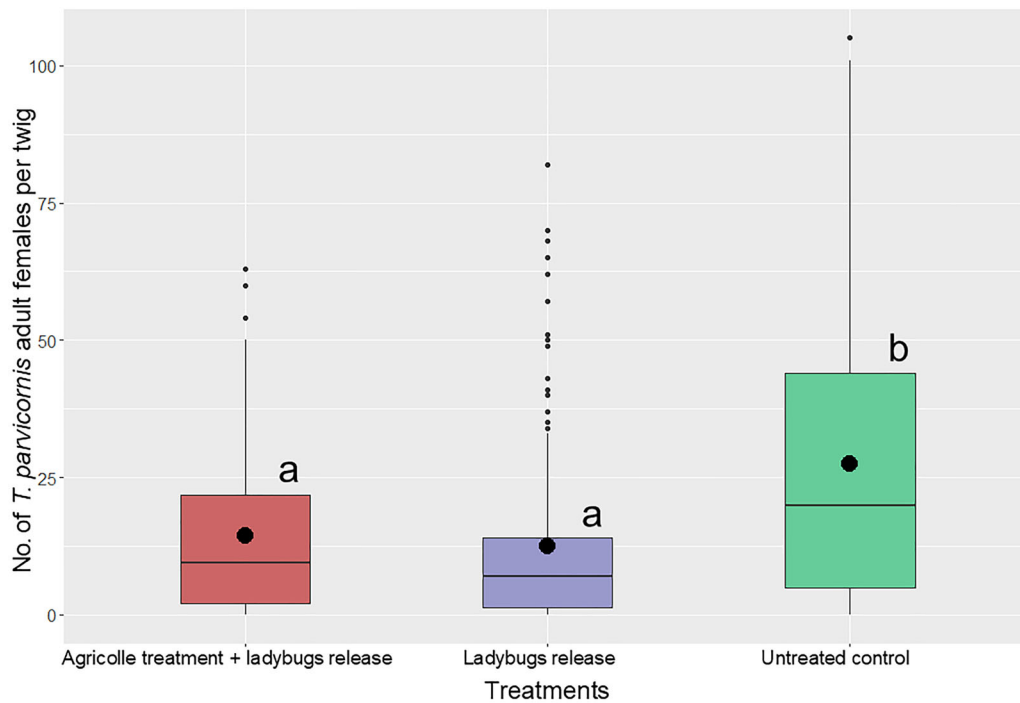


Fig. 2 Infestation level observed during the overall season (number of observation per treatment: 144). *Toumeyella parvicornis* adult females counted on stone pine twigs belonging to the treatment “Agricolle + ladybugs release” (Group I), *T. parvicornis* adult females counted on stone pine twigs belonging to the treatment “Ladybugs release” (Group II) and “untreated control” (Group III). Black dots inside the boxes indicate the mean values of the population, and lines inside the boxes indicate the median values. The whiskers include 95% of the data, while the points represent the outliers. Different letters indicate significant differences assessed through a generalized linear model with mixed-effect (GLMM) followed by the Tukey’s *post hoc* test ($\alpha < 0.05$).

mation of infestation level), and plants as a random variable (block). Calculations were carried out through the R software using the *glmer.nb()* function within the R package *MASS* (Ripley *et al.*, 2013), the *emmeans()* function within the R package *emmeans* (Lenth & Lenth, 2018), the *pairs()* function within the R package *multcompView* (Graves *et al.*, 2015), and the *cld()* function within the R package *multcomp* (Hothorn *et al.*, 2016).

Results

Multiresidue analysis was the starting point of the field survey, and showed a zero level of abamectin on the plants, namely < 0.005 mg/kg. Accordingly, the results of this experimentation are not affected by the previous treatments carried out on plants.

As detailed in Section *Statistical analysis*, the first step of data analysis assessed statistical differences among the treatments and the untreated control in the overall season. Group I and II were statistically different from Group III, in terms of adult female populations (GLMM,

$Z = -4.060$, $P < 0.001$, $NDF = 377$; and GLMM, $Z = -4.951$, $P < 0.001$, $NDF = 377$, respectively). No significant differences were observed between the female populations on plants of Group I and Group II (GLMM, $Z = 0.895$, $P = 0.64$, $NDF = 377$), even if a slightly lower population was observed on group II. A graphical representation of this part of the results is shown in Fig. 2.

The second part of the data analysis further explored the differences between treatments by focusing on the single dates (number of observations for each single date-treatment: 18), so as to highlight the time needed by the treatments to show their efficacy with reference to the untreated control.

Proceeding by order, no statistical differences between the treatments, in terms of adult female population, were assessed between 22 April and 10 of June (Fig. 3), ($P > 0.05$) (Table 1).

Significant differences between the treatments were assessed from 16 of July (Fig. 3), approximately one month after the ladybugs release, suggesting their likely role as biological controller of *T. parvicornis* and confirming the results obtained from the general analysis. On 16 July,

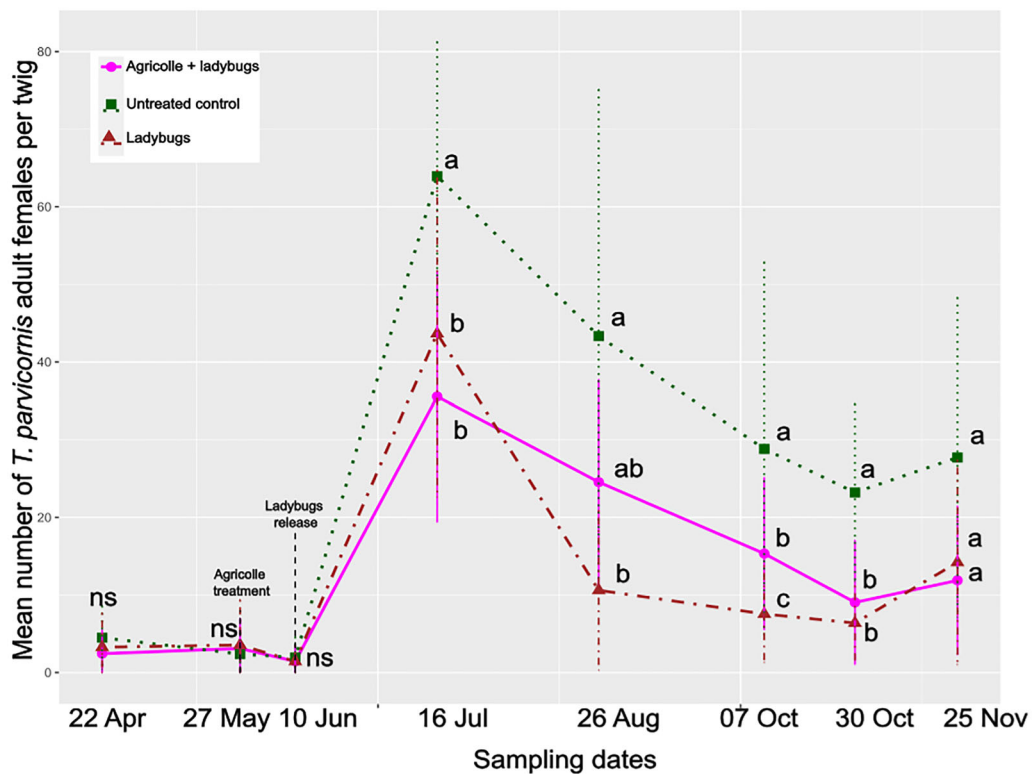


Fig. 3 *Toumeyella parvicornis* infestation level over time (number of adult females counted on stone pine twigs). Pink continuous line represents the Group I treatment, namely “Agricolle treatment + ladybugs release,” the brown dotted-lined line represents the Group II treatment, namely “Ladybugs release,” and the dotted green line represents the Group III treatment, namely the “untreated control.” Whiskers represent the standard errors. Different letters indicate significant differences assessed through a generalized linear model with mixed-effect (GLMM) followed by the Tukey’s *post hoc* test ($\alpha < 0.05$).

statistical differences were observed between Group I and Group III (GLMM, $Z = -4.095$, $P < 0.001$, $NDF = 53$); the treatment induced a reduction of 44% in terms of adult female population. On the same sampling date, differences were assessed between Group II and Group III (GLMM, $Z = 2.680$, $0.01 \leq P \leq 0.05$, $NDF = 53$) with a pest density reduction of 32%. Group I and Group II, instead, were not statistically different (GLMM, $Z = -1.420$, $P = 0.33$, $NDF = 53$).

On 26 August, no statistical differences were observed between Group I and Group III (GLMM, $Z = -0.972$, $P = 0.9$, $NDF = 53$), and between Group I and Group II (GLMM, $Z = 2.161$, $P = 0.08$, $NDF = 53$). The only statistical difference assessed was between Group II and Group III (GLMM, $Z = 3.090$, $0.001 < P \leq 0.01$, $NDF = 53$) where ladybugs led to a reduction of 76% of the pest density.

On 7 October, statistical differences were observed between Group I and Group III (GLMM, $Z = -2.690$, $0.01 \leq P \leq 0.05$, $NDF = 53$), where the combination of canopy spraying and ladybugs release led to a reduction

of 47% of the pest population. The second difference was observed between Group II and Group III (GLMM, $Z = 5.520$, $P < 0.001$, $NDF = 53$) where the ladybugs release led to 74% of pest reduction. Statistical differences were observed between Group I and Group II as well (GLMM, $Z = 2.876$, $0.01 \leq P \leq 0.05$, $NDF = 53$), highlighting a higher efficacy of the Group II treatment (only ladybugs release).

The sampling carried out on 30 October showed similar results: Group I and Group III were statistically different (GLMM, $Z = -3.411$, $0.001 < P \leq 0.01$, $NDF = 53$), with a reduction of 61% of *T. parvicornis* adult female populations, and Group II was statistically different from Group III (GLMM, $Z = 4.628$, $P < 0.001$, $NDF = 53$), with a reduction of 72% of pest population. No statistical differences, instead, were observed between Group I and Group II (GLMM, $Z = 1.255$, $P = 0.42$, $NDF = 53$).

On 25 November, the last sampling date, no statistical differences were observed between the Groups ($P > 0.05$).

Table 1 Results of the statistical analysis, comparing the three treatments based on the different sampling date.

Sampling date	Treatment comparison	<i>T. parvicornis</i> adult females		
		Z	P-value	NDF
22 April	Group I vs. Group II	−0.664	0.78	53
	Group I vs. Group III	−1.397	0.34	53
	Group II vs. Group III	0.737	0.74	53
27 May	Group I vs. Group II	−0.250	0.88	53
	Group I vs. Group III	0.489	0.90	53
	Group II vs. Group III	−0.739	0.74	53
10 June	Group I vs. Group II	−0.078	0.99	53
	Group I vs. Group III	−0.642	0.80	53
	Group II vs. Group III	0.559	0.84	53
16 July	Group I vs. Group II	−1.420	0.33	53
	Group I vs. Group III	−4.095	$P < 0.001$	53
	Group II vs. Group III	2.680	$0.01 \leq P \leq 0.05$	53
26 August	Group I vs. Group II	2.161	0.08	53
	Group I vs. Group III	−0.972	0.59	53
	Group II vs. Group III	3.090	$0.001 < P \leq 0.01$	53
07 October	Group I vs. Group II	2.876	$0.01 \leq P \leq 0.05$	53
	Group I vs. Group III	−2.690	$0.01 \leq P \leq 0.05$	53
	Group II vs. Group III	5.520	$P < 0.001$	53
30 October	Group I vs. Group II	1.225	0.42	53
	Group I vs. Group III	−3.411	$0.001 < P \leq 0.01$	53
	Group II vs. Group III	4.628	$P < 0.001$	53
25 November	Group I vs. Group II	−0.401	0.91	53
	Group I vs. Group III	−2.035	0.10	53
	Group II vs. Group III	1.636	0.23	53

Discussion

This study showed, for the first time, evidence of efficacy of *Exochomus quadripustulatus* in controlling populations of *Toumeyella parvicornis* in natural growing conditions. Our results confirmed and extended the observations and findings of Di Sora *et al.* (2024a) in controlled and semi-field environments. This result is surely promising and encourages biological control programs, in Europe, based on local natural enemies. Such an action is fundamental for the next future, as the front wave of spread of this species is rapidly advancing in many Mediterranean areas.

According to our results, the spraying treatment gave no greater effect than that given by predator action in controlling *T. parvicornis* populations. We can say that the efforts of canopy spraying is not justified in terms of pest control, besides its hard applicability on large scales (e.g., pinewood forests), but at the same time different concentrations of the product and different periods should be investigated.

After the release, the effect of the predators is not immediate (either with or without canopy washing), as shown by the results of this study. However, once their activity started, predators were able to reduce the pest population during the overall vegetative season. As gravid females, *E. quadripustulatus* may have the possibility to establish stable population where released or increase the abundance of populations already present. A decrease of the efficacy was observed in November, when average temperatures were stable below 15 °C and the photoperiod is turning toward longer nights. These environmental conditions could increase the mortality of *E. quadripustulatus* and its overwintering phase. Demographic reduction, in that condition, is known for this species and it was already observed in other studies (Majerus, 2009). Moreover, even the natural demographic reduction of *T. parvicornis* during winter season may had a role on the predator activity. Overall, this study confirmed, for the case of *T. parvicornis*, the impact that *E. quadripustulatus* has on scale insects (adult females). In fact, the same consistent predatory performance has been already observed

on other scale insects, such as *Saissetia oleae* Olivier, 1791 (Katsoyannos & Laudeho, 1975) and *Eupulvinaria hydrangea* (Merlin, 1993), especially by gravid females.

Although they may need time to adapt to new prey or hosts, as could be the case of *E. quadripustulatus*, indigenous control agents might be a quicker solution than non-native controllers coming from pests' native areas. Native natural enemies, in fact, are potentially more specialized, but several studies devoted to understand the potential effects on the ecosystems are needed before their introduction and mass release (Simberloff et al., 2013; Jeschke et al., 2014). This operation usually requires a long time, further extended by local administrations, as it needs the formulation of *ad hoc* regulations and the mass rearing of the natural enemies before the release. Accordingly, even if the introduction of co-evolved natural enemies from native areas might seem the quicker solution, it might not always be the case.

On the other hand, the scarce long-term efficacy of endotherapeutic techniques has been proven in different contexts, from urban to more natural environments (Di Sora et al., 2022; 2023b). Given the high costs, repeated treatments on stone pines is unfeasible, also because endotherapy might compromise the long-term structure of the trees (Aćimović et al., 2016; Berger & Laurent, 2019). Additionally, holes not properly performed might cause infections from secondary hosts (e.g., fungi) (Archer et al., 2022). Biological control actions based on natural enemies are different under this point of view. Besides being safer for human and environmental health, and with no restrictions in urban environments, the release of biological control agents may lead to the establishment of a semi-independent population that could find an equilibrium with the pest. This phenomenon was already observed in other contexts, such as for *Cryptolaemus montrouzieri* used on *Crisicoccus pini* (Kuwana, 1902) (Hemiptera: Pseudococcidae) (Boselli & Pellizzari, 2016) and could be an object of future studies for *T. parvicornis* as well.

It is also worth remarking that *E. quadripustulatus* is a generalist predator that feeds and develops on different hosts, including scale insects (Merlin, 1993). The non-specificity of this natural controller is a great advantage, as it increases the probability of establishing a stable population even if *T. parvicornis* is not always present over the season. Moreover, if *T. parvicornis* lacks nutrients needed, for instance, for egg production or in particular phases of *E. quadripustulatus* life cycle, the presence of the other prey in the environment can further facilitate the development of the predator, compensating the diet (Symondson, 2002).

The successful predation impact of *E. quadripustulatus* has been observed on many scale insect species, such as *Coccus hesperidum* Linnaeus, 1758, *Saissetia oleae* (Olivier, 1791) (Stathas & Skouras, 2013) and *Eupulvinaria hydrangeae* Steinweden, 1946 (Merlin, 1993). This predator is commonly present in Italian, as we observed many times during *T. parvicornis* monitoring surveys (Di Sora et al., 2022; 2023a; 2023b; 2023c; 2024a; 2024b), and European pinewood forests. At the current state of the art, containing actions from *E. quadripustulatus* are limited by the gap in population density that there is between the predator and *T. parvicornis* (Symondson, 2002), probably related to the fact that the predator needs time to adapt to the new prey. Accordingly, there is the need for augmentative release to increase the demographic level and to start effective containing actions.

Considering that so far there are no field studies that investigated biological control for *T. parvicornis*, this study may open new horizons for its sustainable and effective management, even if further studies should be devoted to more precise monitoring of the predator in natural environments, so as to better identify the minimum amount of specimens to release and repetition of these releases over time.

Author contributions

Conceptualization, N.D.S., L.R., G.L., M.C., and S.S. Methodology, N.D.S., G.L., and M.C. Software, N.D.S. and L.R. Validation, N.D.S., G.L., and M.C. Formal analysis, N.D.S., L.R., and M.C. Investigation, N.D.S., G.L., and M.C. Resources, N.D.S., M.C., and S.S. Data curation, N.D.S. and L.R. Writing—original draft preparation, N.D.S., L.R., G.L., and M.C. Writing—review and editing, N.D.S., L.R., G.L., M.C., and S.S. Visualization, N.D.S., L.R., and M.C. Supervision, M.C. and S.S. Project administration M.C. and S.S. Funding acquisition, M.C. and S.S. All authors have read and agreed to the published version of the manuscript.

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Disclosure

The authors declare no conflict of interest.

Data availability statement

The data that support the findings of this study as well as the R script to reproduce the results, are openly available in GitHub at <https://github.com/nicodisora/Exo>

References

- Ćimović, S.G., Cregg, B.M., Sundin, G.W. and Wise, J.C. (2016) Comparison of drill- and needle-based tree injection technologies in healing of trunk injection ports on apple trees. *Urban Forestry & Urban Greening*, 19, 151–157.
- Archer, L., Crane, J.H. and Albrecht, U. (2022) Trunk injection as a tool to deliver plant protection materials—an overview of basic principles and practical considerations. *Horticulturae*, 8, 552.
- Berger, C. and Laurent, F. (2019) Trunk injection of plant protection products to protect trees from pests and diseases. *Crop Protection*, 124, 104831.
- Bertin, S., Ilardi, F., Scapini, C., Simoni, S. and Roversi, P.F. (2022) Alien pest *Toumeyella parvicornis* (Cockerell) (Hemiptera: Coccidae) on *Pinus pinea* L.: short time evaluation of endotherapeutic treatment. *Redia*, 105, 11–16.
- Biogard (2025) <https://www.biogard.it/> (accessed on 19 November 2025).
- Boselli, M. and Pellizzari, G. (2016) First record of the Kuwana pine mealybug *Crisicoccus pini* (Kuwana) in Italy: a new threat to Italian pine forests?. *Zootaxa*, 4083, 293–296.
- Bradley, G.A. (1973) Effect of *Formica obscuripes* (Hymenoptera: Formicidae) on the predator-prey relationship between *Hyperaspis congressis* (Coleoptera: Coccinellidae) and *Toumeyella numismaticum* (Homoptera: Coccidae). *Canadian Entomologist*, 105, 1113–1118.
- Burgio, G., Magagnoli, S., Mondini, R., Guerrieri, E., Casoli, L., Profeta, M. *et al.* (2025) Area-wide augmentation of *Anagyrus vladimiri* and *Cryptolaemus montrouzieri* enhances biological control of mealybugs in Lambrusco vineyards in Northern Italy. *Biological Control*, 206, 105800.
- Carlsson, N.O.L., Sarnelle, O. and Strayer, D.L. (2009) Native predators and exotic prey—an acquired taste?. *Frontiers in Ecology and the Environment*, 7, 525–532.
- Chomnunti, P., Hongsan, S., Aguirre-Hudson, B., Tian, Q., Peršoh, D., Dhami, M.K. *et al.* (2014) The sooty moulds. *Fungal Diversity*, 66, 1–36.
- Cockerell, T. and Quaintance, A. (1897) New and little-known Coccidae from Florida. *Psyche: A Journal of Entomology*, 8, 89–91.
- Di Sora, N., Rossini, L., Contarini, M., Chiarot, E. and Speranza, S. (2022) Endotherapeutic treatment to control *Toumeyella parvicornis* Cockerell infestations on *Pinus pinea* L. *Pest Management Science*, 78, 2443–2448.
- Di Sora, N., Mannu, R., Rossini, L., Contarini, M., Gallego, D. and Speranza, S. (2023a) Using species distribution models (SDMs) to estimate the suitability of European Mediterranean non-native area for the establishment of *Toumeyella parvicornis* (Hemiptera: Coccidae). *Insects*, 14, 46.
- Di Sora, N., Rossini, L., Contarini, M., Mastrandrea, G. and Speranza, S. (2023b) *Toumeyella parvicornis* versus endotherapeutic abamectin: three techniques, 1 year after. *Pest Management Science*, 79, 3676–3680.
- Di Sora, N., Turco, S., Brugneti, F., Rossini, L., Mazzaglia, A., Contarini, M. *et al.* (2023c) Molecular characterization and phylogenetic analysis of the pine tortoise scale insect *Toumeyella parvicornis* (Cockerell) (Hemiptera: Coccidae). *Forests*, 14, 1585.
- Di Sora, N., Rossini, L., Contarini, M., Virla, E.G. and Speranza, S. (2024a) Are the ladybugs *Cryptolaemus montrouzieri* and *Exochomus quadripustulatus* (Coleoptera: Coccinellidae) candidate predators of *Toumeyella parvicornis* (Hemiptera: Coccidae)?. *Pest Management Science*, 80, 2881–2891.
- Di Sora, N., Contarini, M., Rossini, L., Turco, S., Brugneti, F., Metaliai, R. *et al.* (2024b) First report of *Toumeyella parvicornis* (Cockerell) (Hemiptera: Coccidae) in Albania and its potential spread in the coastal area of the Balkans. *EPPO Bulletin*, 54, 160–165.
- EPPO (2021) <https://gd.eppo.int/taxon/TOUMPA/distribution/> (accessed on 30 October 2025).
- EPPO (2023) <https://gd.eppo.int/taxon/TOUMPA/hosts> (accessed on 30 October 2025).
- Furlong, M.J. and Zalucki, M.P. (2010) Exploiting predators for pest management: the need for sound ecological assessment. *Entomologia Experimentalis et Applicata*, 135, 225–236.
- Garonna, A.P., Foscari, A., Russo, E., Jesu, G., Somma, S., Cascone, P. *et al.* (2018) The spread of the non-native pine tortoise scale *Toumeyella parvicornis* (Hemiptera: Coccidae) in Europe: a major threat to *Pinus pinea* in Southern Italy. *Iforest - Biogeosciences and Forestry*, 11, 628–634.
- Graves, S., Piepho, H.P. and Selzer, M.L. (2015) Package “multcompView.” *Visualizations of Paired Comparisons*, <https://cran.r-project.org/web/packages/multcompView/index.html> (accessed on 30 October 2025).

- Hamon, A.B. and Williams, M.L. (1984) Arthropods of Florida and neighboring land areas. In *The Soft Scale Insects of Florida (Homoptera: Coccoidea: Coccidae)*, Vol. 11. Florida Department of Agriculture and Consumer Services.
- Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A., Scheibe, S. et al. (2016) Package ‘multcomp’: simultaneous inference in general parametric models. <http://multcomp.R-forge.R-project.org> (accessed on 30 October 2025).
- Jeschke, J.M., Bacher, S., Blackburn, T.M., Dick, J.T.A., Essl, F., Evans, T. et al. (2014) Defining the impact of non-native species. *Conservation Biology*, 28, 1188–1194.
- Katsoyannos, P. and Laudeho, Y. (1975) Périodes d’activité des principaux insectes entomophages indigènes de Saissetia oleae Bern. sur l’olivier, en Grèce continentale. *Fruits*, 30, 271–274.
- Lenth, R. and Lenth, M.R. (2018) Package “lsmmeans.” *The American Statistician*, 34, 216–221.
- Madadlou, A., O’Sullivan, S. and Sheehan, D. (2011) Fast protein liquid chromatography. In *Protein Chromatography: Methods and Protocols* (eds. D. Walls & S. Loughran), pp. 439–447. Humana Press, Totowa, NJ.
- Majerus, M.E.N. (2009) Ladybugs. In *Encyclopedia of Insects* (eds. V.H. Resh & R.T. Cardé), pp. 547–551. Academic Press, San Diego.
- Mandal, D.S., Samanta, S., Alzahrani, A.K. and Chattopadhyay, J. (2019) Study of a predator-prey model with pest management perspective. *Journal of Biological Systems*, 27, 309–336.
- Merlin, J. (1993) La cochenille Eupulvinaria hydrangeae (Steinw.) (Homoptera: Coccidae) en région bruxelloise: Épidémiologie, ennemis naturels et moyens de lutte. Dissertation, Université libre de Bruxelles, Faculté des sciences, Bruxelles.
- Mulligan, K.J., Brueggemeyer, T.W., Crockett, D.F. and Schepman, J.B. (1996) Analysis of organic volatile impurities as a forensic tool for the examination of bulk pharmaceuticals. *Journal of Chromatography B: Biomedical Sciences and Applications*, 686, 85–95.
- Nikolova, I. (2021) Impact of natural products on *Acyrtosiphon pisum* density on *Pisum sativum* L. and forage quality. *Pesticidi i Fitomedicina*, 36, 15–22.
- Pintor, L.M. and Byers, J.E. (2015) Do native predators benefit from non-native prey?. *Ecology Letters*, 18, 1174–1180.
- Rabkin, F. (1939) Studies on the biology of the Manitoba Jack pine scale *Toumeyella* sp. (Coccidae: Homoptera). Master thesis. University of Manitoba, Winnipeg, MB, Canada.
- Rabkin, F.B. and Lejeune, R.R. (1954) Some aspects of the biology and dispersal of the pine tortoise scale, *Toumeyella numismaticum* (Pettit and McDaniel) (Homoptera: Coccidae). *The Canadian Entomologist*, 86, 570–575.
- Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, A., Firth, D. et al. (2013) Package “mass.” *Cran R*, 538, 113–120.
- Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J. et al. (2013) Impacts of biological invasions: what’s what and the way forward. *Trends in Ecology and Evolution*, 28, 58–66.
- Stathas, G.J. and Skouras, P.J. (2013) Biological control on insect pests in citrus orchards in Greece. *Integrated Control in Citrus Fruit Crops IOBC-WPRS Bulletin*, 95, 1–9.
- Symondson, W.O.C. (2002) Molecular identification of prey in predator diets. *Molecular Ecology*, 11, 627–641.
- Van Lenteren, J.C. (2000) A greenhouse without pesticides: fact or fantasy? *Crop Protection*, 19, 375–384.
- Wilson, L. (1971) *Pine Tortoise Scale*. Department of Agriculture, United States. Forest Service.

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