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Prospects for Biological Control of *Marchalina hellenica* in Australia Using a Silver Fly †

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Abstract: The giant pine scale (GPS), *Marchalina hellenica* (Hemiptera, Margarodidae), is native in Greece and Turkey, where it is not considered a pest of *Pinus* spp.. However, in 2014 GPS was accidentally introduced in Australia and soon its population increased dramatically causing significant damage to *Pinus radiata* plantations. The silver fly, *Neoleucopis kartliana* (Diptera, Chamaemyiidae) was found as the most abundant predator of GPS. To assess the potential use of this species in a classical biological control program, GPS-infested branches were collected from five different areas of northern Greece and examined in the laboratory to study its biology and attempt to rear it.

Keywords: Classical biological control; Chamaemyiidae; Margarodidae

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1. Introduction

The giant pine scale (GPS), *Marchalina hellenica* (Hemiptera, Margarodidae), is a univoltine sap-sucking insect native in the eastern Mediterranean region, particularly in Greece and Turkey. The scale feeds on *Pinus* spp., especially *P. brutia* and *P. halepensis*, but it can also infest *Abies cephalonica* [1]. In its native range, it is considered an economically significant insect for apiculture rather than a major pest of *Pinus* spp., since it rarely causes tree mortality. It excretes a sweet, glutinous substance called honeydew, which is collected and converted by bees into pine honey, that represents 60-65% of annual honey production in Greece [1]. Due to its importance to apiculture, the GPS has been deliberately introduced in new areas of Greece and the Italian island of Ischia [3]. On several occasions it has become a pest reaching high population densities that are associated with the decline of tree health and insect biodiversity reduction of pinewoods [4]. In 2014 GPS was accidentally introduced in Australia (Melbourne and Adelaide), spotted on a novel host, the North American species *Pinus radiata*, which represents 74.5% of the nation's softwood plantation estate [5]. Soon, its population increased dramatically causing significant damage to *P. radiata* in urban and peri-urban settings, threatening the pine forest industry of Australia [6]. A recent research on the scale's natural enemy complex suggests the the silver fly, *Neoleucopis kartliana* (Diptera, Chamaemyiidae) is the most abundant predator among the natural enemies of GPS in its native range, supporting the potential of *N. kartliana* in a classical biological control program [6]. This beneficial species has already been used successfully as a biological control agent against GPS in the Italian island of Ischia [7]. Therefore, the aim of this study is to investigate the biology of *N. kartliana* in Greece in order to evaluate its suitability as a biological control agent against *M. hellenica* in Australia.

2. Materials and Methods

To study the biology of the predator *N. kartliana*, we regularly collected GPS-infested pine tree twigs and branches (every 7-10 days) from six sampling sites of northern Greece, namely Thessaloniki, Stratoni-Stratoniki, Arnea, Parthenonas, Katerini-Makriyalos and Pyrghetos. The samples were then transferred to the Laboratory of Forest Entomology (Forest Research Institute, Hellenic Agricultural Organization Demeter) at Vassilika (Thessaloniki, Greece) and examined under a stereoscope. The abundance of the fly, expressed as count per GPS individual, and its developmental stage were evaluated according to the descriptions of Gaimari et. al. [8]. The life stage of GPS was also estimated according to of Hodgson and Gounari [9]. Any species associated with GPS encountered was collected to further contribute to the estimation of natural enemy complex of GPS.

Subsequently, *N. kartliana* larvae and pupae as well as intact GPS-infested branches were collected. The material was transferred in well ventilated cages (3.5 cm and diameter2 cm, and 60 x 60 x 60 cm respectively) and placed inside a climate chamber set at 23 °C, 60% relative humidity and 18:6 (L:D) with a one-hour long transition from 0% to 100% light and vice versa. The cages were inspected every 1-2 days in search of any *N. kartliana* adults. Several adults that emerged from the cages were transferred in cages (30 x 30 x 30 cm), provided only with artificial food sources (water, honey and honey with dry yeast diluted in water, milk, sugar with dry yeast and sugar with fresh yeast mixtures in different rates and liquidity), to determine the best substitute of the natural food source (honeydew) of adults.

After emergence, adults were individually collected in small falcon tubes (6 cm, diameter 1.5 cm) and their sex was identified using a stereoscope, according to the descriptions provided by Gaimari et. al. [8]. They were then transferred in large cages (60 x 60 x 180 cm) installed in environmental conditions, protected by direct sunlight and adverse weather conditions. These cages were provided with young, alive *Pinus brutia* trees, infested by GPS, as a natural food source. The GPS infestation was amplified by introduction of various developmental stages of *M. hellenica*, collected from the small cages containing the branch samples, installed in the climate chamber. GPS ovisacs were inspected under a stereoscope in search of any stage of the predatory fly before their introduction to the large cages, while GPS adults were solely collected. First instar nymph collection was conducted by using an aspirator. Second and third instar nymphs were collected when the branches desiccated, and the nymphs voluntarily removed their mouthparts from the branches and roamed in search of a new feeding spot. Apart from its natural food source, *N. kartliana* was provided with artificial food sources consisting of water, and dry yeast and sugar mixture (10-30%) placed on cotton as droplets on a petri dish (diameter 8.5 cm), to resemble its natural food source (cotton-like wax and honeydew excreted by GPS), which was renewed every 2-3 days. The artificial food sources selected were the ones *N. kartliana* showed immediate response to, surviving for two weeks in captivity.

For the estimation of prey specificity of *N. kartliana* to GPS, *Pittosporum tobira* branches, infested by *Icerya purchasi* (Homoptera, Margarodidae) were collected from two sites of Greece (Thessaloniki and Athens), adjoining GPS-infested pine trees. *Icerya purchasi* is a scale insect producing wooly secretions and honeydew, similarly to GPS. The branches were examined under a stereoscope in pursuit of any *N. kartliana* individuals, prior to their transportation in small cages (30 x 30 x 30 cm) and installation in the climate chamber (23 °C, 60% relative humidity, 18L:6D) to observe any *N. kartliana* adults emerging.

Regarding natural enemies of *N. kartliana*, pupae are known to be parasitized by an unidentified parasitoid of the genus *Chartocerus* (Hymenoptera, Signiphoridae) [6,8]. To assess the complex of natural enemies of *N. kartliana* and estimate their abundance, pupae were collected during branch examination, placed in the climate chamber and were frequently checked for any presence of the parasitoid. After a period of 3 months, nymphs were removed and inspected under a stereoscope for the presence of parasitoid-exit holes. The parasitoids encountered were collected and stored for identification, while *N. kartliana* nymphs that carried the parasitoid's exit holes, were counted and compared to the total number of nymphs to estimate the parasitism rate of *N. kartliana*.

3. Results

Neoleucopis kartliana was present in all sites studied, in various abundances, reaching an 50% abundance in GPS ovisacs (Table 1), with a highest record of 6 larvae per GPS ovisac.

Table 1. Neoleucopis kartliana abundance.

Neoleucopis kartliana **abundance**			
Abundance %	**Highest**	**Lowest**	Mean
	50	0.01	9.92

Neoleucopis kartliana was observed in every developmental stage during branch examination (eggs, larvae, pupae) and adult collection from the cages. The eggs are about 0.5 mm long, greyish-white colored, and carry a strip pattern. Pupae are 2-2.5 mm long, reddish-brown colored and carry two distinct prominences. Larvae are white with a size ranging from 0.5 to 2 mm, depending on their level of development. The eggs were located inside or close to the cotton-like wax produced by GPS. Larvae were spotted either inside the ovisacs of GPS or close to other developmental stages (1st,2nd, 3rd instar nymphs and adults). Nymphs were found either inside the wax of GPS or in bark crevices with the presence of GPS, seemingly unnecessary.

One year data suggest that, unlike its prey (univoltine), *N. kartliana* has at least three generations per year, although there is a possibility of a fourth one, or overlapping generations existing (Figure 1).

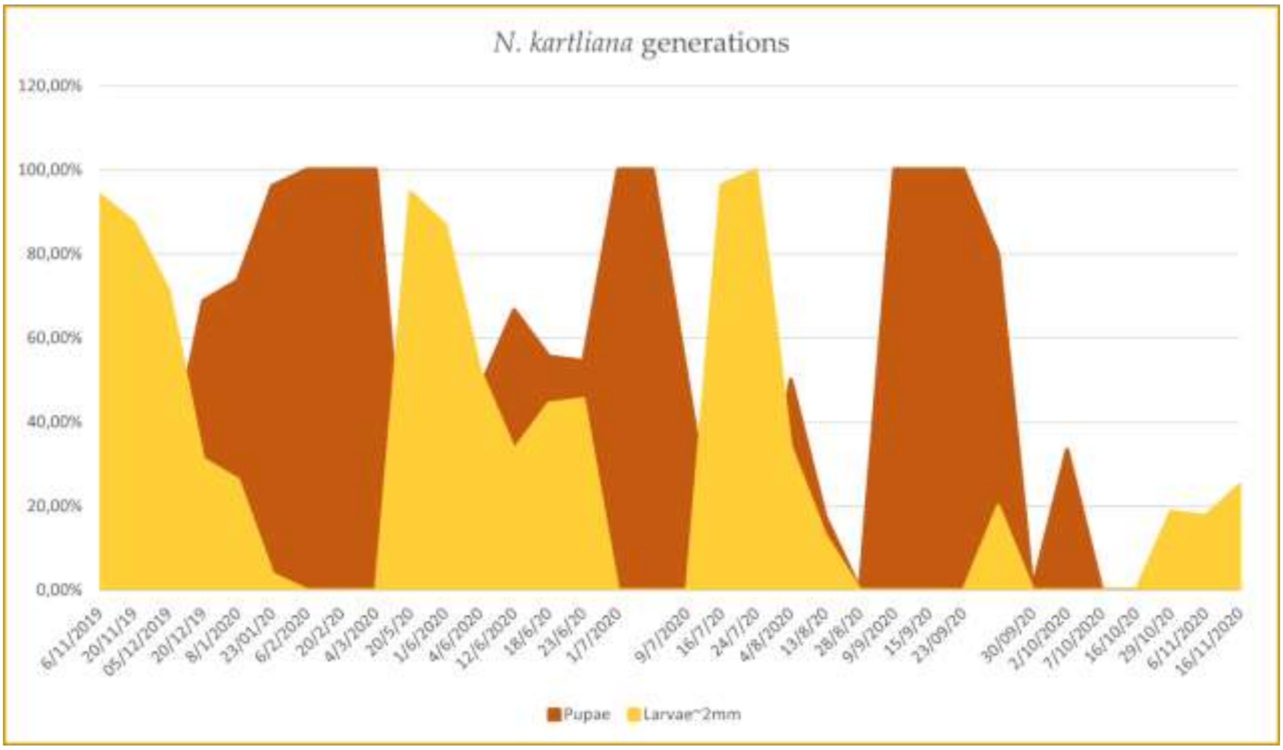

Figure 1. *Neoleucopis kartliana* generations.

A total of 5738 *N. kartliana* adults were used to estimate its sex ratio, which is apparently 1:1 (51.22:48.78). The chamber conditions chosen (23 °C, 60% relative, 18L:6D) accelerated the development *N. kartliana*, since pupae and larvae reached the adult stage one and two weeks faster than in the environment, respectively. Adults that were introduced in cages containing a variety of artificial food sources exhibited immediate response only to two of them (water, and dry yeast with sugar mixture), surviving for two weeks in captivity with a sole providence of artificial food sources. Adults that were introduced to environmental conditions, provided with alive GPS-infested pine trees and artificial food sources, resulted to 5133 individuals in total. They were mated in these cages without direct sunlight reaching the cages, at 21 °C, 60-65% relative humidity, and 13:11 (L:D), for a minimum of 34 min.

The *Pittosporum tobira* branches did not show any presence of the fly. Similarly, no *N. kartliana* adults emerged from the *P. tobira* branches in the cages installed inside the climate chamber.

Concerning the natural enemies of *N. kartliana*, a parasitoid of the genus *Chartocerus* was observed emerging from the pupae during branch examination and from those kept in the containers. The parasitism rate, calculated by count of pupae carrying the parasitoid's exit holes versus the total number of pupae (404), varied considerably. In fact, during spring, summer, and fall (May-December) the parasitism rate remained low to null, with a mean of 1.13%, while during the winter (January-April) it arose dramatically, forcing the general parasitism rate to reach 11.14%.

4. Discussion

Our results suggest that classical biological control using *N. kartliana* as a biocontrol agent is a plausible option for minimizing the impact and spread of *M. hellenica* in Australia. The fly appears to be the most abundant predator of GPS, preying indiscriminately on every developmental stage of the scale. It was present in every site studied and appears to have at least three generations per year, an attribute that can considerably support a faster study of its biology, as well as assist its adaptation to novel environments, due to being capable of surviving and reproducing in various environmental conditions.

Moreover, *N. kartliana* is capable of surviving for two weeks in captivity, provided with artificial food sources alone (water, and dry yeast and sugar mixture), as well as accelerate its development at 23 °C, 60% relative humidity, and 18L:6D. Additionally, when provided a spacious cage and its natural food source, apart from the artificial food sources, it is capable of mating. Additionally, the fly appears to be prey specific, considering its absence in *I. purchasi*-infested *P. tobira* branches, an issue that further supports the potential of *N. kartliana* to become a successful biological control agent against GPS.

The number of *N. kartliana* pupae examined for the presence of the parasitoid belonging to the genus *Chartocerus* is insufficient, and the parasitism rate varies significantly, depending on the season and the fly's generation. Consequently, further investigation of the biology of this parasitoid is requisite in order to draw credible inferences about the exact parasitism rate of *N. kartliana.*

References

1. Gounari, S. Studies on the phenology of *Marchalina hellenica* (gen.) (Hemiptera: Coccoidea, Margarodidae) in relation to honeydew flow. *Journal of Apicultural Research*. 2006, 45, 8–12.
2. Mendel, Z.; Branco, M.; Battisti, A. Invasive sap-sucker insects in the Mediterranean Basin. *Insects and Diseases of Mediterranean Forest Systems*. 2016, 261–291.
3. Petrakis, P. V.; Spanos, K.; Feest, A. Insect biodiversity reduction of pinewoods in southern Greece caused by the pine scale (*Marchalina hellenica*). *Forest Systems*. 2011, 20, 27. doi.org/10.5424/fs/2011201-8924
4. Downham, R.; Gavran, M. Australian plantation statistics 2017 update. *Australian Goverment Department of Agriculture and Water Resources Canberra, Australia*. 2017, 12.
5. Avtzis, D. N.; Lubanga, U. K.; Lefoe, G. K.; Kwong, R. M.; Eleftheriadou, N.; Andreadi, A.; Elms, S.; Shaw, R.; Kenis, M. Prospects for classical biological control of *Marchalina hellenica* in Australia. *BioControl*. 2020, *65*(4), 413–423. https://doi.org/10.1007/s10526-020-10012-3
6. Garonna, A.P.; Viggiani, G. The establishment in Italy of *Neoleucopis kartliana* (Tanasjtshuk) (Diptera: Chamaemyiidae), predator of *Marchalina hellenica* (Gennadius) (Hemiptera: Margarodidae). *XXIII Italian National Congress of Entomology Genoa*. 2011, 346 (in Italian)
7. Gaimari, S.D.; Milonas, P.; Souliotis, C. Notes on the taxonomy, biology and distribution of *Neoleucopis kartliana* (Diptera: Chamaemyiidae). *Folia Heyrovskyana Ser A*. 2007, 15(1):7–16
8. Hodgson, C. J.; Gounari, S. Morphology of *Marchalina hellenica* (Gennadius) (Hemiptera: Coccoidea: Marchalinidae) from Greece, with a discussion on the identity of *M. caucasica* Hadzibeyli from the Caucasus. *Zootaxa*. 2006. *1196*(1), 1–32.