RESEARCH

Unusual Occurrence of Cocoons in Population of *Haplodiplosis marginata* (Diptera: Cecidomyiidae) in Belgium

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ABSTRACT. The saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae), is a phytophagous species that develops in saddle-shaped galls on stems of wheat *Triticum vulgare*, barley *Hordeum sativum*, rye *Secale cereale*, and some other species of Poaceae. Only one generation develops per year. Full-grown larvae leave galls and drop onto the soil where they remain up to the springtime of the following year. Larvae do not usually spin cocoons. However, formation of cocoons by larvae was observed in populations developing in western Europe: in England in 1954, in the Netherlands in the 1960s, and in Belgium in 2011. On the basis of our analysis, a part of the larval population forms cocoons as protection against unfavorable weather conditions, especially drought.

Key Words: salivary gland, life cycle, development, Europe

The cocoon is a case produced by some animals during the larval stage. The cocoon may protect larva or pupa or eggs. It is known that some butterflies (Lepidoptera), lower flies (Diptera: Nematocera), and worms (Hirudinea and Oligochaeta) form cocoons. Some spiders (Araneae) also spin a fibrous mass, to cover their eggs. Larvae (caterpillars) of the domesticated silkmoth *Bombyx mori* (Lepidoptera: Bombycidae) form cocoon, the fiber of which is used to weave silk.

In Diptera, cocoons protect larvae or pupae against the attack of predators or parasitoids and under changes in climatic conditions. It is constructed by the previous larval instar. The cocoon may also include soil particles, small stones, particles of leaves, or may only be made of silk. The formation of cocoons is frequent in Chironomidae, Keratoplatidae, Mycetophilidae, and Cecidomyiidae. This phenomenon has been described in detail by Darvas and Fónagy (2000).

Larvae of the gall midge family (Cecidomyiidae) use various strategies and adaptations to survive unfavorable environmental conditions (Skuhravý et al. 1996). One of them is creating or spinning cocoons during their life cycle. They use the secretion of salivary glands to create the cocoon. During this period, the salivary glands change and gradually reduce in size (Skuhravý 2007). Considerable variation occurs in cocoon making. In nature, this variation may be a response to the environment (Gagné 1989).

The saddle gall midge, *Haplodiplosis marginata* (von Roser, 1840), is a polyphagous species belonging to the family Cecidomyiidae that develops on various cereals (wheat *Triticum vulgare,* barley *Hordeum sativum*, and rye *Secale cereale*) and grasses (mainly on *Argopyron repens*). It is a minor pest in cereals in northern Europe, but a major pest in central Europe (Skuhravý et al. 1993, Skuhravá 2000). *H. marginata* mainly affects wheat and barley. Larvae cause saddle-shaped galls on the stems covered by the leaf sheaths. The full-grown larvae are 2.5–4-mm in length and rosa red. They have a strongly sclerotized organ called "sternal spatula" on the ventral side of the prothoracic segment.

Only one generation of *H. marginata* develops per year. The life cycle starts in springtime. Adults generally emerge in May and at the beginning of June, sometimes the flight of adults may be prolonged up to the end of June. After mating, the females lay eggs on the leaves of cereals. The life of an individual is very short, lasting 1–4 d. Larvae hatch from the eggs and move to the stem where they feed under the leaf sheaths and cause saddle-shaped galls. A single larva develops in each gall. There are usually two or three galls per stem but during outbreaks up to 60 larvae develop on one stem. The larvae quickly develop in galls from June to mid-July. The full-grown larvae leave galls and drop onto the soil where they spend the majority of their life from July to April of the following year. Most larvae hibernate at depth of 10–20 cm, and some larvae were found as deep as 40 cm. In the springtime, the larvae move up from deeper layers to the surface of the soil where they pupate. Pupation lasts 14–25 d and then adults emerge (Skuhravá 2000, Skuhravá and Skuhravý 2013).

Forming a cocoon during the life cycle of *H. marginata* has not been previously described in detail. Only Barnes (1956) mentioned that pupation of *H. marginata* takes place in a cocoon in the springtime not long before the emergence of the midges. One larva in a cocoon and eight larvae without cocoons were observed in soil samples at Harpenden (Hertfordshire, England) in the springtime of the year 1954.

The unusual formation of very thin cocoons in the population of *H. marginata* was observed in the Netherlands in the 1960s by Nijveldt and Hulshoff (1968). They explained this phenomenon as a layer produced by larvae, by their own slime, to protect themselves against severe drought, when the climatic conditions were very unfavorable. They mentioned that such coverings are rarely observed by researchers because they can be quickly destroyed as they are extracted from the soil samples.

In the Czech Republic, the larvae of *H. marginata* enveloped by cocoons have never been found although many soil samples including about 100 specimens of larvae were taken and examined during outbreaks of this gall midge species from 1971 to 1989 (Skuhravý et al. 1993).

In 2011, *H. marginata* larvae enveloped by thin cocoons were observed in Belgium during investigations in areas where wheat is cropped intensively (Censier et al. 2012).

In this article, we describe the cocoon of *H. marginata,* its shape in color photographs, and its occurrence in Belgium. We try to elucidate

the reasons why a cocoon is formed on the basis of the analysis of environmental conditions and the comparison of occurrence of this phenomenon in other gall midge species.

Materials and Methods

Occurrence of *H. marginata* in the studied areas was evaluated by taking soil samples and counting larvae from soil surface up to a depth of 15 cm. Soil samples were taken from wheat fields in two localities in Belgium: Sauvenière and Meetkerke.

Sauvenière is a village near Gembloux, in the province of Namur, at about 127 km southeast from the North Sea. The geographical coordinates are 50° 34′30″ N and 4° 44′28″ E, at an altitude of 153 m a.s.l. The field, of a size of 5 acres with a loamy soil, has not been plowed before setting up the trial. A part of the trial was sown on 14 October 2010, and the other on 23 November 2010, using several varieties of wheat.

Meetkerke is a village located in the Belgian polders near Bruges, in the Province of West Flanders. The geographical coordinates are 51° 14′51″ N and 3° 08′55″ E. The altitude is 1 m a.s.l. The 8-acres field has a clay soil with a high content in organic matter (>10%). It was sown with spring wheat (variety Marin) on 2 February 2011 without prior tillage.

Meteorological data for surveyed sites come from the stations of Gembloux and Jabbeke, which are, respectively, 4 and 8 km away from the fields of Sauvenière and Meetkerke (Meteobelgique 2012).

In the field of Sauvenière, one soil sample was taken in the middle of the field on 22 April 2011. The sampled area was 0.16 m^2 and 5 cm in depth. The soil sample had been frozen at −17.5°C until the larvae extraction.

Four soil samples were also taken in the middle of the field in Meetkerke on 8 June 2011. Three layers of 0.02 m^2 and 5 cm deep (0–5, 5–10, and 10–15 cm depth) were taken for each sample. In this case, the larval extraction took place just after sampling.

For the extraction of larvae, each soil sample was placed in a 5-liter bucket filled with water and then kneaded until the full dispersion of soil particles. Water containing soil was poured on three successive sieves with openings of 3 mm, 1 mm, and 500 µm. This operation was repeated until the entire sample was filtered. The larvae were extracted from the sieves with a paintbrush and finally counted.

Regarding the soil samples taken in Meetkerke, the vertical distributions of free larvae and larvae in cocoons were statistically compared by using a chi-square test of independence ($\alpha = 0.05$).

In Prague, a picture of cocoons with larvae of *H. marginata* was taken using the Canon D550 camera with a reverse MP-E 65 mm lens. Cocoons containing larvae of *H. marginata* were mounted on microscope slides to allow their structure description.

Results

In 2011, a total of 1,359 larvae of *H. marginata* were extracted from all the soil samples: 13 larvae in Sauvenière and 1,346 larvae in Meetkerke (Tables 1 and 2). In both study areas, most of the larvae of *H. marginata* were found without cocoon, the usual phenomenon connected with the life cycle of this gall midge species. Only a small proportion of larvae were enveloped by thin cocoons, a rare phenomenon observed in this species.

The cocoons were ball shaped, globular or spherical formations, about 2 mm in diameter. The larva inside a cocoon is bent or rolled into the shape of a crescent (Fig. 1). One can see the dark-brown-colored sternal spatula situated on the ventral side of the larval prothorax. The cocoon is quite translucent and is formed of a very thin layer, without any structures, and it seems that it is not spun from fibers (as cocoons usually are), but it includes very small particles, small stones, or very small grains of sand.

Larvae of *H. marginata* enveloped by cocoons were observed for the first time in Sauvenière on 22 April 2011. In one soil sample, 13 larvae of *H. marginata* were found. Seven of them were in cocoons. The ratio between larvae enveloped with cocoon (54%) and larvae without cocoon (46%) was ∼1:1. In this soil sample, the number of larvae enveloped by a cocoon was high, but the total number of larvae was very low.

The larvae of *H. marginata* enveloped by cocoons were also observed in soil samples taken in Meetkerke on 8 June 2011. In total, 1,346 larvae were found in four soil samples on a depth of 15 cm (Table 1). The majority of them, 1,238 larvae (92%), were without cocoon, and 108 larvae (8%) were enveloped by thin cocoons.

At the beginning of June, the largest number of larvae of *H. marginata* (∼48%) occurred in the upper soil layer at the depth of 0–5 cm (Table 2). The number of larvae decreased with increasing depth. In the deep soil layer of 10–15 cm, only 8% of the larval

Table 1. Occurrence of larvae of *H. marginata* in soil samples in Meetkerke (Belgium) on 8 June 2011

Sample	Number of larvae	Larvae in cocoon	Percentage of larvae in cocoon
A	57	7	12%
B	559	35	6%
C	517	46	9%
D	213	20	9%
Total	1,346	108	8%

Average proportion of cocoons (A–D): 9.2 ± 2.5%.

Table 2. Distribution of larvae of *H. marginata* in different soil depths in Meetkerke (Belgium) on 8 June 2011

Depth of sample	Number of larvae	Free larvae	Larvae in cocoon
0–5 cm	647 (48%)	585 (47%)	62 (57%)
5–10 cm	586 (44%)	550 (44%)	36 (33%)
10–15 cm	113 (8%)	103 (8%)	10 (9%)
Total	1,346	1,238	108

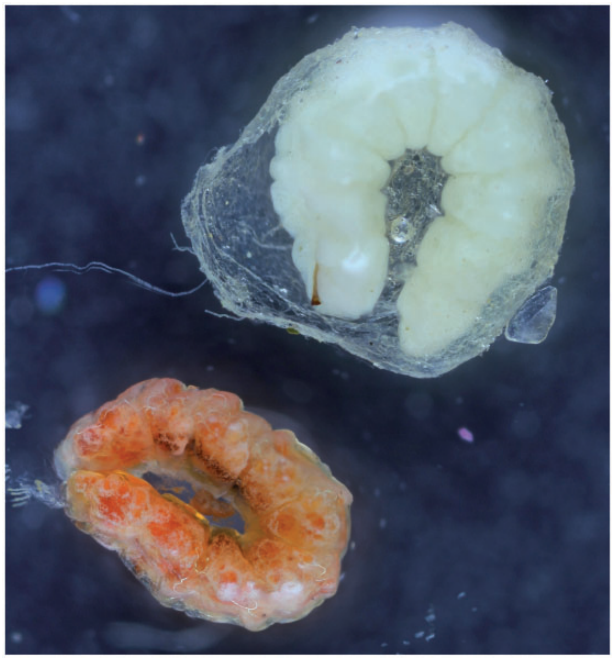

Fig. 1. Two larvae of *H. marginata* enveloped by thin and translucent cocoons. Rose/orange colored larva of future female (below), and white larva of future male with brown colored sternal spatula on the anterior body part (above).

population occurred. A high proportion of larvae in the upper soil responded to the state before the emergence of adults. A part of the population of *H. marginata* occurring in deeper soil layers (8%) will probably remain in this layer, they will not move up, and will hibernate there.

In these samples, several larvae in cocoons were found in each soil layer, ranging from 10 to 62 (Table 2). The number of larvae enveloped by cocoons, as well as the number of free larvae, decreased with increasing depth. The Chi-square test did not demonstrate a significant difference between the vertical distributions of larvae in cocoons and free larvae ($\chi^2 = 5.04$; df = 2; $P > 0.05$).

Discussion

Larvae of gall midges form cocoons in response to changes in their environment. Depending of the species, creating a cocoon may be a permanent part of the life cycle, or a cocoon is formed only under certain unfavorable environmental conditions. It gives physical protection against bad conditions or natural enemies. The formation of a cocoon is a permanent part of the life cycle of the orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin 1857). The larvae spin cocoons to overwinter, but this can only take place under some conditions. This is also the case of *Resseliella dizygomyzae* (Barnes 1933), which larvae hibernate in a cocoon if they are in the soil, while the larvae remaining in burrows on the rods of *Salix viminalis* hibernate without spinning cocoon (Urban and Skuhravá 1982).

Some gall midges can also adapt their behavior according to altitude. Larvae of *Thecodiplosis brachyntera* (Schwägrichen 1835) spin cocoons in the soil at low altitudes but hibernate at the base of pine needles without creating cocoons in mountains at altitudes over 1,200 m a.s.l. (Skuhravý 1991).

It seems that the formation of thin cocoons by larvae of *H. marginata* is correlated with environmental conditions. Nijveldt and Hulshoff (1968) considered it as a mechanism of resistance to drought. Our observations reported here support this hypothesis. Indeed, in 2011, cocoons including larvae of *H. marginata* were only found during drought conditions, resulting from the combination of higher average monthly temperatures and a lack of rainfall, when compared with the average of the last 10 yr (Fig. 2).

H. marginata occupies a large distribution area in Europe. It occurs in 20 countries (out of 40 countries from which data are available), and it occurs from western Europe (e.g., the southern part of United Kingdom) to eastern Europe (e.g., Romania and Bulgaria), reaching up to the Black Sea. It is a serious pest of cereals mainly in central and eastern Europe: in Germany, Poland, Czech Republic, Slovakia, and Romania (Skuhravá and Skuhravý 2013).

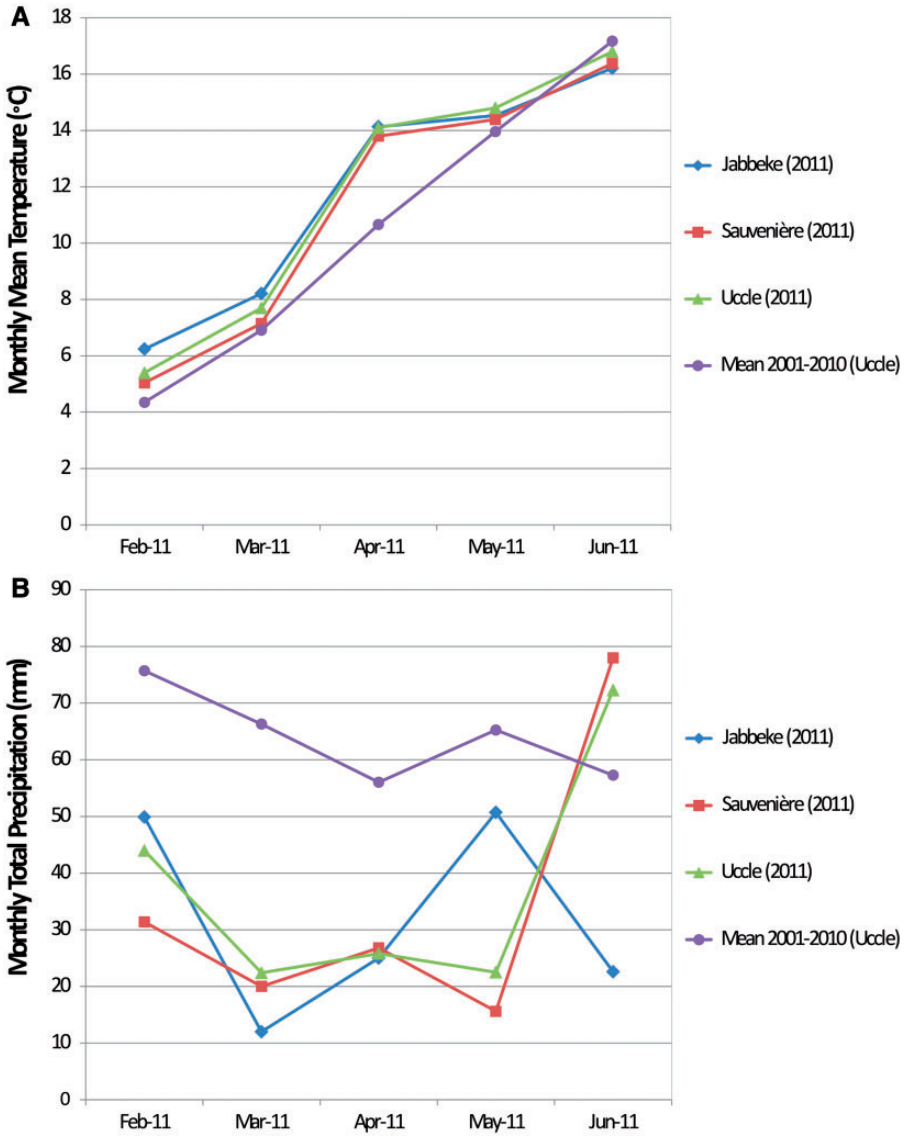

Fig. 2. Monthly mean temperature (A) and monthly total precipitation (B) between February and June 2011 for the two studied sites and for the reference meteorological station in Belgium (Uccle), and monthly means of the last 10 yr for Uccle.

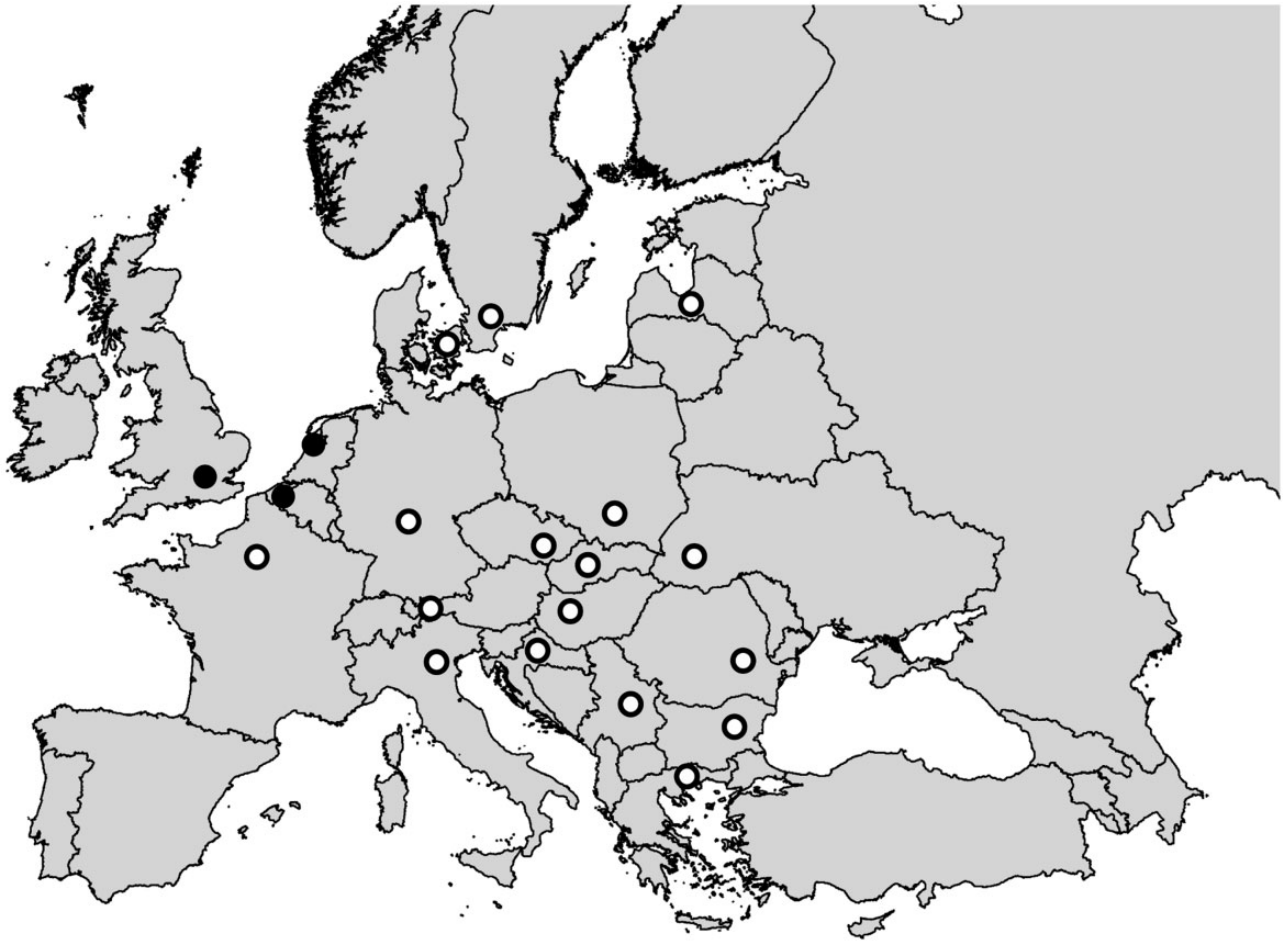

Fig. 3. Occurrence of *H. marginata* in countries of Europe: black circles, population including larvae of both types, with and without cocoons; white circles, population including larvae without cocoons.

It is interesting to notice that the larvae of *H. marginata* enveloped by cocoons were discovered in soil samples only in three countries of western Europe, all situated near the sea: Harpenden (United Kingdom), Arnhem-Nijmegen (the Netherlands), and recently in Belgium (Fig. 3). They have never been found in soil samples collected from countries of continental Europe. The formation of thin cocoons by larvae in the soil might thus be connected with the geographical position of localities and their degree of oceanity-continentality. Future research should focus on central Europe and the Mediterranean area to validate this hypothesis.

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