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Establishment and honeydew honey production of *Marchalina hellenica* (Coccoidea Margarodidae) on fir tree (*Abies cephalonica*)

Nicolaos BACANDRITSOS
Institute of Veterinary Research of Athens, Agia Paraskevi, Athens, Greece

Abstract

Marchalina hellenica (Gennadius) (Coccoidea Margarodidae) is an important honeydew producing scale insect useful to apiculture and endemic in the pine (*Pinus halepensis*) forests of Greece.

Up to 1995 this insect was known to be monophagous on certain species of the genus *Pinus*. From 1995 onwards several attempts were made to establish *M. hellenica* on fir trees, *Abies cephalonica* (Loudon), on Mt. Helmos (Greece). Two different inoculation treatments, either with 2nd instar insects or with adult individuals, were used for the establishment of the insect on fir trees.

These trials were successful and for the first time in 1999 was collected honey, the physicochemical characteristics of which represents a good quality honey. These results create the necessary preconditions for the increase in the honey production derived from the honeydew of *M. hellenica*.

Key words: *Marchalina hellenica*, *Abies cephalonica*, establishment, honeydew honey.

Introduction

In Greece there are approximately 1,280,000 beehives, tended by 24,800 beekeepers. Annual honey production amounts roughly to 15,000 tons (Bacandritsos, 1998) of which about 65% is derived from insects honeydew (Santas, 1983; Thrasyvoulou and Manikis, 1995; Bacandritsos, 1998, 2002; Zografou *et al.,* 2002).

Conifers are affected by several insects' species. These insects infest mainly conifers in many European countries, such as Austria, the former Czechoslovakia, France, Germany, Italy, Poland, Romania, Sweden, Turkey, the former USSR and Yugoslavia (Ermin, 1950; Santas, 1983, 1988).

In Greece 5% of honey production comes from honeydew produced by insects such as *Physokermes hemicryphus* (Dalman), *Eulecanium sericeum* (Lindiger) and *Mindarus abietinus* (Koch), which infest *Abies cephallonica* (Loudon) and *Abies borisiiregis* (Mattf) (Santas, 1983; Thrasyvoulou and Manikis, 1995). The remaining 60% comes from the honeydew produced by the insect *Marchalina hellenica* (Gennadius) (Coccoidea Margarodidae) (Santas, 1983; Thrasyvoulou and Manikis, 1995; Bacandritsos and Papadopoulou, 1999; Bacandritsos, 1998, 2002), which infests *Pinus brutia* (Tenore), *Pinus halepensis* (Miller) (Bodenheimer, 1953; Nikolopoulos, 1959,1964; Kailidis, 1965; Pollini, 1998), more rarely *Pinus pinea* L. (Nikolopoulos, 1964; Pollini, 1998) and to a lesser extent *P. nigra* (Yfantidis, 1983; Avtzis, 1985) and *Pinus silvestris* (Nikolopoulos, 1965). According to Vayssiere (1926) pine-trees sustain serious damage from *M. hellenica*, while Pollini (1998) says that it has not been determined that pine trees sustain any damage.

Apart from Greece, *M. hellenica* has been recorded in Turkey, Italy and the islands of the Eastern Mediterranean (Kailidis, 1965; Nikolopoulos, 1965; Santas, 1983; Tranfaglia and Tremblay, 1984; Fimiani and Sollino, 1994; Tremblay, 1995; Pollini, 1998).

M. hellenica has one generation per year (Nikolopoulos, 1964, 1965; Pollini, 1998). Male insects are very rare (Nikolopoulos, 1964; Fimiani and Sollino, 1994; Erlinghagen, 2001; Minachilis, 2002). The female lays its eggs on pine trees, parthenogenetically, once a year (Nikolopoulos, 1965; Pollini, 1998). It lives in crevices of the pine trees bark, concealed under white cottonish secretions. It feeds by sucking sap from the tree and discharges through its anus a viscous honey-like substance. This transparent pinkish-red honeydew is collected and converted by bees into honey.

According to Nikolopoulos (1965), the female's biological-cycle consists of four stages: egg, 1st instar, 2nd instar and adult (table 1). The criterion for the classification of the individual insects is the number of segments of their antennae. The number of the segments of

Table 1. Physicochemical characteristics, sucrose, fructose and glucose content of the three Greek honey types. The data are reported as average values ± standard error.

Honey type	Fructose + Glucose (%)	Sucrose (%)	Water (%)	El. conductivity (mS/cm)	Total acidity (meq/kg)	Diastase (DN)	HMF (mg/kg)
I *M. hellenica*- fir forest	49.0±0.2	0.30±0.05	15.2±0.2	1.3±0.1	33.3±0.8	27.1±0.3	5.0±0.3
II other coccids- fir forest	44.5±2.5	0.14±0.02	14.0±0.2	1.4±0.1	29.4±0.6	14.2±0.5	1.8±0.5
III *M. hellenica*- pine forest	48.5±2.2	0.16±0.05	16.2±0.1	1.3±0.1	36.1±4.0	20.8±1.2	2.9±1.0
EU Limits	*>45.0*	*<5.00*	*<20.0*	*>0.8*	*<50.0*	*>8.0*	*<40.0*

the antennae are 6 in the 1st instar, 9 in the 2nd instar and 11 in the adult . In Greece the insect overwinters, according to Nikolopoulos (1965), at the 2rd instar. During this stage the insect exhibit vigorous feeding activity so that its weight greatly increases. The most honeydews have been observed during this stage. By contrast no feeding takes place at the adult stage, which is a short-lived migratory stage confined to egg-laying (Nikolopoulos, 1964, 1965).

The main purpose of this study was to examine the establishment of the coccid on fir forest, a new habitat for the insect, with two different ways and to collect possible honeydew honey obtained from *M. hellenica* (figure 1). Additionally, for having a more complete overview, a comparison with Greek (*M. hellenica*) honeydew honey from pine trees and Greek (other coccids) honeydew honey from fir trees was carried out.

Figure 1. Individuals of *M. hellenica* established on fir tree. (In colour at www.bulletinofinsectology.org).

Materials and methods

To investigate the possibility of the establishment and the ability of the development of *M. hellenica* on fir trees, 270 trees of *A. cephalonica* were used in 1995 and 270 in 1996. The trees were of 50-60 cm in circumference and 10-15m in high. The experiment was carried out on Mt. Helmos at the location of the ski centre (longitude 22° 11'33"E, latitude 37 °59'30"N, altitude 2,000 m).

Two different inoculation treatments were used based either on the use of the 2nd instar female individual (treatment I) or the use of adult female individual (treatment II). For each treatment 90 fir trees were used. The attempted inoculations (placements) were carried out with the aid of pine twig cuttings not more than 10 cm in diameter and 30 cm in length. Each of them had more than five female insects. The infested pine twigs came from the Mt. Fteri (longitude 22°4’40’’E, latitude 38°9’10’’N, altitude 700m).

The inoculation attempts took place during April (treatment I) and May (treatment II) of 1995 and 1996 three times per year. Each time, 30 pine twigs infested by 2nd instar stage of *M. hellenica* and other 30 infested by adult female individual were placed in the axillas at an equal number of fir trees. Shady parts of the tree were chosen for these placements. The distance between the three groups of trees was more than 200 metres.

The trees were appropriately marked so to draw a clear distinction between the different treatments. Observations on the establishment of the insect *M. hellenica* on the fir trees were carried out during August, September and October of the years 1995 and 1996.

In order to examine if there was any production of fir honeydew honey, two years later and when the establishment had been verified, one apiary of 10 beehives was placed near the infested fir trees on Mt. Helmos. The beehives had empty frames in the honey chamber. Ten honey samples were gathered during August when the other coccids and aphids of the Greek fir trees did not produce honeydew (Santas, 1983; Thrasyvoulou, 2001). Additionally 10 samples of *M. hellenica* honeydew honey from pine forest and 10 samples of other coccids honeydew honey from fir forest (Mt. Helmos) were gathered. All the samples have been examined for sucrose, fructose, glucose, water, electrical conductivity, total acidity, diastase and hydroxymethylfurfural (parameters of the EU Directive, 2001).

The determination of the saccharides were done following the gas-chromatography technique (Sabatini *et al*., 2001) with a flame ionization detector on honey solutions derived with an oximating and a silylation reagent. The identification and quantification of the single carbohydrates were obtained by comparing a known concentration reference mixture. The chromatographic conditions were: Oven temperature cycle: initial temperature 70°C, increased by 5°C per min until a temperature of 140°C, increased by 6°C per minute up to 300°C, maintained for 15 minutes; Carrier gas: hydrogen, flow velocity 47.3 cm/sec; Apolar capillary column: SE52, 25m, 0.32 mm id, 0.1-0.15 µm film thickness.

The water content, electrical conductivity, total acidity, diastase and HMF were determined according to Codex Alimentarius Commission methods (1989) and Bogdanov *et al.* (1997). The samples were stored at 4-6°C until the end of the analysis.

The concentrations of fructose+glucose, sucrose as well as the physicochemical characteristics of the tree honeydew honey types were statistically tested using t-test (MS Excel).

Results and discussion

For the trees inoculated in 1995, the first clearly successful establishment of *M. hellenica* was determined in October 1996, while for those inoculated in 1996 it was observed in October 1997. The verified establishment encompassed the success of all life stages including reproduction (Bacandritsos, 2002).

Results are showed in figure 2. The level of insect’s establishment on fir trees is considered to be very high and ranged between 76.7-86.7%. Between the two years the lowest effectiveness was observed in 1997 concerning the treatment I and the highest in 1996 concerning the treatment II. Within the year the highest effectiveness was observed for the treatment with adults.

Relevant data exist on *Pinus* spp inoculation where the effectiveness of insect establishment varied between 96.0 and 97.9% (Bikos, 1998).

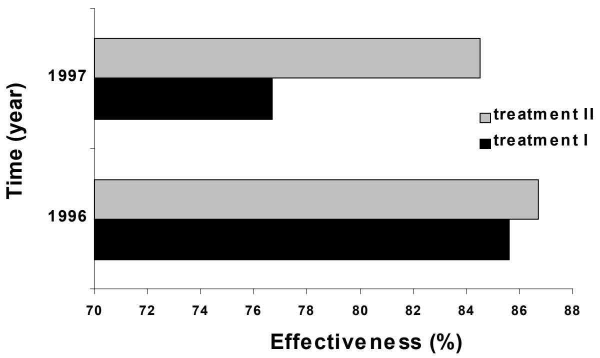

Figure 2. Mean value of the effectiveness of the establishment for the two treatments. Time concerns the observation year. (treatment 1: 2nd instar individual, treatment 2: adult individual).

At the sites of infestation there were large amounts of a white, cotton-like, waxy substance. The trees on which *M. hellenica* was successfully established continued to be infested to a greater extent. The infestation spread to neighbouring trees. As result of that infestation, the first collection of honeydew honey in 1999 occurred.

A preliminary analysis was carried out for the tree honey types: *M. hellenica* honeydew honey from fir forest (type I), other coccids honeydew honey from fir forest (type II) and *M. hellenica* honeydew honey from pine forest (type III). The results of the seven parameters in reference to the EU Directive / 2001 are summarized in table 1 including the EU limits for comparison purpose. Also the results of a comparison (t-test at level of significance a=0.05) among the three types of Greek honeydew honeys are given in table 2.

The sum of fructose and glucose content for the type I honeydew honey ranged from 48.1 to 49.7% with a mean value of 49.0%. For the type II the mean value was 44.5% with a range between 35.7 and 57.0%. The mean value for the type III was 48.5% and ranged from 40.9 to 61.0%. Sucrose content of the type I ranged from 0.10 to 0.60% with a mean value 0.30%. For type II it was 0.14% and ranged between 0.10 and 0.20% while the type III had mean value 0.16% ranged between 0.10 and 0.60%.

Concerning the water content of the type I, it ranged from 13.9 to 16.3% with an average of 15.2%. The type II had mean value 14.0% with a minimum of 13.0% and a maximum of 15%, while the type III had had mean value 16.2% with a minimum of 15.9% and a maximum of 17.0%.

Electrical conductivity represents a new criterion for the quality of honeys in Council Directive of 20th December 2001. Type I had values which ranged from 0.85 to 1.71 mS/cm with an average value of 1.3 mS/cm. Type II ranged from 0.9 to 1.8 mS/cm with an average value of 1.4 mS/cm and type III ranged from 1.1 to 1.6 mS/cm with an average value of 1.3 mS/cm.

The range for total acidity concerning the type I was from 30.1 to 38.3 meq/kg and had a mean value 33.3 meq/kg. For the type II was from 26.0 to 32.0 meq/kg with a mean value 29.4 meq/kg and for the type III was 27.0 to 62.0 meq/kg with a mean value 36.1 meq/kg.

In case of diastase for the type I the range was found to be from 25.1 to 28.1 DN with a mean value 27.1 DN. Type II was from 11.7 to 16.8 DN with a mean value 14.2 DN and type III from 12.3 to 25.8 DN with a mean value 20.8 DN.

Hydroxymethylfurfural (HMF) which constitutes a quality control criterion for the type I ranged from 3.1 to 6.5 mg/kg with a mean value 5.0 mg/kg. Type II ranged from 0.3 to 4.3 mg/kg with a mean value 1.8 mg/kg and type III from 0.5 to 9.7 mg/kg with a mean value 2.9 mg/kg.

As mentioned above, the mean values of the seven parameters of the three examined honeydew honeys were compared with the EU limits (Council Directive of 20th December 2001). Regarding the type I and type III, the mean values for the seven examined parameters were inside the EU limits. Regarding the type II, six of the seven parameters were inside the EU limits. The only exception was the sum of fructose+glucose content which had a mean value beyond the limit. In that case the 50% of the examined samples had values out of the EU limit (>45%). This result agrees well with that of Thrasyvoulou *et al*. (2002).

The comparison among the three types of honeydew honeys revealed some differences. In the first group (comparison between types I and II) the sucrose content, the water content, the total acidity and the H.M.F. were statistically different. In the second group (comparison between types I and III) and third group (comparison between types II and III) results showed statistically different only in water content and diastase ($p \leq 0.05$) (table 2).

It seems that, the responsible factors for the observed differences in the three types of honeydew honeys are: the honeydew insect, the host plant on which the insect feeds, the annual production of honey per hive and finally the weather conditions (temperature, relative humidity, wind speed-direction) during the honeydew collection. Maurizio (1976) referred that the chemical composition of honeydew varies with different honeydew insects. Also Sabatini and Barbattini (2003) re ported that honeydew honeys coming from an insect that infects both wild and cultivated plants, the flatid *Metcalfa pruinosa* (Say), had different physicochemical characteristics.

Table 2. Differences among the three Greek honey types. Values in bold indicate significant differences ($p \leq 0.05$).

	Fructose + Glucose (%)	Sucrose (%)	Water (%)	El. conductivity (mS/cm)	Total acidity (meq/kg)	Diastase (DN)	HMF (mg/kg)
I - II	0.0909	**0.0186**	**0.0011**	0.4370	**0.0011**	**0.0000**	**0.0000**
I - III	0.8179	0.0885	**0.0004**	0.6060	0.4990	**0.0001**	0.0547
II - III	0.2516	0.7077	**0.0000**	0.6533	0.1146	**0.0001**	0.3234

The present research indicates that *M. hellenica*, known, to date, as a monophagous species (feeding solely on pine trees), is a stenophagous species (feeding both on pine and fir trees) and plays an important role as honey producer. The establishment and the development of *M. hellenica* on fir trees is likely to represent an important factor in the effort of increasing the honey production in Greece. Also the preliminary examination of *M. hellenica*'s honeydew honey from fir forest showed a type of honey which meet the composition criteria of Council Directive 2001/110/EC. However, many issues related to the impact of this insect on our country's fir forests have to be examined in the future.

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Author's address: Nicolaos BACANDRITSOS (e-mail: bac.ivra@nagref.gr), Institute of Veterinary Research of Athens, 25 Neapoleos Street, Agia Paraskevi, 153 10 Athens, Greece.

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