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ALIEN PEST *TOUMEYELLA PARVICORNIS* (COCKERELL) (HEMIPTERA: COCCIDAE) ON *PINUS PINEA* L.: SHORT TIME EVALUATION OF ENDOTHERAPIC TREATMENT

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Bertin S., Ilardi F., Scapini C., Simoni S., Roversi P.F. – Alien pest *Toumeyella parvicornis* (Cockerell) (Hemiptera: Coccidae) on *Pinus pinea* L.: short time evaluation of endotherapic treatment.

The Pine Tortoise Scale *Toumeyella parvicornis* (Cockerell) is native to North America and was found for the first time in Italy in 2014, in Campania Region. This alien species gradually colonized woods and urban areas in Latium and subsequently moved northward in Tuscany and Abruzzo, and southward in Apulia. The scale was responsible for severe damages on the Stone Pine *Pinus pinea* L., a conifer representing an element of great importance in the Italian landscape and included in the Area Natura 2000 Network for its significant naturalistic interest. Heavy attacks of *T. parvicornis* on *P. pinea* cause a progressive defoliation on the canopies, and the plants quickly decline and are often irreversibly compromised. Such severe symptoms prompted studies for seeking control strategies with a low environmental impact that are suitable in urban areas. In this context, the National Reference Institute for Plant Protection was commissioned by the Italian Ministry of Agricultural, Food and Forestry Policies to verify the potential of endotherapic methods against *T. parvicornis*. The effect of abamectin distributed by trunk injection technology (Nuovo Metodo Corradi®) was experimentally evaluated from April to October 2021 in a park in Rome (Central Italy) on 72 pine trees that showed strong and homogeneous scale infestations. 0.9 ml of a commercial product containing abamectin at 18.37 g/l concentration was injected per cm of plant diameter at breast height (dbh) through holes that were 6 mm in diameter and 6-7 cm in depth. This treatment provided an efficient control of *T. parvicornis* infestations on the pine trees throughout the growing season. The presence of mature females on the one-years-old twigs significantly decreased in treated pines from April to July (about 2 females/twig on average) and to October (about 1.8 females/twig) compared to the control untreated pines (about 40 and 14 females/twig in July and October, respectively). These results showed that a single endotherapic treatment with abamectin can efficiently reduce the populations of *T. parvicornis* at low density levels at least 25 weeks after the trunk injection. The treated pines also visibly appeared to recover from the severe infestation symptoms and no injury and bark lesions ascribable to the trunk drilling were observed.

Key Words: Pine Tortoise Scale, trunk injection, abamectin, urban area.

INTRODUCTION

The growth of international trades and in particular the exchange of plant material across distant geographic areas resulted in an exponential increase of alien species introductions (Roques, 2010; Roques *et al.,* 2016). Several insect pests were found to be able to develop biological invasions worldwide and caused severe infestations on horticultural, ornamental, fruit and woody crops. The scale insects (Hemiptera: Coccomorpha) represent one of the largest groups of alien insects that have been established in the Mediterranean basin in the last decades. Some scale species have strongly affected woods and ornamental trees in parks and avenues, drastically modifying the urban landscapes.

The Tortoise Pine Scale *Toumeyella parvicornis* (Cockerell) (Hemiptera: Coccidae) is native to North America where it is widespread from Southern USA (i.e. Florida and Georgia) to Canada (Clarke, 2013). This species is highly polyphagous, being recorded on at least twenty species of *Pinus* (Clarke, 2013). In the home range, this species is not considered as a primary pest, being responsible for few damages mainly limited to young pine plantations and seed orchards. In the first 2000 decade, the Tortoise Pine Scale was accidentally introduced in Puerto Rico (Segarra-Carmona and Cabrera-Asencio, 2010), and later in Turks and Caicos Islands where it caused the loss of more than 90% of the endemic *Pinus caribaea* trees (Malumphy *et al.,* 2012). More recently, this invasive species was introduced in Italy where it was firstly identified in 2014 in Campania Region (Garonna *et al.,* 2015). Then, it gradually colonized woods and urban areas in Latium and subsequently moved northward in Tuscany and Abruzzo, and southward in Apulia. The infestations were recorded mainly on *Pinus pinea* L. trees located in urban zones where severe symptoms were observed, such as reduction in shoot development, desiccation and yellowing of the needles and lack of vegetative renewal. The damage is related to the scale feeding activity and the subsequent release of a huge amount of honeydew that in turn favours the development of sooty moulds (Garonna *et al.,* 2018). The *T. parvicornis* populations reach high density levels in a short time, causing heavy attacks that gradually in-

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duce pine decline and dieback. The damage phenology is similar to that determined by another pine alien pest, *Matsucoccus feytaudi* (Ducasse), introduced in Italy in the '90s: also in this case, the infested pines present yellowing and reddening of the foliage, and ultimately the loss of needles.

In the northern parts of its distribution range, *T. parvicornis* has one generation per year whereas four generations are typical for southern USA (Cooper and Cranshaw, 2004; Clarke, 2013). The warm climate of the Mediterranean basin allows at least three generations per year, and the scale overwinters in the adult female stage. Oviposition periods usually occurred from late April to end of May, from July to first half of August, and from mid-September to November, and the different generations can overlap (Garonna *et al.,* 2018*).*

In Italy, the entity of the infestations of *T. parvicornis* in the urban areas of Naples and Rome prompted studies for seeking control strategies with a low environmental impact and that are suitable in densely inhabited environments. The endotherapy can be considered as an environmentally friendly strategy to reduce the risks associated with spraying. Moreover, the trunk injection of insecticide products already showed to be efficient in controlling different pests in urban environments, such as *Rhynchophorus ferrugineus* (Olivier) infesting palm trees (Dembilio *et al.,* 2015) and *Cameraria ohridella* (Deschka & Dimić) infesting horse-chestnut trees (Ferracini and Alma, 2008). Promising results were obtained also when endotherapy was performed on conifers against *Crisicoccus pini* (Kuwana) (Boselli *et al.,* 2018).

In the present study, an experimental trial was conducted by trunk-injecting an abamectin-based insecticide on the *P. pinea* trees of an urban park of Rome (Central Italy). Visual inspections of the pine trees as well as microscope observations of sampled branches were carried out to assess the abamectin effect on the population density of *T. parvicornis* until 25 weeks after the treatment.

MATERIALS AND METHODS

The study was carried out in 2021 in the urban park of the Scuola Ufficiali Carabinieri, Caserma "Ugo De Carolis", located in the Northern part of Rome (41°90'N, 12°42'E). The park includes 72 *P. pinea* trees, that were about 50-80 years old and were grown in the same environmental and pruning conditions. Four lots of pines were identified within the park, being separated each other by physical barriers (i.e. school buildings and athletic field). The first three plots (plot 1 with 17 plants, 2 with 27, 3 with 17) were located on the northern, eastern and south-western side of the park, respectively. The fourth plot, located in the southern and bottom boundary of the park, accounted for 11 control trees that were not treated.

Trunk injection using abamectin

On the 27th-28th of April 2021, the trunks of trees of the plots 1-3 were injected with abamectin (Vargas, Rotam Agrochemical) using the Nuovo Metodo Corradi® technique (Boselli *et al.,* 2018). Five to seven equidistant (about 30 cm) holes/tree were performed, depending on the circumference and number of branches of each tree. The holes were performed in the trunk, about 1 m above the ground, using a drill, and were 6 mm in diameter and 6-7 cm in depth. 0.9 ml of commercial product with abamectin (a.i.) concentration of 18.37 g/l was injected per cm of diameter at breast height (dbh). The solution was manually injected in each hole by exerting a low pressure into xylem flow and by exploiting the absorption capacity of the plant to distribute the a.i.. After each treatment application, the injection pump was cleaned with a 15% quaternary ammonium salt solution and the pump tip replaced. The holes in the trunk were disinfected with a 4% quaternary ammonium salt solution and plugged with biodegradable stoppers.

Visual inspection

Before and after treatment time, to come monitor crown conditions, series of photos were acquired. As the injection process involved trunk drilling, after 25 weeks from the treatment, the injection woundes were inspected to evaluate possible bark lesions (hole closure, resin overemission, injury to adjacent woody emission).

Twig sampling and laboratory scale population control.

The level of *T. parvicornis* infestation was estimated on the 18th of April 2021, just before the treatment, and on the 15th of July and 22th of October 2021 on three pines in the control plot and on three pines in each treated plot. For each tree, a one- to three-years-old portion of four branches were collected, for a total of 12 untreated and 36 treated branch samples per survey. For each sample, the infestation level was assessed by observing at the stereomicroscope the presence/absence of eggs, first instars and male cocoons and by counting the number of live adult females. The observations were focused on the twigs, given the rare presence of *T. parvicornis* on needles (Orr, 1931). Presence of honeydew and sooty mould was also recorded.

Statistical analysis

The effect of the trunk injection treatments was tested by comparing population levels in treated *vs* untreated trees after each survey. Data on the number of active adult females were analysed by analysis of the variance (ANOVA). Before endotherapic treatment, on the considered area, evaluation concerning homogeneity of the infestation was performed: a two-way ANOVA was applied to consider effect of plot and single plant on the abundance of the scale females. Then ANOVA was adopted to analyse the possible effect of treatment and sampling data on the number of *T. parvicornis* females.

RESULTS

In April 2021, all the 72 pine trees of the park showed clear symptoms of a severe infestation of *T. parvicornis*.

Pre-treatment check, the 18th of April, 2021



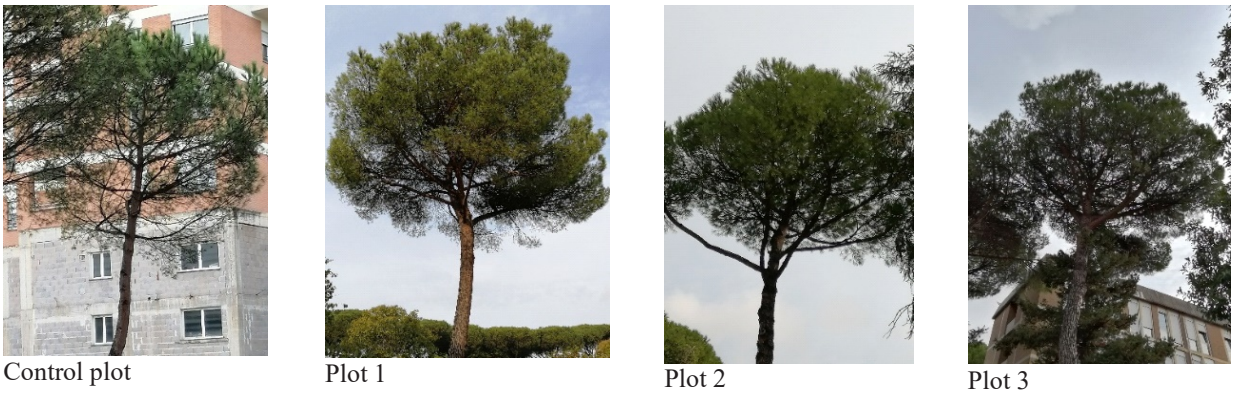
Control plot | Plot 1 | Plot 2 | Plot 3

Post-treatment check, the 15th of July, 2021



Control plot | Plot 1 | Plot 2 | Plot 3

Post-treatment check, the 22th of October, 2021



Control plot | Plot 1 | Plot 2 | Plot 3

Fig. I - Symptoms of the infestation of *Toumeyella parvicornis* on *Pinus pinea* observed ten days before, and 11 and 25 weeks after endotherapic treatment in the control plot and in the treated plots 1-3.

Abundant phylloptosis and needle yellowing as well as huge amounts of honeydew and sooty mould were observed. The pine canopies appeared to be declining and the lowest branches were defoliated (Fig I). Large colonies of egg-laying females of *T. parvicornis* were found on the pine branches that were sampled throughout the urban park. The scale density was homogeneous in the whole area, independently of the considered plant ($F_{2,47}$=0.01; P=0.991) and plot ($F_{3,47}$=0.248; P=0.862) (Fig. II). In the post-treatment evaluations, performed in July and October, significant differences in female density were detected, determined by the treatment ($F_{1,92}$=113.8; P<0.0001) and sampling time ($F_{1,92}$=6.4; P=0.013) (Fig. III).

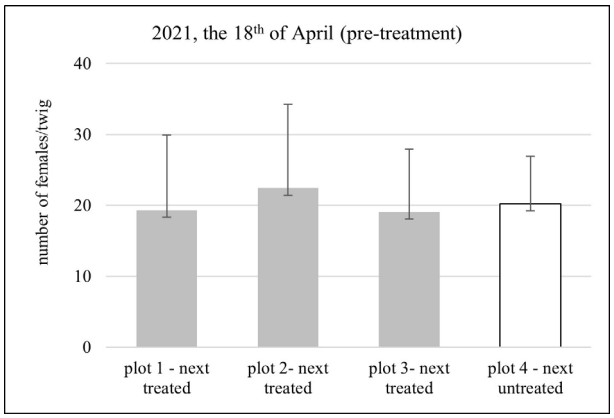


Fig. II – Mean number of *Toumeyella parvicornis* females (mean±SD) detected per twig in the four plots before the endotherapic treatment.

In July 2021, 11 weeks after treatment, at visual inspection the pines of the control plot still showed a general decline: the new sprouts were limited to the highest part of the canopy, and abundant sticky honeydew was covering the surfaces under the pine crowns (Fig. I). At the same sampling time, the trunk-injected plants appeared to be recovering and new sprouts were present also on the lowest branches (Fig. I). Microscope observations showed that eggs and first instars, as well as both male and female adults of *T. parvicornis*, were present on most of the one-year-old portions of the branches sampled within the control plot. Small colonies were found also on the two-year-old and sporadically on the three-year-old portions (Fig. IV). Huge amounts of honeydew and sooty moulds were present on the twigs sampled within the control plot. In the treated plots 1-3, the scale infestation was moderate and mostly restricted to the one-year-old portion of the twigs (Fig. IV), which were often free of fresh honeydew and moulds. The mean number of live females counted on the one-year-old twigs significantly differed between the treated (1.5 ± 3.53SD in plot 1, 0.17 ± 0.58SD in plot 2, 4.75 ± 9.23SD in plot 3) and untreated pines (38.67 ± 10.86SD) (t test value = 7.64, P<0.001).

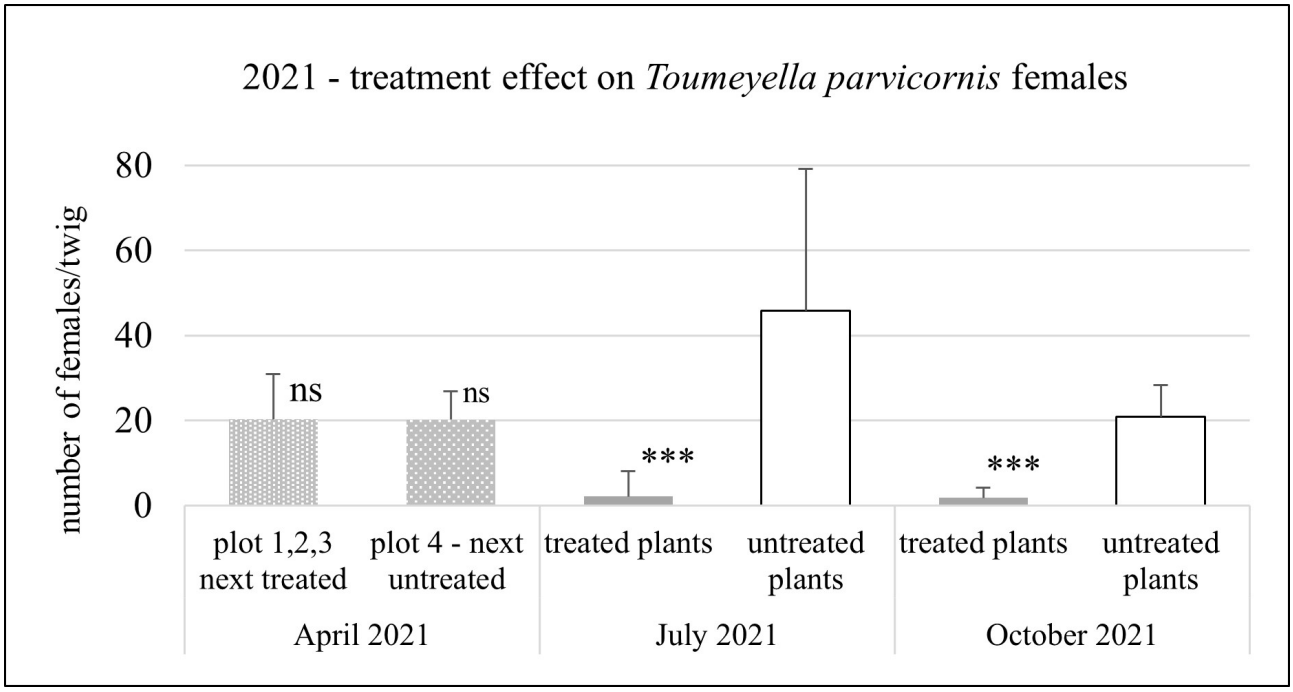


Fig. III – Density of *Toumeyella parvicornis* females (mean±SD) per one-year-old twig by endotherapic treatment and by sampling time (April: pre-treatment; July and October: post-treatment; t-test P<0.001).

The survey carried out in October 2021 at 25 weeks after treatment confirmed that the level of scale infestation greatly differed between treated and untreated pines. The crown of untreated plants showed a further decline, with needle desiccation and abundant phylloptosis, especially on the lowest part of the canopy, whereas the treated plants noticeably showed vigour and vegetative growth even at the lowest branches (Fig. I). First instars, females and males, mostly belonging to the third generation of *T. parvicornis*, were observed on the one-year- and two-year-old portions of all the untreated twigs (Fig. IV) together with abundant fresh honeydew. Sporadically, eggs, first instars and adults were observed also on the twigs of the treated pines sampled within the plots 1 and 3, on the one-year-old portion only (Fig. IV). The mean number of live females was 2.83 ± 2.66SD and 2.66 ±

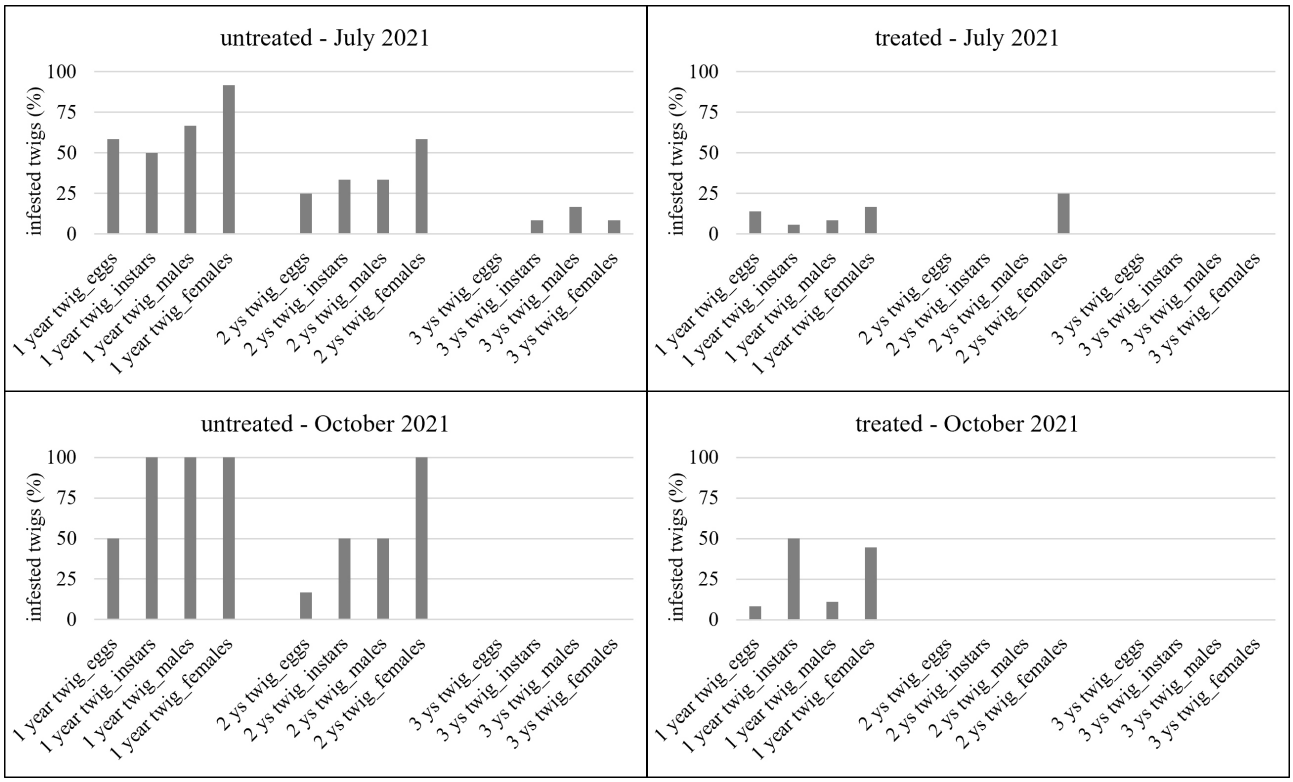


Fig. IV – Percentage of presence of the different life stages of *T. parvicornis* on twigs of different age in treated and untreated plots in July and October 2021.

2.46SD in plot 1 and 3 respectively whereas no specimens were found within the plot 2. These values were significantly lower than the mean number of females counted on the untreated twigs (14.48 ± 0.69SD) (t test value = 13.66, P<0.001).

During the survey carried out in October 2021, the injection holes were visually inspected on all the treated plants within the park. No crack or exudates were observed, and the holes showed a complete scarring in all cases (Fig. V).

DISCUSSION

Since the first outbreak in 2018, *T. parvicornis* rapidly spread in the whole urban area of Rome and heavily attacked the *P. pinea* trees which are widespread in every park, garden, and roadside tree of the city (Griffo and



Fig. V – Absence of crack or exudates around the injection holes that were visually inspected on the trunk-injected pine trees 25 weeks after treatment.

Fig. V – Absence of crack or exudates around the injection holes that were visually inspected on the trunk-injected pine trees 25 weeks after treatment. 2021; Tornini and Gozzi, 2021). Several factors may have contributed to the development of such impressive scale populations. As already observed in Naples, the host species *P. pinea* resulted to be extremely susceptible to the pest, and the large distribution of aged stone pines regularly planted along the main routes of communication allowed *T. parvicornis* to complete its rapid invasion between adjacent pines and by wind (Garonna *et al.,* 2018). Moreover, the warm Mediterranean climate allowed several generations per year, as already occurred in Campania region and in the Southern range of the native area of the pest (Cooper and Cranshaw, 2004; Camacho and Chong, 2015; Garonna *et al.,* 2018).

The ever-frequent pine dieback due to *T. parvicornis* attacks is already resulting in visible changes in the public green of Rome and can radically alter the typical landscape of the city, as already occurred in the Turks and Caicos Islands with the decline of *P. caribaea* pinewoods (Malumphy *et al.,* 2012). For this reason, several reactions are rising from the citizenship and a prompt control strategy against *T. parvicornis* is becoming mandatory. The results of the experimental trial carried out in an urban park of Rome showed that the trunk injection of *P. pinea* trees with abamectin-based insecticide was able to efficiently control high population density of *T. parvicornis* at 11 and 25 weeks after treatment. The treated pines visibly appeared to recover from the severe infestation symptoms, and the microscope observation confirmed that the scale colonies significantly decreased or disappeared on these trees. Indeed, very few specimens of *T. parvicornis* were found on some treated pines in July, and even more sporadically in October when the plants within the plot 2 resulted to be still free of scale infestation. Thus, the populations of *T. parvicornis* were reduced at low density levels or were even absent 25 weeks after a single endotherapic treatment with abamectin. This is

according with preliminary observations made after pilot treatments carried out in Italy inVatican gardens and in Ischia island (Tornini and Gozzi, 2021; Vinciguerra, 2021). Similar encouraging results were also obtained with the trunk injection by Nuovo Metodo Corradi® technique of the stone pines in Central Italy for the control of another pine scale, *C. pini* (Boselli *et al.,* 2018). Further studies are currently ongoing to define both the absorption and persistence levels of the abamectin in *P. pinea* and the efficacy of treatments for periods longer than 25 weeks.

Endotherapy is likely representing the immediate measure to limit the damage of *T. parvicornis* in urban Mediterranean environments. Moreover, the visually inspection of the treated trees showed that the injection technique used in this study did not cause any injury and bark lesions ascribable to the trunk drilling. However, the endotherapic approach is time- and labour-consuming and entails huge efforts to cover large areas such as a metropolis, and long-term control measures are indeed necessary. The lack of efficient natural enemies, already assessed in other areas in Italy (Garonna *et al.,* 2018), prompted the research for implementing possible classical biological control programmes. Studies aiming at selection and introduction of natural enemies from the native area of *T. parvicornis* and from other recently colonized territories have started at CREA-DC as well, in the frame of the national project PROTEGGO 1.3 granted by the Italian Ministry of Agricultural, Food and Forestry Policies.

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