Rossini Luca (Orcid ID: 0000-0003-2558-7111)
Speranza Stefano (Orcid ID: 0000-0003-0106-3938)
Contarini Mario (Orcid ID: 0000-0001-8348-2970)

Toumeyella parvicornis versus endotherapic abamectin: three techniques, one year after

Nicolò Di Sora[1], Luca Rossini[2,1,*], Mario Contarini[1,*], Giovanni Mastrandrea[3]. and Stefano Speranza[1]

[1] Dipartimento di Scienze Agrarie e Forestali, Università degli Studi della Tuscia, Via San Camillo de Lellis snc, 01100 Viterbo, Italy

[2] Service d'Automatique et d'Analyse des Systèmes, Université Libre de Bruxelles, v. F.D. Roosvelt 50, CP 165/55, 1050, Brussels, Belgium

[3] Segretariato generale della Presidenza della Repubblica - Servizio Tenuta di Castelporziano, Via Pontina 690, 00128 Roma, Italy

Corresponding author: luca.rossini@unitus.it (L.R.), contarini@unitus.it (M.C.)

ORCID:

Nicolò Di Sora: https://orcid.org/0000-0003-2125-9137

Luca Rossini: https://orcid.org/0000-0003-2558-7111

Mario Contarini: https://orcid.org/0000-0001-8348-2970

Giovanni Mastrandrea: Record in progress

Stefano Speranza: https://orcid.org/0000-0003-0106-3938

Abstract

BACKGROUND

Toumeyella parvicornis is an invasive soft scale insect native to North America that is rapidly spreading in Italy and France, provoking severe infestations on *Pinus pinea* L. To date, the control of this pest is entrusted to three endotherapic techniques whose short-term efficacy is partially known. No information on long-term efficacy is currently available, although fundamental. This work aims to firstly report the long-term effect that abamectin-based insecticides, injected with the three different techniques, have on adult female populations.

RESULTS

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The study was carried out in an infested *P. pinea* forest in the area of Rome, Italy. Results showed that the tested methods had a similar long-term effect, and only in one case there were differences with the untreated control. Multiresidue analysis reported a zero level of abamectin in plant tissues 14 months apart from injection, except for one treatment where pesticide concentration was just above the limit of quantification.

CONCLUSIONS

This study represents a first long-term evaluation about endotherapic control strategy against *T. parvicornis*. In fact, despite the pest may quickly bring the stone pines to the death and to the fall, representing this a concerning risk for the citizens, control actions to manage it are still partially known and deserve more in-depth investigations.

Keywords: Tortoise scale insect; Stone pine; Biological invasion; Integrated pest management; Alien species.

1. Introduction

Outbreaks of *Toumeyella parvicornis* (Cockerell) (Hemiptera: Coccidae) are increasing all over the European continent,[1] seriously endangering stone pine trees (*Pinus pinea* L.) forests and urban parks. This pest was firstly detected in Campania and Lazio regions of Italy, but in 2021 its presence has been ascertained in other Italian regions[2,3] and in France as well.[1]

Since the first Italian outbreak, the scientific community and representatives of authorities started analysing the methods of controlling the infestations and their consequences on stone pine plants, and the response of the species to the Italian climate conditions.

The species in its place of origin, North America, has one generation per year while the south European climate conditions lead the species to close up to 4 overlapping generations, strongly increasing its damaging potential.[4–6] *T. parvicornis* is a sap sucking pest whose feeding activity causes an initial canopy dieback with a subsequent death of stone pine plants. The noxious effect is ulteriorly extended by the abundant production of honeydew, optimal substrate for a plethora of secondary hosts such as black moulds.[5–8]

The absence of natural enemies in non-native environments, and the highly damaging potential assessed in Europe on stone pines led the scientific community to test control strategies that: *i)* can be applied in urban areas, where restrictive laws often limit treatments, and *ii)* long lasting protection of the stone pine trees. The recent studies of Bertin et al.[9] and Di Sora et al.,[6] stated that the injection of active ingredients was identified as an optimal, considering both efficacy and ease of application in urban areas. Large-scale control actions were conducted in the most infested areas of Lazio region (Central Italy), located mostly in the urban and semi urban areas of Rome, using the three different stone pine plant treatment endotherapic techniques.

While some information about the short-term efficacy of endotherapic treatments has been recently published,[6,9] there is no information about the longer-term coverage of a single treatment. Endotherapy is an invasive technique of application of plant protection products, since it requires long application times, and has a varying cost depending on the company which is in charge of applying (in this case) the acaro-insecticide. The economical threshold of its use may be justified if the plant protection against *T. parvicornis* is ensured for a long time. Accordingly, knowing the long-term effects of the three endotherapic techniques currently applied to control this pest is fundamental to better understand the prospects of future planning of control actions in terms of techniques and costs.

The endotherapic campaign carried out in 2021 involved the Tenuta Presidenziale di Castelporziano, one of the biggest *P. pinea* forests of Lazio, Italy. In 2021 a high level of infestation was ascertained, and three different endotherapic *T. parvicornis* control techniques commonly applied in Italy were tested. Given the severity of the emergence, as well as the need of controlling the further spread of the pest, we aim to report the results of the survey and represent helpful information for further urgent control actions. One year and two months after the application, we analysed the field situation in terms of infestation level and persistence of the active ingredient into plants, in order to compare which was the most effective and durable treatment.

2. Materials and methods

2.1. Study area

The experimentation was carried out in the “Tenuta Presidenziale di Castelporziano" located in Rome, Lazio region, Italy (41°44'03.1"N 12°23'54.0"E) where *P. pinea* covers a surface of 750 hectares. We focused on a group of plants in the north-central part of the park, distributed in rows and separated by the main road. Plants are approximately 80 years old and have a height of 20 m, a diameter of 60-65 cm, and are separated six metres apart. A preliminary inspection was carried out in April 2021, when the presence of the pest in all the plants was confirmed. Typical symptoms were detected through inspections of the canopy using binoculars, and of the leaves on the ground. After the inspection of 2021, groups of stone pine plants were treated with different endotherapic techniques. The second part of the study was carried out in July 2022 according to the protocol detailed below.

2.2. Sampling design and treatment

To evaluate the long-term effect of endotherapic abamectin treatment, we selected in 2021, two stone pine rows along the main avenue of the park. Along the rows, we considered 4 treatments, each composed of 20 stone pines, separated from each other by a buffer zone of 10 untreated plants. The four treatments were divided as follows: untreated plants, hereafter denoted as C, and three abamectin-based treatments using different endotherapic techniques, hereafter denoted as T1, T2, and T3, respectively.

The T1, T2 and T3 endotherapic techniques considered in this study were applied by private companies according to the endotherapic kit indications, to limit as much as possible any bias in the results. The VARGAS® abamectin-based pesticide was the same for all the techniques, being the only product registered for *T. parvicornis*, to date. VARGAS® is an Emulsifiable Concentrate (EC) with a concentration of 18.37 g/l of abamectin (1.8 %) subsequently combined with a co formulant solution.

The first technique (T1) was the Arborjet® manual equipment, specifically the Arborjet "QUIK jet kit" ® with VIPER microinjection interface technology. The application provided for the creation of injection points to directly reach the plant vascular system of the trees. The trunks were perforated at a height of 1.5 m using a needle (8 mm diameter and 20 cm length), at an angle of 30° and spacing the injection points horizontally of 20 cm. The injection of the product was carried out firstly inserting, in each injection point, the Arborplugs® and subsequently injecting the solution through the VIPER needle and with a high-pressure. Each tree treated with this technique received a total solution of 50 ml VARGAS® + 50 ml carrier formulation through the plugs.

The second technique (T2) was the Nuovo Metodo Corradi® technique. This technique consists of a low-pressure insecticide equipment that actively inject, in each tree, a total solution of 55 ml VARGAS® + 55 ml carried formulation. Injections were carried out at a height of 1.5 m using a syringe in previously drilled holes (4 mm diameter and 5 cm length), directly reaching the lymphatic vessels of the plant. Perforations were horizontally spaced 40 cm.

The third technique (T3) was the Ynject Go® (Fertinyect, Spain) low pressure-high volume natural uptake technique. This technique consists of self-pressurised bags containing abamectin that are inserted through plugs in plant trunks. Plants were previously drilled (6.5 mm diameter and 14 cm length) at a height of 1.5 m, at an angle of 30° and respecting a horizontal distance of 30 cm between each perforation. Each stone pine received a total solution of 25 ml VARGAS® + 225 ml carrier formulation.

Because of industrial copyright, no information about carrier formulations of T1, T2, and T3 treatments was accessible to us. Plants received a single treatment in the second half of April of 2021. After the treatment, the injection points of T1 did not receive any disinfection, while T2 and T3 have been disinfected with quaternary ammonium salts and ethyl alcohol, respectively. Injection points of T1 were sealed leaving the Arborplugs® in the holes, while T2 were sealed with biodegradable plugs. T3 was not sealed.

2.3. Samples collection and laboratory analysis

The trial was conducted following a double-blind analysis, avoiding the “experimenter effect”. The treatment associated with the code (C, T1, T2, T3) was revealed at the end of the data analysis.

Plants belonging to each treatment (C, T1, T2 and T3 respectively) were selected and labelled by external technicians the day of the injection. Given the purpose of this study, plants were inspected one year and two months after, to assess the permanence and the entity of the infestation at larger time ranges.

On 7 July 2022 a sampling collection was carried out applying the same methodology published by Di Sora et al.[6] as follows: five plants belonging to each treatment were inspected as follows, reaching the plant canopy through a basket crane. Six 20-cm long twigs were collected, sealed in single plastic bags and brought to the laboratory. On the same day, the number of adult females contained in each twig was counted after an appropriate identification.

Two more twigs per plant were collected to carry out a multiresidue analysis in all the C, T1, T2, and T3 treatments, to assess the average level of abamectin still present in plant tissues after 14 months. Following the protocols available in Madadlou et al.[10] and Mulligan et al.,[11] the level of insecticide was assessed, on the twenty additional twigs, by chromatography.

2.4. Statistical analysis

Experimental data were analysed through R software as follows: GLM with mixed-effect (GLMM) with a negative binomial distribution and the Bonferroni adjusted as a post hoc test ($\alpha = 0.05$), considering treatment as an independent variable, while plants as a random variable (block). Calculations were carried out through the *glmer.nb()* functions within the R package lme4, the *emmeans()* function within the R package emmeans, the *pairs()* function within the R package multcompView, and the *cld()* function within the R package multcomp. The R code and the dataset to fully reproduce the results of this study are publicly available at https://github.com/nicodisora/toumpa2022.

3. Results

3.1. Population abundance and abamectin residue analysis

Results are plotted in Fig. 1. No significant differences were observed among the adult female population abundances on stone pine plants treated with the three endotherapic techniques (T1 – T2 GLMM, $Z = 2.145$, $P = 0.31$, NDF = 149; T1 – T3 GLMM, $Z = 1.157$, $P = 1$, NDF = 149; T2 – T3 GLMM, $Z = -1.088$, $P = 1$, NDF = 149). In addition, adult female populations sampled on T1 and T3 plants were not statistically different from untreated plants (C – T1 GLMM, $Z = 1.554$, $P = 1$, NDF = 149; C – T3 GLMM, $Z = 2.610$, $P = 0.09$, NDF = 149). The only difference was assessed between adult female populations sampled on T2 and untreated plants (C – T2 GLMM, $Z = 3.697$, $0.01 < P \leq 0.001$, NDF = 149).

Multiresidue analysis indicated a zero level of abamectin on the treatments T1 and T3, while a concentration of 0.007 ± 0.004 mg kg^{-1} was assessed on T2 treatment (Table 1).

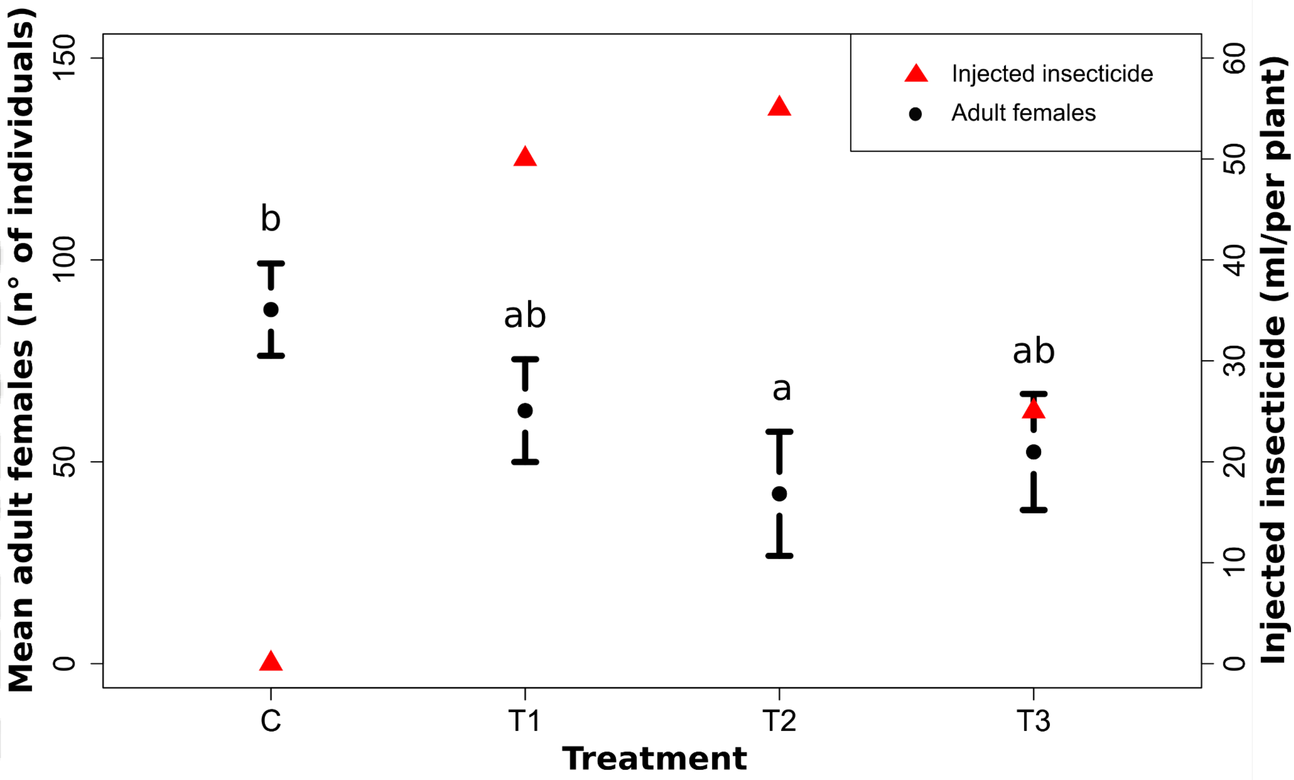

Figure 1. *Toumeyella parvicornis* adult females counted on stone pine twigs. Plants belong to four tested treatments: untreated control (C), and abamectin treatments through Arborjet® (T1), Nuovo Metodo Corradi® (T2), and Fertinyect® (T3) endotherapic techniques. Red triangles indicate the quantity of the injected product per tree. Different letters indicate significant differences assessed by a GLMM followed by the Bonferroni *post hoc* test ($\alpha = 0.05$).

Table 1. Results of the multiresidue analysis carried out on the 4 treatments. Abamectin concentration is calculated as the sum of Avermectin B1a, Avermectin B1b and delta-8,9 isomer of Avermectin B1a, expressed as Avermectin B1a. Abamectin concentration values are associated with the respective extended uncertainty (confidence interval of 95 %, coverage factor k=2). Limit of Quantification = 0.005; Limit of Detection = 0.003.

Treatment	Injection technique	Abamectin concentration (mg kg^{-1})

C	Untreated control	< 0.005
T1	Arborjet®	< 0.005
T2	Nuovo Metodo Corradi®	0.007 ± 0.004
T3	Fertinyect®	< 0.005

4. Discussion and Conclusion

The results of this ongoing study present for the first time the long-term effect that endotherapic abamectin injected through three different techniques have on *Toumeyella parvicornis* populations. We believe that this information is fundamental in contrasting the rapid spread of this pest through the Italian peninsula,[2] and the European continent.[1] The recent first detection in France[1] and the abrupt changes that infested and dying stone pines are provoking to Italian landscapes are increasing even more the need of scientific knowledge about methods to control *T. parvicornis*. Although endotherapy represents, to date, the only method of contrasting the infestation of this pest, there is a need to widen the knowledge that can delineate both its potential of use and its limits.

is study takes a step in this direction, showing that the only three techniques of abamectin injection (Arborjet®, Nuovo Metodo Corradi®, and Fertinyect®) currently applied in Italy to control *T. parvicornis* infestations did not show long-term differences between each other. On the other hand, plants treated with the Nuovo Metodo Corradi® were significantly different, in terms of adult female populations, from untreated plants. Interestingly, the abamectine level, although in extremely low concentration, was detected in plants treated with Metodo Corradi® technique, 14 months after the injection as proven by the multiresidue analysis. This low level of active ingredient may be related with the composition of the insecticide carrier formulation.[12] Carrier formulations are usually an exclusive of the producers, for this reason they vary depending on the employed techniques and likely affect the translocation of abamectin throughout stone pines. Similar observations have been already reported by Ferreira et al.[13] on coconut palms, and we may suppose a similar effect on stone pine plants as well, until proven otherwise. The low presence of abamectin observed in plants treated with Nuovo Metodo Corradi® may be also related to the greater initial concentration of active ingredient (55 ml), over the total amount of product injected at the beginning of the experiment, than Arborjet® (50 ml) and Fertinyect® (25 ml) techniques. Certainly, the concentration of abamectin may conditionate the pest control efficacy, as stated by James et al.[14] and by Fettig et al.[15] even concerning different pests and hosts. Fourteen months after the treatment, besides the technique applied, the pest population showed a damaging level similar to the untreated control plants that did not receive the abamectin injection at the beginning of the experiment. The adult female populations observed on stone pine plants implies that the insecticidal action exerted by abamectin has a shorter duration as well.

Bertin et al.[9] assessed the efficacy of the Nuovo Metodo Corradi® technique in controlling *T. parvicornis* infestations. The authors observed an effectiveness of 25 weeks, even though no information about the persistence of insecticide into plant tissues, and after how many weeks it becomes effective, were reported. This leaves us to suppose that endotherapy may be valid as short-term treatment, but further studies should better investigate the absence of long-term effects we showed in this study.

Our results confirm, on a longer time scale, what Di Sora et al.[6] observed on stone pines treated with Fertinyect® technique. In a shorter time range, the authors reported a limited persistence of abamectin into plant tissues, with a subsequent effect on *T. parvicornis* populations for approximately 60 days.

Although with limited time validity, endotherapic abamectin is, to date, the most effective treatment to control *T. parvicornis*. Given the entity of the emergence it may be convenient above all in urban contexts. Endotherapy indeed has been employed as a control strategy for several pests, often introduced, since it couples high efficacy in reducing pest populations with low adverse effects on the environment, non-target species, and humans.[16,17] Since no differences were observed between the different control techniques of *T. parvicornis*, it is possible to choose the most suitable one in terms of maintenance cost, that in stone pines is already high,[18] easiness of application, work effort, and effects that the injection has on stone pine plants. This compromise may be considered if an average of one treatment per year is required, according to the information available to date.

The actual Italian national regulation allows only one treatment per year, usually carried out in spring time. In this framework, additional studies should be directed also on the timing of injection, in relation with different factors such as the generations that usually occur, the stone pine's physiology or the environmental conditions.

Overall, we believe that the information provided with this study will help in finding an effective direction in both further studies and spread reduction of *T. parvicornis*.

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Author contribution

NDS, LR, MC, GM, and SS conceived research. NDS, LR and MC wrote the manuscript. NDS and GM conducted the experiments. NDS and LR conducted the statistical analyses. SS secured fundings. All the authors read, contributed to and approved the manuscript.

Competing interests

The authors declare no competing or financial interests

Data availability statement

The data associated with this publication, as well as the R script to reproduce the results, are publicly available at https://github.com/nicodisora/toumpa2022.

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