The spread of the non-native pine tortoise scale *Toumeyella parvicornis* (Hemiptera: Coccidae) in Europe: a major threat to *Pinus pinea* in Southern Italy

Antonio Pietro Garonna (1), Alessandro Foscari (1), Elia Russo (2), Giovanni Jesu (1), Silvano Somma (1), Pasquale Cascone (2), Emilio Guerrieri (1-2-3)

Invasive pests are considered a major threat to biodiversity, conservation and agriculture. The Italian peninsula is a major site of intensive commercial exchange and transport of plants and goods, being consequently one of the European countries most invaded by alien insects. Hemiptera Coccomorpha are the largest group of non-native species recorded in Europe. For example, in the last 70 years more than 50 scale insect species have been accidentally introduced into Italy, 50% of which are now well established. This study was conducted to investigate the biology and the damage of the non-native pine tortoise scale *Toumeyella parvicornis* Cockerell (Hemiptera: Coccidae) accidentally introduced a few years ago into southern Italy. *T. parvicornis* is multivoltine in the invaded territories, being able to complete at least three generations per year, overwintering in the adult female stage. Oviposition periods during 2015-2017 surveys occurred from late April to end of May, from July to first half of August, and from mid-September to November. Fecundity was positively correlated to body size of gravid females and varied among the genera. Investigations on natural control by autochthonous species showed a seasonal activity of *Metaphycus flavus* (Hymenoptera: Encyrtidae), parasitizing mainly immature male individuals. The morpho-molecular approach confirms the hypothesis of an ongoing shift of parasitoid populations from other indigenous soft scales to the invasive one. Unfortunately, the low level of natural control was ineffective in hampering the spread of *T. parvicornis*, and preventing the dieback of local pine species, *Pinus pinea*, as observed in all invaded areas.

**Keywords:** Invasive Pest, Europe, *Toumeyella parvicornis*, Life History, *Pinus pinea*, Natural Control

The Italian peninsula, in the centre of the Mediterranean basin, is a major site of commercial exchange and transport of plants and goods. Therefore, Italy is one of the European countries most invaded by alien insect species (Roques 2010, Roques et al. 2016). The accidental introduction of exotic insect species has become a common event given the extreme difficulty in controlling incoming plant material at the borders. A constant linear trend of accidental introduction was found in the Italian peninsula since 1945 (Pellizzari et al. 2005, Inghilesi et al. 2013) due to the wide range of climatic conditions allowing the acclimation of tropical and subtropical species in most of its regions. This scenario is further exacerbated by global warming directly enhancing the survival of several potential pests and indirectly affecting the trophic relationships between the phytophagous insects (Battisti & Larsson 2015), their host plants and their natural antagonists (Ka-linkat & Rall 2015). Finally, the altered conditions of urban zones, characterized by severe air pollution, warming, and disruption of biological control, favour the rapid adaptation of invasive species to new areas (Meinke et al. 2013, Dale & Frank 2014). Invasive pests are considered a major threat to biodiversity, conservation and agriculture (Early et al. 2016) and, among these scale insects (Hemiptera: Coccomorpha) form the largest group of alien insects arrived in Europe in the last 70 years, with more than 50 species recorded for Italy, of which 50% are now well established (Mazzeo et al. 2014).

The genus *Toumeyella* Cockerell (Hemiptera: Coccidae: Myzolecaninae) currently includes 18 species mostly distributed in the Nearctic and Neotropical Regions (Kon do & Pellizzari 2011, García Morales et al. 2016). Among these, 4 species are associated with conifers belonging to the genus *Pinus* in the Nearctic region: *T. parvicornis* Cockerell, the pine tortoise scale (PTS); *T. pini* King, the striped pine scale; *T. pinicola* Ferris, the irregular pine scale; and *T. virginiana* Williams and Kosztarab, the Virginia

© SISEF http://iforest.sisef.org/
**Parasitoid characterization**

A combined approach was used for the correct identification of the parasitoid collected and to understand the origin of its populations.

**Molecular analysis**

Before card mounting, whole specimens were subjected to total DNA extraction following a non-destructive protocol using a Chelex 100 Resin (Biorad, Hercules, CA, USA) according to Cascone et al. (2015). Three individuals were placed into homogenisation solution containing 5 ml of Proteinase K (20 mg/ml) and 80 ml of 5 % Chelex, incubated at 55 °C for 60 min, then for 10 min at 99 °C and centrifuged for 5 min at 13,000 rpm. We sequenced mitochondrial Cytochrome Oxidase 1 (COI) and the internal transcribed spacer (ITS2) region obtained by PCR performed in 15 μl volumes of distilled water containing: 1X GoTaq™ buffer (Promega, Madison, WI, USA), 2.5 mM of each dNTP, 10 μM of both forward and reverse primer, 1 unit of GoTaq™ DNA Polymerase and 1 μl of template DNA. COI amplifications were achieved using a Biorad thermocycler Mycycler™ (Bio-rad) programmed for: 1 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 90 s at 48 °C, and 60 s at 72 °C, and a final step of 7 min at 72 °C (Guerrerio et al. 2016). Gene was amplified using the following primer combinations: LCO/HCO (Folmer et al. 1994), MlepF1/HymR (Hebert et al. 2004, Hajibabaei et al. 2006) and HCoextb/C1J2195 (Simon et al. 1994, Schulmeister 2005). PCR fragments were sequenced directly in both direction at Macrogen (Korea) using Big-Dye Terminator ver. 3.1. The obtained chromatograms were edited and assembled in Chromas ver. 2.6.4 (Technelysium, South Brisbane, Queensland, Australia). The sequences were deposited in GenBank under accession numbers MG946790-MG946792. Similarly, to amplify and to sequence the ITS2 region, thermocycler was programmed as following: 3 min at 94 °C, followed by 33 cycles of 45 s at 94 °C, 45 s at 53 °C and 45 s at 72 °C, and a final step of 5 min at 72 °C. ITS2Fw and ITS2revb primer were used (Stouthamer et al. 1999). The sequences were deposited in GenBank under accession numbers MG946793-MG946795.

**Morphological analysis**

Card and slide mounted material of the parasitoid were prepared following Noyes (1982). The specimens were compared with a long series of individuals authoritatively identified and deposited at the Natural History Museum, London (UK).

**Statistical analysis**

The relationship between the fecundity and the combined body size of the females and egg load was determined through
Pearson’s correlation and linear regression. Analysis of variance (ANOVAs) was used to determine differences in both adult female size and the chorionated egg load in four subsequent generations during 2016-2017. Differences in treatment means were tested using post-hoc multiple comparisons Tukey HSD test at α=0.05. All data satisfying conditions of normality and homoscedasticity, both untransformed or after appropriate transformation, were analyzed by the software Statgraphics Plus® (Statgraphics Technologies Inc., The Plains, VA, USA).

Results

Life history of PTS

PTS distribution and life history in 2015-2016 are summarized in Fig. 1 and Fig. 2. Unless otherwise specified, life history refers to scale population sampled in the urban zone.

In Campania PTS completed at least three generations, partially overlapping, on Italian stone pine P. pinea (Fig. 2) with little differences observed among sampled sites. An early emergence (5-7 days) was recorded in 2015-2016 on urban pines in Naples in respect to pine forests on Mt. Vesuvius. The minimum time gap between two subsequent ovipositions (May-July and July-September) was on average 65 ± 3.46 days (n=200). Adult females of the third generation were recorded from the second half of November to the end of December, 73 ± 5.61 days (n=200) after the first egg hatch. The species overwinters as fecundated females mainly on twigs (bark form: 92.15%, n=26,370) and rarely on pine leaves (leaf form: 7.85%, n=207). In the following generations, the bark form represented 100% of adult females, mainly due to the high mortality of immature females settled on pine leaves during summer and autumn. Conversely, male instars were scattered on both pine leaves and twig axes, where they usually formed clusters around female stages.

The species is clearly ovoviviparous: females laid 20-25 eggs daily and hatching of the crawlers occurred in a few hours. Oviposition period lasted 35-40 days. Eggs of the first generation of the year were recorded from the second half of April to end of May. The following oviposition periods occurred from July to early August and from mid-September to November. First crawlers were recorded on May 5th in 2015 and April 24th in 2016. Mild winter and early spring temperatures during 2015-2016 led to an earlier emergence of crawlers (~12 days) in 2016 in respect to 2015.

After eclosion, the crawlers dispersed on pine shoots and settled along twig terminals or on leaves nearby. The peak density of first instars varied from year to year. In 2015 three clear peaks were recorded in mid-May, mid-July and from end of September to mid-October (Fig. 2). Conversely, during 2016 first instar presence was recorded over a longer period due to longer overlapping of subsequent generations. Sexual dimorphism became evident during the third week after the settlement of the crawlers, when immature females and males can be readily separated by their shape, oval and convex in the former, much more elongate in the latter. Adult male flight peaks were clearly distinguishable in 2015, but not in 2016 when a larger overlapping of subsequent generations occurred. Males of the first generation appeared in June 15th in 2015 and a week earlier in 2016, with flight curves peaking in the first week of July and in the last decade of June, respectively. Adult male flight peaks of the following summer and autumn generations were recorded in the second half of August and beginning of November during 2015 and in mid-August and end of October in 2016. Huge amounts of honeydew were produced during feeding activity of late instars.

After mating, the female’s body greatly increased in size with the last molt, ovary development and reproduction. The females began to produce eggs in their ovaries 5-7 days after reaching the adult stage. Adult female densities on P. pinea reached 10 females cm\(^{-1}\) of twig in the overwintering population (average density: 5.51 ± 2.17 females cm\(^{-1}\), n=100 twigs). We deter-
mined that the fecundity of PTS (Fig. 3) correlated significantly to female body size (p<0.05). Pearson’s correlation coefficient varied from 0.59 (overwintering generation 2016-2017) to 0.78 (spring generation 2017). Linear regression analysis corroborated the results of Pearson’s correlation analysis.

The number of eggs laid per female varied among the generations and ranged between 198.82 ± 11.61 for the summer generation 2016 and 730.36 ± 19.51 for the overwintering generation 2016-2017. The highest recorded fecundity was 1014 eggs female\(^{-1}\) and the lowest 90 eggs female\(^{-1}\). The average number of eggs laid by female of the sampled generations differed significantly (p<0.01).

**Host plants**

In the invaded area, *P. pinea* resulted the most distributed species, followed by *P. pinaster*, *P. halepensis* and *P. nigra*. Few other pine species used as ornamental trees have been recorded. The most susceptible host plant resulted *P. pinea* followed by *P. pinaster* and *P. nigra*. No signs of PTS infestation were found on a dozen *P. halepensis* and a few *P. roxburghii* trees. To date, strong decline and dieback in field was recorded only for the *P. pinea*, due to the impressive populations of PTS hosted on a single tree.

These results were confirmed by laboratory tests on *P. pinea* where all saplings died after 8 months and three generation of infestation by PTS. Four pine species reared at the same conditions, namely *P. pinaster*, *P. nigra*, *P. nigra* var. *laricio* and *P. sylvestris*, survived and showed lower levels of infestations. Finally, PTS was unable to infest Aleppo pine saplings in laboratory.

**Natural control**

*Metaphycus flavus* Howard, a polyphagous parasitoid known to attack several scale insects species, emerged from PTS samples collected in the Vesuvio National Park. The first parasitized scale instar was recorded in September 2015. Fig. 4 summarized the parasitization trend during 2015-2017. Parasitization rate peaked from November to January in 2015-2016 (=18%) and 2016-2017 (=38%). Parasitized samples revealed that male instars were preferred over female ones (range: 60-90% – Fig. 5). Parasitization rates showed a seasonal activity of *M. flavus*, concentrated during autumn and winter months. Living larval instars of the parasitoid were found in dissected scales until February in 2016 and March in 2017. The samples collected from March to September 2016 and during late spring 2017 showed only a scattered presence of emergence holes.

Sex-ratio of the encyrtid parasitoid recorded from October to December 2016 was strongly male-biased (1.0 M : 0.53 F - n=270 M : 141 F). Longevity of emerged *M. flavus* specimens fed in laboratory with di-
Several aspects of PTS life history in Campania confirmed previous investigations carried out in USA and Turks and Caicos Islands (Malumphy et al. 2012, Clarke 2013). Similarly, PTS behaviour here recorded is consistent with what was reported for the Southern range of the native area on Nearctic pines (Cooper & Cranshaw 2004, Clarke 2013, Camacho & Chong 2015). The noteworthy environmental plasticity of the species is exemplified by the variable number of generations completed per year coupled with the acceptance of new host species (Malumphy et al. 2012). In Campania, three main periods of oviposition were recorded on P. pinea. Our survey (2015-2017) evidenced the ability of the invasive scale to spread across new territories. From the first official record (Garonna et al. 2015), in a few months PTS colonized large areas and undermined a possible eradication program. To date over 2000 km² are interested by heavy infestations favored by the large distribution of the highly susceptible P. pinea. The widespread presence of aged stone pines regularly planted along highways and other main routes of communication, allowed PTS to complete this rapid invasion. Active and passive dispersal behavior proved to be extremely successful. Crawlers spread from infested trees to adjacent pines where branches intertwine, but can also be carried by wind for longer distances, as already demonstrated in the Caribbean and North America (Rabkin & Le Jeune 1954, Malumphy et al. 2016). Indeed, airborne dispersal of T. parvicornis crawlers has been reported to occur at a distance up to 4.8 km (Rabkin & Le Jeune 1954), rendering virtually impossible the protection of uninfested trees.

Our results confirm that the body size of gravid females of PTS is a valid parameter to predict their fecundity. The significant correlation between body size and fecundity has been reported for PTS by Rabkin & Le Jeune (1954) in Canada and for soft scales of the genus Parthenolecanium spp. by Camacho et al. (2017). The fertility values of PTS in Italy are in line with those recorded in North America (Rabkin & Le Jeune 1954, Clarke 2013). Whilst egg load of univoltine populations of PTS reaches 500 eggs per female (Rabkin & Le Jeune 1954), in Southern Italy (Campania) it varied among generations. The highest values were recorded in the overwintering generation, resulting up to 2-3 times higher than those of spring or summer ones. Apart from body size, fecundity could also depend on different factors including environmental conditions, population density and host plant (Camacho et al. 2017). Earlier than expected, it took only a couple of years after PTS introduction in Campania to find the first presence of the autochthonous parasitoid M. flavus attacking its colonies. This species is a common parasitoid of preimaginal stages of soft scales including C. hesperidum, the citrus scale C. pseudomagnoliaria and the black scale Saissetia oleae and is considered cosmopolitan (Guerrieri & Noyes 2000, Noyes 2003). The environmental conditions and the habitat composition in the Vesuvian area, with citrus and olive trees together with spontaneous shrubs, resulted particularly favorable to M. flavus. Indeed, this species has been collected regularly in this area since 1970s (Vigiani & Guerrieri 1988, Guerrieri & Noyes 2000). M. flavus is included in the list of the parasitoid complex of PTS in North America, along with a few species of Coccophagus (Aphelinidae) and Microtarsus flavus (Toryridae) (Cooper & Cranshaw 2004, Malumphy et al. 2012, Clarke 2013). Unfortunately, few data are available about the level of natural control realized by these parasitoids in the native area of the pest (Orr 1931, Clarke 2013), as recently confirmed by Myartseva et al. (2016) in relation to New World parasitoids of Toumeyaella. The parasitization rate reported by M. flavus in Campania reached high values only during the second year of observations, indicating a possible positive trend. The period of parasitoid activity was strictly limited to September-February in both years of observations, in accordance with the presence of susceptible host stages. However, the level of parasitization resulted unable to control the scale due to a clear "sexual" preference (Kapranas & Zena 2013). Indeed, the sex ratios of each winter stage were by far preferred over immature and adult females (Fig. 5). Furthermore, the simplification of the host population during winter and the presence of adult females only from the end of December to the beginning of April, did not allow the parasitoid to maintain a continuous relationship with its new host. As a consequence, M. flavus disappeared at the end of each winter season from the sampled areas, both in 2016 and 2017. A new colonization process started again during summer of 2016 with parasitoids coming from other plant-scale systems. Indeed, only 5 months later new signs of parasitization were found on the new generation of PTS. The identification of the parasitoids collected posed the question whether they were introduced with the invasive host or they belonged to local populations that adapted to it. The molecular characterization of M. flavus populations collected from different hosts in the Vesuvian area, confirmed the second hypothesis. The arrival of the invasive pest represented a large reproductive opportunity for the Vesuvian population of M. flavus but considering the biological features of both species, it is likely that a continuous passage from local scale species to PTS will happen at the beginning of the season every year. The strong male-biased sex ratio of M. flavus collected on PTS possibly indicated an incomplete adaptation to the new host and this aspect is worthy to be investigated in depth. Unfortunately, in July 2017 a devastating crown fire destroyed the monitored forested areas making it impossible to complete these obser-
Malumphy C, Hamilton MA, Sanchez MD, Green PWC (2016). Trapping confirms aerial recruitment of pine tortoise scale (Toumeyella parvicornis Cockerell) (Hemiptera, Coccidae) in the Turks and Caicos Islands. Entomologist Monthly
Toumeyella parvicornis threatens European pines


