Bulletin of Insectology **62** *(1): 85-91, 2009*
ISSN 1721-8861

Observations on the biology of *Parthenolecanium rufulum* in northeastern Italy, with a redescription of the first and second instar females

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Abstract

This paper presents some observations on the life history, phenology and morphology of *Parthenolecanium rufulum* (Cockerell) (Sternorrhyncha Coccidae), a Palaearctic soft scale species, widely distributed in European countries and especially common on *Quercus*. The study was carried out in northeastern Italy on *Quercus robur* L. trees between July 2006 to June 2008. *P. rufulum* has one generation per year and overwinters as the 2nd instar nymph. Moulting to adult female occurs from mid April onwards. Egg laying occurs between late April and late May. Egg hatching occurs from the end of May. First instar nymphs settle on the under surface of the leaves and moult to 2nd instar nymphs in August. Starting from mid September, the 2nd instar nymphs gradually migrate from the leaves to the twigs to overwinter. This migration is completed during the first 10 days of December. The pattern of phenology in Italy is about a month earlier than in central Europe. The 1st and 2nd instar females of *P. rufulum* are also redescribed and illustrated; no males were noted and reproduction was parthenogenetic. In addition, some observations on the predator *Anthribus nebulosus* Forster (Coleoptera Anthribidae) are reported.

Key words: Sternorrhyncha, Coccidae, oak soft scale, phenology, morphology, *Quercus robur*, *Anthribus nebulosus*.

Introduction

Parthenolecanium rufulum (Cockerell) (Sternorrhyncha Coccidae) the oak soft scale, is a Palaearctic species, described from specimens collected in France, department of Vienne, July 25, 1896 (Cockerell, 1903) and redescribed by Boratynski and Williams (1964). It is common on *Quercus*, but also recorded on *Carpinus*, *Castanea*, *Corylus*, *Fagus*, *Sarothamnus*, *Robinia*, *Rosa*, *Ulmus*, *Vaccinum* and *Rubus* (Kosztarab and Kozár, 1988). It is distributed throughout Europe, with the exception of Albania, Ireland and Portugal where it is until now unrecorded (Ben-Dov *et al*., 2009). *P. rufulum* is also unrecorded in the northern European countries, where deciduous oaks are at the northern border of their range.

Some other soft scales also live on European deciduous oaks, namely *Eulecanium ciliatum* (Douglas), *Eulecanium tiliae* (L.) and *Parthenolecanium corni* (Bouché), but *P. rufulum* is the most common and widespread species. Until a few years ago, *P. rufulum* was considered to be of no economic importance, but recently it has been reported as a pest of hazelnut (*Corylus avellana* L.) in Turkey (Saruhan and Tuncer, 2001) and as infesting deciduous oaks in urban environments in Georgia (Tbilisi) and Turkey (Ankara) (Japoshvili, 2001; Ülgentürk and Canakcioglu, 2004). Deciduous oaks are becoming of increasing economic importance as ornamental trees in Italy where they are currently largely used in urban parks and gardens. Despite its wide distribution, the only known biological data on *P. rufulum* relates to central Europe, namely Germany and Poland (Schmutterer, 1954; 1972; Dziedzicka, 1968). Observations on its phenology and biology were therefore carried out in northeastern Italy.

Among the natural enemies of *P. rufulum*, *Anthribus nebulosus* Forster (Coleoptera Anthribidae) is regarded as an effective predator in central Europe. Its phenology is closely related to that of its prey, which consists of monovoltine soft scales and their eggs. According to Kosztarab and Kozár (1983) and Ponsonby and Copland (1997), adults of *A. nebulosus* appear in mid April and feed on all stages of the soft scales, plus their honeydew and fungi. The eggs are laid singly from early May to early June, under the body of the female soft scale. The beetle larvae are active from mid June to July, mainly feeding on the soft scale eggs, and pupate then under the soft scale cover. The newly emerged adults enter diapause in August, in sheltered places, where they remain until the following spring. Because of its potential importance as a natural enemy, observations were also made on the activity of *A. nebulosus* in the monitored area.

Species identification of scale insects is usually based on general adult female morphology. In the absence of adult females, slide mounted young instars, which may be present on the host plant for most of the year, can help in identifying the species. Among the species of *Parthenolecanium* recorded in Europe, *P. corni* is highly polyphagous and shares with *P. rufulum* several host plants, on which both the species can live together (Stepaniuk and Lagowska, 2006). Young instars of *P. corni* have been described by Schmutterer (1954), Canard (1958), Kawecki (1958), Dziedzicka (1968) and Tereznikova (1981), but those of *P. rufulum* have only been briefly described (Schmutterer, 1954; Rehacek, 1960; Dziedzicka, 1968). With the collection of fresh specimens, the opportunity is here taken to redescribe and illustrate the 1st and the 2nd instar nymphs of this species and to discuss the differences from those of *P. corni*.

Materials and methods

This study was carried out in the park of the Faculty of Agriculture, University of Padua, northeastern Italy (45°20'50"N - 11°57'35"E), between July 2006 and June 2008. Random samples were collected from 50 twenty-year old *Quercus robur* L. trees, each about 7 m high. Samples were collected weekly in April, May and June and every 10-15 days during the other months. A total of about 3 m of 1-2 years old branches were sampled and all the soft scales, settled on the leaves of the 3m length of branches or on the wood (according to the season) were counted. Frequencies were referred to one metre of branch and ten days periods; the population dynamics were plotted on logarithmic scale graphs in order to show the abundance of each instar occurring on the branches at lower frequencies than those on leaves. Data on temperature and rainfall were provided by the local weather station. Fecundity of 10 adult females and the egg hatching time were checked in laboratory. Fully grown adult females (which had therefore stopped feeding), were collected in the field at the beginning of oviposition, and placed on Petri dishes under laboratory conditions (25 ± 0.5 °C, 70 ± 3% RH, and photoperiod of 16:8 (L:D). Eggs were counted every day until the end of oviposition. The incubation period and hatching rate were recorded.

The occurrence of the predator *A. nebulosus* was monitored between May and July in 2007 and 2008, and egg laying and post reproductive females of *P. rufulum* were randomly collected on the infested oaks and observed in the laboratory.

With regard to the morphological description, all specimens were slide mounted according to the procedures of Ben-Dov and Hodgson (1997). Measurements and numbers are given as ranges, followed by the mean in parentheses. Terminology follows that of Hodgson (1994) and Williams (1997).

Results

Observation on biology of *P. rufulum*

Year 2006 (figure 1)

Sampling started in mid July, when the 1st instar nymphs were already settled on the undersurface of the leaves. First instars scored a mean frequency of 2124 specimens/m. Moulting to the 2nd instar was first noted in late August and, by early September, about 60% of the population were 2nd instar nymphs and by late September, the whole soft scale population was 2nd instars, with a mean frequency of 602 nymphs/m. From mid September, the 2nd instar nymphs gradually moved from the leaves to the twigs to overwinter. By late September, at least 70% of the 2nd instars were already settled on the branches and this migration was completed by mid December. An average frequency of 30 nymphs/m was recorded on branches during winter.

Year 2007 (figure 2)

After overwintering, the 2nd instar nymphs moulted to adult females, starting in mid April, with a peak adult female abundance in mid April, when they constituted 58% of the population. After mid May, the entire soft scale population was adult females, with an average frequency of 11 females/m. No males were observed, so it

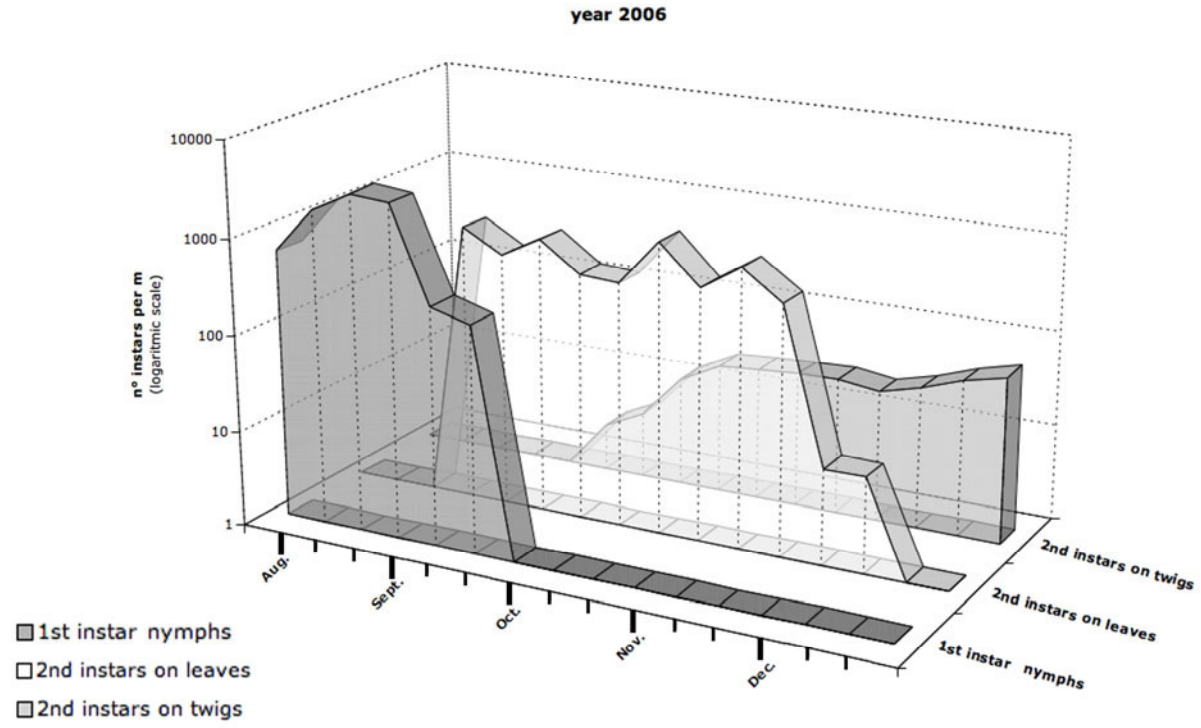

Figure 1. Population dynamics of *P. rufulum* on *Q. robur*, in the Padua district, Italy, year 2006.

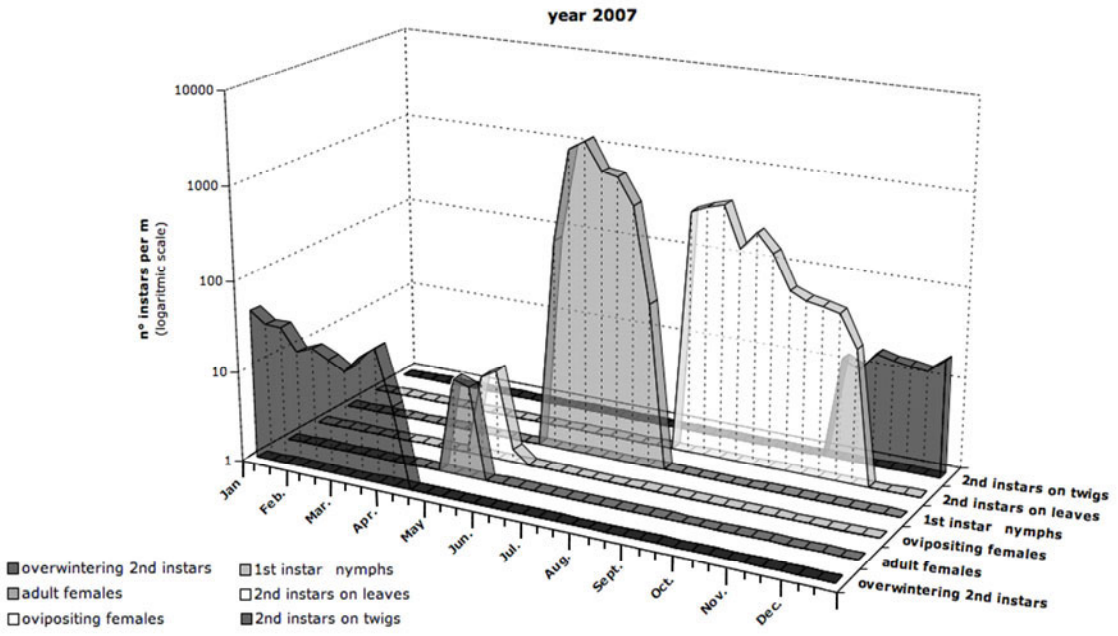

Figure 2. Population dynamics of *P. rufulum* on *Q. robur*, in the Padua district, Italy, year 2007.

is confirmed that reproduction is parthenogenetic. Just after the last moult, the adult females were flat, 1.6-1.8 mm long and 0.9-1.2 mm wide, but they then matured rapidly, became convex, and about 6 mm long and 4 mm wide. The first egg laying females were observed in late April. In early May, egg laying females constituted about 53% of the population and, from mid May onwards, all adult females were egg laying. Egg hatching started in late May and ended a week later. After emergence, the crawlers moved from the twigs to the under surface of the leaves, where they settled near the main leaf veins. First instars scored an average frequency of 1830 instars/m. Second instars were observed from August 10th, and the whole population was 2nd instars by August 24th, when they had an average frequency of 536 instars/m. The migration from leaves to twigs started in late October and the whole population was settled on branches by mid November, with a mean frequency of 24 nymphs/m. In the laboratory, an average fecundity of 1892 eggs/female was observed (minimum 425; maximum 2410 eggs/female).

Year 2008 (figure 3)

After overwintering, the 2nd instar nymphs began moulting into adult females in mid April, as in the previous year, and adult females constituted 80% of population by early May. Moulting was complete by mid May, with an average frequency of 8 adult females/m. The first eggs were observed in mid May and all the females were egg laying, from late May onwards. Crawlers started to emerge in early June and hatching lasted about 10 days. The 1st instar nymphs settled on the leaves, attaining an average frequency of 1319 nymphs/m. In the laboratory, an average fecundity of 333 eggs/female was recorded (minimum 226; maximum 487 eggs/female). Oviposition lasted on average 6.8 days; the incubation period was 6.7 ± 2.8 days, with a hatch rate close to 100%.

Observations on *A. nebulosus*

Between May and July in 2007, a total of 270 egg laying and post reproductive *P. rufulum* females were observed. Of these, 106 had a larva, pupa or an adult *A. nebulosus* within the egg chamber, with a predation rate of 39%. Larval and pupal *A. nebulosus* were recorded from May 25th until mid June. Adult predators were observed on June 5th and 18th and on July 3rd. In the same period in 2008, a total of 178 female *P. rufulum* were observed but only 26 had a larva, pupa or an adult predator within the egg chamber, giving a predation rate of only 14.6%. *A. nebulosus* pupae and adults were observed from June 12th until the end of the month.

Morphology of *P. rufulum*

Material examined

Italy:

Padua, *Q. robur*, 24. VII. 2007: 11 1st instar nymphs; Padua, *Q. robur*, 24. VIII. 2006: 6 2nd instar nymphs; Padua, *Q. robur*, 22. III. 2007: 29 2nd instar nymphs.

Description of 1st instar nymph (figure 4)

Note:

Described from 5 specimens in good condition, details checked on the remaining specimens.

Mounted material:

Body oval; body length of youngest specimens: 370-389 (379) µm, width: 207-215 (211) µm; body length just prior to moulting: 673-685 (679) µm; anal cleft short.

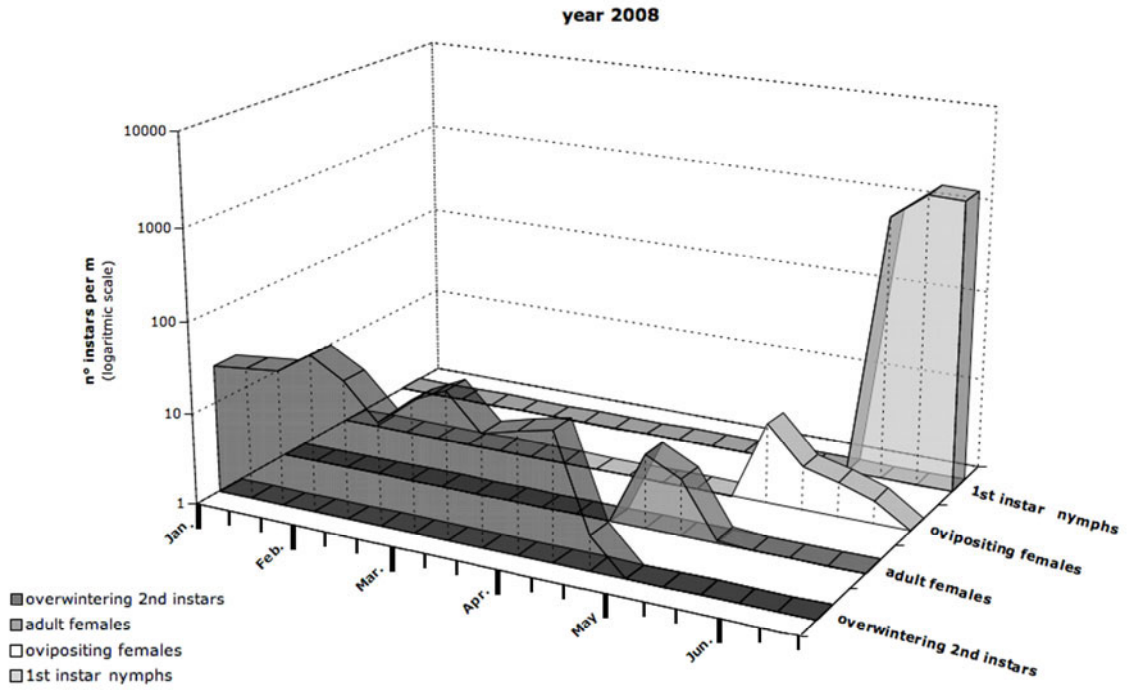

Figure 3. Population dynamics of *P. rufulum* on *Q. robur*, in the Padua district, Italy, year 2008.

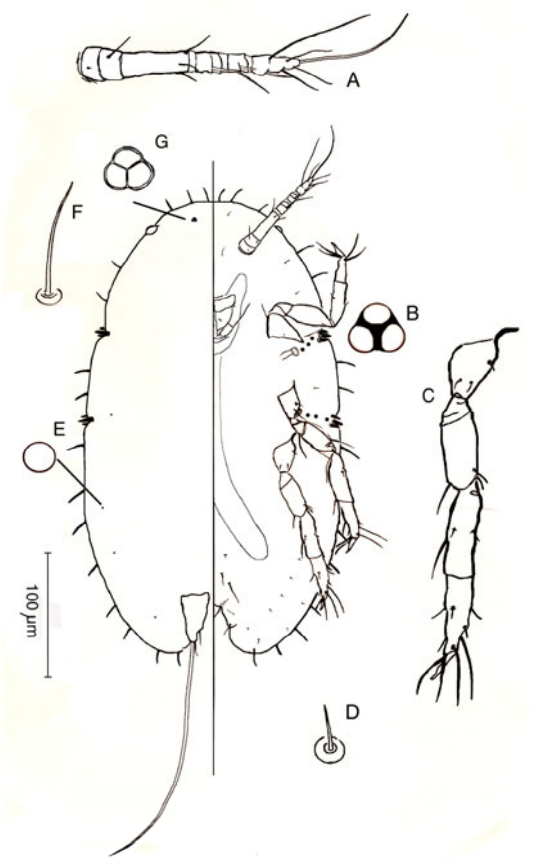

Figure 4. *P. rufulum*, 1st instar nymph. Where A = antenna; B = spiracular trilocular disc-pore; C = metathoracic leg; D = abdominal ventral seta; E = dorsal simple pore; F = marginal seta; G = dorsal trilocular pore.

Dorsum:

Derm membranous. Dorsal simple pores, each less than 1 µm wide, scattered along body margin except anterior 1/3 of margin where they are absent. One pair of trilocular pores, each about 2 µm wide, situated on head apex. Anal plates each sub-triangular, broad, with inner margins slightly diverging; each plate with 1 posterior margin seta, 10-16 (13) µm long, 2 inner margin setae, each seta 6-9 (6) µm long, with one flagellate apical seta 174-196 (187) µm. Ano-genital fold with 1 pair of anterior margin setae, each 10-30 (18 µm) long, and 1 pair of lateral margin setae, each 10-13 (11) µm long. Anal ring with 3 pairs of setae.

Margin:

Marginal setae setose, each 10-13 (12) µm long, seta set in a basal socket 2-4 (3) µm wide, distributed along body margin as follows: 6-8 anteriorly between eyespots; 2 between eyespot and anterior stigmatic area; 2 between stigmatic areas; 8 between posterior stigmatic area and anal cleft. Stigmatic spines: 3 per cleft, each slightly shorter and more spinose than marginal setae, set slightly onto dorsum, with median spine longer than laterals.

Venter:

Derm membranous, segmentation obscure. Ventral setae, each 3-6 (4 µm) long, present in submarginal and submedial rows; setae mostly present on last abdominal segments; also with one pair of inter-antennal setae, each 17-26 (22) µm long; with 1 pair of pregenital setae, each 23-25 (24) µm long. Antennae 6-segmented, each 122-137 (129) µm long, third segment longest. Spiracles: peritremes 5-7 µm wide. With 3 spiracular disc-

pores, each usually with 3 loculi, 3-5 (4) µm wide, forming a short band from each spiracle to body margin. Legs well developed, without a tibio-tarsal sclerosis; claw denticle small; with claw digitules setose, 1.6-2 (1.8) µm long; tarsal digitules each 23-36 (31) µm long.

Description of 2nd instar nymph (figure 5)

Note:

Described from 11 specimens in good conditions, details checked on the remaining specimens.

Mounted material:

Body elongate oval; dimensions of youngest specimens: length: 760-969 (837 µm), body width: 447-566 (501) µm; dimensions of oldest specimens: length: 1669-1788 (1746) µm, body width: 938-1147 (995) µm; anal cleft short.

Dorsum:

Derm membranous. Dorsal microducts minute, present throughout, each 1.5 µm wide, with a filamentous inner ductule and sclerotised pore, appearing bilocular when viewed from above. Anal plates each subtriangular, broad, with inner margins slightly diverging; anal plate setae as follows: 1 posterior margin seta, 9-13 (12) µm long, 2 inner margin setae, latter in a subapical position, each 7-12 (9) µm long, 1 apical seta, 9-15 (12) µm long. Anogenital fold with 2 pairs of anterior margin setae, 12-15 (13) µm long and 16-22 (20) µm long respectively, and 1 pair of lateral margin setae 15 µm long. Anal ring with 3 pairs of setae.

Margin:

Marginal setae sharply spinose, each 10-13 (12) µm long, 1.5-2 (1.7) µm wide at base, each seta set in a basal-socket 4-6 (5) µm wide, distributed along body margin as follows: 13-14 anteriorly between eyespots; 5-7 between eyespot and anterior stigmatic area; 6-7 between stigmatic areas; 16 between posterior stigmatic area and anal cleft. Stigmatic spines: 3 per cleft, each more spinose and longer than marginal setae, median spine bluntly spinose, 22-25 (23) µm long and 3-4 µm wide at base, set slightly onto dorsum, lateral setae shorter, each 12-17 (14) µm long and 2-4 µm wide at base. With 1 seta on each anal lobe significantly longer than other marginal setae, each 23-35 (29) µm long. Width of each eyespot lens 15-16 µm.

Venter:

Derm membranous, segmentation obvious medially on abdomen, obscure elsewhere; with minute dermal spinules appearing most frequent medially around anal cleft. Short ventral setae, each 4-9 (6) µm long, present in submarginal and submedial rows along body margin, mainly distributed on abdominal segments; also with 3 pairs of pregenital setae 42-58 (50) µm long; plus two pairs of inter-antennal setae: one pair 22-33 (27) µm long and the other 104-118 (113) µm long. Ventral microducts forming a band 2-3 pores wide along body margins, each microduct with a sclerotised pore 1.5-3 (2) µm wide, with short and broad inner ductule. Antennae 6-segmented, each 170-204 (191) µm long, 3rd segment longest, usually with 1 distinct pseudo-segmentation, causing antenna to appear 7-segmented; apical segment with 3 or 4 antennal bristles, 3 or 4 fleshy setae and 3 or 4 flagellate setae, longest 61-62 µm long. Spiracles: peritremes 12-15 (13) µm wide. Spiracular disc-pores quinquelocular pores, each 3-4 µm wide, forming a band of 9-12 (10) pores between each spiracle to body margin. Other ventral pores: with one pair of preantennal simple pores, each about 3 µm wide, near each scape. Legs well developed, without tibio-tarsal sclerosis; claws denticles small; claw digitules unequal: one broad, 20-25 (23) µm long, and other setose, 22-28 (23) µm long; tarsal digitules capitate, each 33-39 (36) µm long.

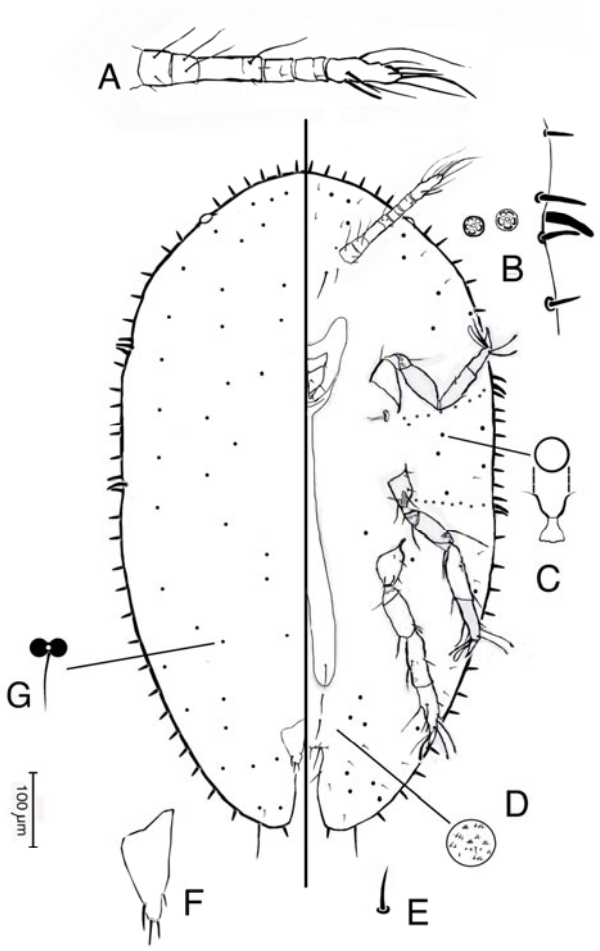

Figure 5. *P. rufulum*, 2nd instar nymph. Where A = antenna; B = anterior stigmatic area with quinquelocular disc-pores and stigmatic setae; C = ventral microduct; D = ventral dermal spinules; E = abdominal ventral seta; F = dorsal view of anal plate; G = dorsal microduct.

Comments on morphology, *P. rufulum* vs *P. corni*

The first instar nymphs of *P. corni* and *P. rufulum* can be easily separated: *P. corni* 1st instars have two longitudinal lines of bilocular pores dorsally which are absent on *P. rufulum* 1st instars. Second instars *P. corni* have usually dorsal submarginal tubercles, although their number

may be variable or also they may be absent (Canard, 1958; Dziedzicka and Sermak, 1967); submarginal tubercles are always absent on 2nd instars *P. rufulum*. In case submarginal tubercles are absent, *P. corni* and *P. rufulum* 2nd instars can be separated by comparison of some morphological characters. According to different authors (Canard, 1960; Dziedzicka, 1968; Schmutterer, 1954; Kawecki, 1958) marginal setae number 70-105 (average 89) in *P. corni*, whereas they are 67-74 (71) in *P rufulum*. Moreover, according to Canard (1960), *P. corni* has 16 marginal setae between eyes and 22 marginal setae between posterior stigmatic area and anal cleft, whereas *P. rufulum* has respectively 13-14 and 16 setae. Furthermore, the anal lobe seta is distinctly longer in *P. corni* 2nd instar: this seta is 40-65 µm long (Canard, 1960) whereas it is 23-35 (29) µm long in *P. rufulum*.

Discussion

This survey confirms that *P. rufulum* is a parthenogenetic species that has one generation per year and overwinters as 2nd instar nymphs. These 2nd instars move from the leaves towards the branches in the autumn, where they settle. Once settled, they do not move for the remaining part of their life, with the final moult to adult female and oviposition taking place where the nymphs settled in the past autumn. There is no spring migration of 2nd instars from the overwintering sites as happens with *P. corni* (Canard, 1958).

As was predictable, the phenology pattern of *P. rufulum* in northern Italy appears earlier than that reported for central Europe by Schmutterer (1954, 1972) and Dziedzicka (1968). In Italy, egg laying started in late April in 2007 and in mid May in 2008, rather than between the end of May and the end of June, as reported for central Europe. In Italy, the eggs hatched in late May in 2007 and in early June in 2008, rather than between the end of June and July, as in central Europe. The delay in oviposition and eggs hatching observed in 2008 with respect to 2007 is probably due to the unfavourable weather conditions during the spring 2008, as reported below.

The mean fecundity was noticeably higher in 2007 (average 1892 eggs/female), than in 2008 (average 333 eggs/female). The lower fecundity observed in 2008 may be due, in part, to the smaller body size attained by adult females in comparison with 2007. In 2007, the preovipositing females reached about 6 mm in length and 4 mm in width, while in 2008, they were on average 4.3 mm long and 2.7 wide. It is hypothesized that the smaller size and subsequent lower fecundity observed in 2008 were affected by the unfavourable spring weather conditions during the growing period of females. The average monthly minimum of temperature in April and May 2007 was 9.6 °C and 13.8 °C, whereas it was 7.7 °C and 12 °C for the same months in 2008; moreover, the average maximum of temperature in April and May 2007 was higher, 22.3 °C and 24.5 °C, whereas it was 17 °C and 22.6 °C) for the same months in 2008. With regard to rainfall, April and May 2007 were sunny, with only 1 and 6 rainy days/month respectively, whereas these months were colder, unusually overcast and rainy in 2008, with 13 and 22 rainy days respectively. The data for fecundity differ considerably not only between years but also with regard to that observed by Schmutterer (1972) in Germany (about 800 eggs/female) and by Dziedzicka, (1968) in Poland (about 700 eggs/female).

According to our data, the population levels appears rather stable, with an average frequency of 11 females/m in 2007 and 8 females/m in 2008. A high mortality was observed during the first instar, but this is quite normal. A further high mortality occurred during the migration of 2nd instar nymphs from the leaves to the twigs.

Predation by *A. nebulosus* differed in the two years: in 2007, the predation rate was about 40%, whereas it reached only 14.6 in 2008. However, a two year observation period is too short to assess the incidence of this predator on the soft scale population.

Further observations are needed to obtain reliable data on female fecundity in north Italy and on the impact of predation.

Acknowledgements

The authors thank C. J. Hodgson, Danièle Matile Ferrero and two anonymous reviewers for their valuable comments and suggestions.

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Received January 23, 2009. Accepted March 12, 2009.