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Interrelationship between the raspberry cane midge, *Resseliella theobaldi* (Diptera: Cecidomyiidae) and its parasitoid, *Aprostocetus epicharmus* (Hymenoptera: Eulophidae)

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Abstract

The raspberry cane midge *Resseliella theobaldi* (Barnes) is one of the most important pests of cultivated red raspberry *Rubus idaeus* L. throughout Europe. For the last 50 years several studies have been made on the biology, life cycle and control of the pest. Some data can also be found on its natural enemies, but among these species only the chalcidoid *Tetrastichus inunctus* Nees turned out to be important in controlling raspberry cane midge populations. However, this species name is now ambiguous as the type is lost. In the present study, *Aprostocetus epicharmus* Walker was the chalcidoid species that parasitized the larvae of the raspberry cane midge, and its biology seems to be very similar to that of *T. inunctus*. It is therefore probable that the eulophid species earlier referred to as *T. inunctus* in the literature is *A. epicharmus*. Besides discussing this problem, particular consideration and detailed data are given on the biology and life cycle of *A. epicharmus* in relation to *R. theobaldi*. Different factors having effect on the population dynamics of both species are also discussed as results of a survey on several red raspberry cultivars, carried out in Hungary between 2002 and 2005.

Keywords: *Resseliella theobaldi*, *Aprostocetus epicharmus*, *Tetrastichus inunctus*, parasite, raspberry, cultivar

Introduction

The raspberry cane midge *Resseliella theobaldi* (Barnes) (Diptera: Cecidomyiidae) was discovered by Theobald in the southeastern part of England in 1920 (Barnes, 1926). Since then, it has become widely distributed throughout Europe, and numerous papers about cane damage associated with infestations of the midge have been published in different countries. Detailed data are given on the history and distribution of the pest by Woodford & Gordon (1978). In Hungary, it was first reported by Hódosy *et al.* in 1964. The biology and life cycle of raspberry cane midge under different conditions were examined by Barnes (1948), Pitcher (1952), Bachmann (1953), Fritzsche (1958), Labruyère & Nijveldt (1959), Stoyanov (1960, 1963), Nijveldt *et al.* (1963) and others. The raspberry cane midge has three or four overlapping generations a year. *Resseliella theobaldi* larvae overwinter in the soil near the surface. The pupae develop in the soil, and after emergence and mating, females usually lay their eggs in natural splits or wounds on the lower parts of stems, where splitting usually occurs first. After hatching, larvae start feeding on the periderm layer under the outer cortex. The fully-fed midge larvae drop to the soil to pupate. However, due to the damage of *R. theobaldi* larvae, dark brown, clearly defined spots appear on the green surface of the bark of the cane, and subsequently the tissue close to the midge feeding sites become discoloured. The damaged

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canes, particularly those attacked by first generation midge larvae are weakened, and a range of fungal pathogens, including *Leptosphaeria coniothyrium* (Fuckel) Sacc., often infect the wounded stems at larval feeding sites, which may result in bud failure, wilt of lateral shoots or even cane death. These characteristic symptoms are the results of a disease complex also called midge blight, which term refers to the connection between the damage of the larvae of *R. theobaldi* and the infestation of numerous fungal pathogens that attack the vascular tissues through the larval feeding sites (Williamson, 1987).

Among the natural enemies of *R. theobaldi* several species belonging to different families have been identified, but *Tetrastichus* spp. are considered to have the most important role in controlling raspberry cane midge populations. The first report of an unidentified hymenopterous species parasitizing the larvae of the pest was given by Barnes (1948), who also found that dead midge larvae remained on raspberry canes, while unparasitized ones dropped to the soil to pupate. Some years later Pitcher (1952) pointed out that the parasitoid noted by Barnes (1948) was *Tetrastichus inunctus* Nees (Hymenoptera: Eulophidae). He found that adults of this species emerged before other parasitoids (28 May to 3 June, 1947) and attacked the first larval generation of the midge causing a considerable reduction in the second and third midge generations at Lingfield, Surrey, England in 1948. *Tetrastichus inunctus* females located the host larvae under the bark by using their antennae and drove their ovipositor perpendicularly through the plant tissue to reach them and deposit their eggs. The midge larvae were soon immobilized and metamorphosis of the parasitoid took place within their bloated and blackened skins. A second peak of emergence was found in August. According to Bachmann (1953) the rate of parasitized midge larvae is often high but this is of low importance because of the numerous larval feeding sites present at the time of parasitoid emergence. In the paper by Bachmann & Fischer (1950), *T. flavovarius* Nees and *T. rosellae* De Geer are named as important parasitoid species of *R. theobaldi*. In the studies of Fritzsche (1958) *T. inunctus* Nees and *T. brevicornis* Nees, the latter as a synonym of *T. flavovarius* Nees, are mentioned as parasitoid species of great importance. Parasitized raspberry cane midge larvae were found by Fritzsche from the beginning of August, and the rate of parasitism reached 45%.

However, as far as the names of the aforementioned parasitoid species are concerned, *T. rosellae* is a misidentification, and as the types of *T. flavovarius* and *T. inunctus* are lost, these names are ambiguous. Stoyanov (1963) also found *T. inunctus* to play an important role in controlling raspberry cane midge populations. The author observed that the parasitoid, overwintering in the larval stage, largely emerged as adults from 20 to 25 May in Bulgaria. The peaks of subsequent generations of adult wasps were at the time of midge larval emergence. Because of that, the number of parasitoid generations was found to be equal to the number of midge generations. Stoyanov (1963) also recommended to growers to leave the dried fructocanes in the plantation after harvest until the end of next May to let the parasitoid adults emerge from dead midge larvae present on stems. According to Balázs (1968), infected canes should be cut and burnt after harvest, but not later than the middle of September because after this time fully-fed midge larvae have already left them and only parasitized ones are present under the outer cortical tissue. The end of July and August are the best periods for eliminating infected canes in order to kill the still young, hardly mobile larvae of the pest (Balázs, 1968). Ambrus (1973) also described *T. inunctus* as an important natural enemy of *R. theobaldi*, which attacked mainly the first generation of the midge and caused a decrease in the population of its subsequent generations.

Materials and methods

Research was carried out in the north of Hungary at Nagyréde and Berkenye, which are both traditional raspberry growing areas having suitable climatic and soil conditions for large-scale raspberry production. Three summer-fruiting cultivars, bearing ripe fruits in July, were examined, Malling Exploit, Rubaca and Fertődi Zamatos, and a single autumn-fruiting cultivar, Autumn Bliss.

Sampling of second-year canes, examination of the overwintering generation of the parasitoid species and the rearing experiment

At Nagyréde, in 2002 and Berkenye, in 2004, dead second-year canes of Malling Exploit and Rubaca, respectively, were collected from the beginning of May until the middle of July to assess parasitized midge larvae under the dried bark. The aim was to follow the appearance of round holes (always only one per parasitized larva) on the dorsal side of dead, dark brown raspberry cane midge larval skins. The holes were the signs of emergence of the parasitic wasp adults. Two sampling methods were used:

1. At the beginning of the vegetation period of 2002, 50–50 dead Malling Exploit canes were collected randomly from a raspberry plantation at Nagyréde each time the plantation was visited. In the laboratory, using a stereomicroscope, all dead, brown midge larval skins found on the 50 canes collected were counted. Inside some skins of dead, parasitized midge larvae, fully-fed parasitoid wasp larvae could be observed. Midge larval skins with holes were also found. In the latter case, the skins were empty as the adults of the parasitoid wasp had already left them by the time of examination. Establishing the rate of empty and not empty parasitized midge larval skins every time of the examination period, the approximate emergence time of the parasitoid wasp could be assessed. The sharp rise in the number of empty parasitized midge larval skins indicated the main time of emergence of the first generation of the adult wasps.
2. In 2004, dead Rubaca canes were collected from another plantation at Berkenye. During this survey, only some second-year canes were cut every second week, and these were studied in the laboratory. At each examination time, a total of 100 dead, brown midge larval skins were counted randomly on the collected canes, and the rate of empty (already left by the adult wasp) and not empty (still parasitized by the larva of the wasp) midge larval skins was again established. The main time of the parasitoid wasp emergence was assessed in the same way as in 2002.

To extend this survey, special insectaries in a rearing experiment were also used under laboratory conditions to find the time of emergence of adult wasps and to obtain specimens for identification. For the study of the emergence of parasitoid adults, parasitized midge larvae were collected in the raspberry plantations of Berkenye on 17 May, 2004.

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These larvae were placed in glass cylinders 18 cm high and 15 cm in diameter covered with cloth sheets. The cover was fixed with an elastic band in each case. These types of insectaries made it possible for us to observe the tiny wasps emerge, and also hindered their escape. For the identification, parasitized midge larvae collected in plantations of Nagyréde and Berkenye between 2002 and 2005 were placed and reared in vials closed with cotton-wool. The identification of the adult wasps took place in the Systematic Parasitoid Laboratory of Kőszeg, Hungary.

In early May and in the second half of June, 2004, the rate of hymenopterous larvae and pupae in the overwintering parasitoid generation was also observed.

Sampling of primocanes and the method of population studies of R. theobaldi *and its parasitoid*

From the end of May, every second week during the vegetation period of 2004 and 2005, 25–25 split primocanes per cultivar were cut randomly, and collected for laboratory work from Berkenye. Cultivars Rubaca, Fertődi Zamatos and Autumn Bliss were observed in this survey. The tendency of primocane splitting during the vegetation periods was also examined in case of each cultivar. As the rate of split primocanes and the size of splits were very small until the middle of June in case of Rubaca and Fertődi Zamatos in 2004 and 2005, and as this was typical of Rubaca in both years and of Fertődi Zamatos in 2005 even at the end of June, primocanes of these cultivars were not collected during these examination periods. Hence, at the beginning of the vegetation period, samples were taken only from the early-splitting Autumn Bliss primocanes. Counts of young and mature living and parasitized midge larvae present on the lower 50 cm of the collected stems were made in order to follow the population dynamics of both raspberry cane midge and its parasitoid. The aim was to reveal the interaction between the host and the parasitoid more accurately. It is important to note that no insecticides were used in the Rubaca and Fertődi Zamatos plantations, while Autumn Bliss was fertilized and sprayed with a synthetic pyrethroid against pests once in early June.

Results

The solitary endoparasitoid species reared from the larva of *R. theobaldi* was *Aprostocetus epicharmus* Walker (Hymenoptera: Eulophidae). Altogether, 824 chalcidoid parasitoid specimens were identified. The sex-ratio was 1 : 82 in favour of the females.

Emergence of the overwintering generation of A. epicharmus

In connection with the emergence of the first (overwintering) wasp generation, a characteristic upward trend could be observed in the number of dead, parasitized midge larval skins with holes from the middle of May until the middle of June. However, based on the observations of holed skins the first parasitoid adults appeared as early as April, and some of them continued to emerge in the first weeks of July (fig. 1).

In the insectary, the peak of emergence, on the basis of 209 *A. epicharmus* specimens, was at the very beginning of June, but a few adults could be seen to emerge in July. The average rates of parasitoid larvae and pupae of the overwintering generation were approximately 1 : 2 in favour of the pupae in the first two weeks of May and 1 : 3 in the second half of June. Young (yellow) and mature (dark brown) pupae could always be found between May and July.

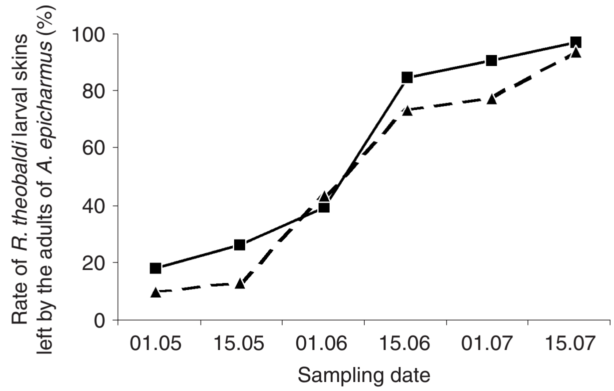

Fig. 1. Emergence of the overwintering generation of *Aprostocetus epicharmus* on the basis of *Resseliella theobaldi* larval skins left by the adults of the parasitoid wasp in two Hungarian raspberry growing regions (■, Nagyréde 2002; ▲, Berkenye 2004).

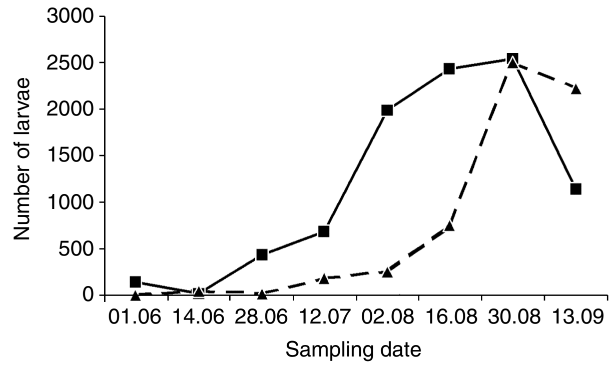

Fig. 2. Larval emergences of *Resseliella theobaldi* (■) and *Aprostocetus epicharmus* (▲) at Berkenye, Hungary in 2004.

Population dynamics of A. epicharmus *in relation to* R. theobaldi

The larval emergences of *R. theobaldi* and its parasitoid, *A. epicharmus* in 2004 and 2005 are shown in figs 2–3.

The first parasitized midge larvae occurred in June in 2004, but many parasitized midge larvae could be found as early as May in 2005 due to the larger rate of split primocanes (62% at the end of May in 2005 compared to 12% in 2004, in case of Autumn Bliss). These *A. epicharmus* larvae were the progeny of the adults which emerged between early April and early July, reaching a maximum between the middle of May and the middle of June. The adults of the first wasp generation developed from overwintering larvae, which were present under the split bark of second-year canes, and parasitized the larvae of the first midge generation, the adults of which started to emerge earlier than the

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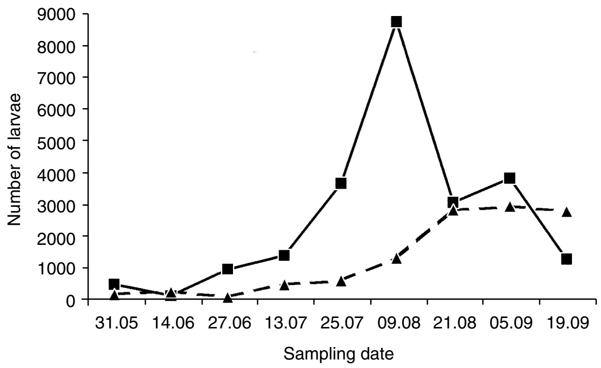

Fig. 3. Larval emergences of *Resseliella theobaldi* (■) and *Aprostocetus epicharmus* (▲) at Berkenye, Hungary in 2005.

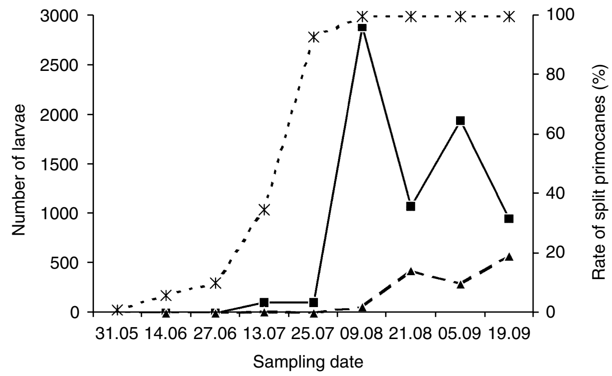

Fig. 5. Larval emergences of *Resseliella theobaldi* (■) and *Aprostocetus epicharmus* (▲) and the rate of split primocanes (⁎) in the case of cultivar Rubaca at Berkenye, Hungary in 2005.

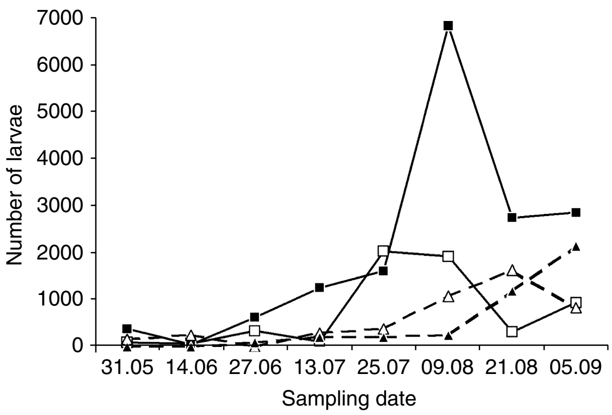

Fig. 4. The rate of different developmental stages of both *Resseliella theobaldi* (□, young; ■, mature) and *Aprostocetus epicharmus* (△, young; ▲, mature) at Berkenye, Hungary during the vegetation period of 2005.

wasps. The second peak of *A. epicharmus* emergence, on the basis of a considerable increase in the number of newly parasitized midge larvae, was in the first weeks of July both in 2004 and 2005. The emergence of the third generation of adult wasps occurred from the beginning of August, and it was prolonged to some extent because of the large midge larval population present on primocanes at this time of the year when almost all stems had splits. Since a fourth generation of *R. theobaldi* was able to develop in both years, *A. epicharmus* had the chance to parasitize this generation as well, but the larger part of the progeny of the third wasp generation remained on canes in larval stage to overwinter.

As far as changes in the rate of young and mature larval stages of both the midge and the wasp are concerned, the data collected every second week provided information on the biology and life cycle of the species (fig. 4).

The first mature larvae of *A. epicharmus* appeared in June, meaning an evidently prolonged emergence of the first generation of the wasp. In early June, the average degree of parasitism was 20% in 2004 and 40% in 2005. Newly immobilized midge larvae containing young parasitoid larvae could be seen again in the middle of July, indicating a second emergence of adult wasps having started before. At this time of the vegetation period, the rate of parasitized larvae was 15% in 2004 and 20% in 2005. In August, there was a clear increase of the parasitoid population, reaching 33% and 23% in the degree of parasitism in 2004 and 2005, respectively. As it is shown in fig. 4, many mature parasitoid larvae of the third generation were present on canes in September (overwintering generation), but young ones were still present as a sign of a partial fourth generation emergence of *A. epicharmus*, the adults of which parasitized the progeny of the last midge generation emerged in early September. In the beginning of autumn, there was no sense in assessing the rate of parasitism, as most *A. epicharmus* larvae remained present on canes to overwinter, whilst the larvae of *R. theobaldi* were leaving the stems to overwinter in the soil. Regarding the degree of parasitism, an assessment at that time would have resulted in an extremely large and false figure.

As far as the tendency of primocane splitting during the vegetation periods is concerned, the results show that this character also had an effect on the population dynamics of the host and the parasitoid. Of the cultivars observed, Rubaca had the fewest splits in the period up to June. Only 9% of its primocanes had broken epidermis at that time, compared to Fertődi Zamatos, which split the second slowest, but had 21% of its primocanes split on an average in late June, 2004 and 2005. Because of the small number of splits during spring, the first generation midges of *R. theobaldi* found it difficult to locate oviposition sites on Rubaca primocanes, hence only a few larvae developed and could be parasitized by *A. epicharmus* until the end of May. In spite of this, a large midge larval population was able to invade the primocanes of Rubaca in August (fig. 5), due to the cultivar's character of having stems with a readily peeling outer cortex under which the larvae could feed, and the neighbouring plantations of different cultivars from where midges could infest these stems as well. In this case, larval populations of the first and second generations of both species were quite small and difficult to observe, and only the factors mentioned above could help the species to reach large populations in the Rubaca plantation.

Discussion

According to the studies on both *A. epicharmus* and its host, *R. theobaldi* in Hungary, between 2002 and 2005, *A. epicharmus* turned out to be the principal parasitoid of raspberry cane midge. It is a widely distributed parasitoid of species of Cecidomyiidae in Europe. *Aprostocetus epicharmus* was reported from the Czech Republic, Slovakia, Germany, France, Greece, Hungary, Ireland, Italy, Poland, Sweden, UK and the former Yugoslavia by Graham (1987). *Aprostocetus epicharmus* specimens were obtained from, e.g. *D. brassicae* Winnertz, *Dasineura papveris* Winnertz, *Jaapiella medicaginis* Rübsaamen, *Contarinia medicaginis* Kieffer (Graham, 1987) and *D. gleditchiae* Osten Sacken (Del Bene & Landi, 1993). *Resseliella. theobaldi* was mentioned by Domenichini (1964) to be a host of *A. vincius*. This species was later synonymized as *A. epicharmus* by Graham (1987), but raspberry cane midge was not listed among its hosts. This contradiction now seems to have been solved by our studies, so *A. epicharmus* is definitely the principal parasitoid of *R. theobaldi*. Since the biology of *T. inunctus*, earlier referred to by other authors as the most important natural enemy of raspberry cane midge, bears many similarities to the biology of *A. epicharmus*, it is probable that *T. inunctus* is the same species as *A. epicharmus*, or they could also be sibling species. However, this supposition cannot be proved, as the type of *T. inunctus* is lost. The sex-ratio of *A. epicharmus* specimens reared from *D. gleditchiae* by Del Bene & Landi (1993) was 1 : 1, but this ratio was 1 : 86 in favour of the females in our studies, so the parasitoid species is assumed to be able to propagate parthenogenetically.

Population studies show *A. epicharmus* to have at least three generations a year under average continental climatic conditions. According to Stoyanov (1963), the parasitoid conspicuously emerges in late May and can be characterized by the same number of generations as its host, the raspberry cane midge. We found that the peak of wasp emergence of the overwintering generation was between late May and early June but it was prolonged. In case of *R. theobaldi*, four generations were observed in 2004 and 2005, but specimens of a partial fifth one were probably able to develop in 2004. On the basis of occurrence of young and mature larvae, it can be summarized that each peak of emergence of *A. epicharmus* adults takes place about two weeks after *R. theobaldi* emergence, due to which the parasitoid cannot produce the same number of generations as its host. Fritzsche (1958) found that the rate of parasitism reached 45%. However, as he observed parasitized midge larvae only from the beginning of August, it can be supposed that the rate of parasitism was assessed to be higher than it actually was during the whole year on average. The explanation for this statement, on the basis of our observations, is the fact that midge larvae have already dropped to the soil to overwinter by this time of the vegetation period, and this might cause a larger figure at the end of the year contrary to the real rate of parasitism. According to our results the maximum rate of parasitism was 63% on 13th September, 2004 and 68% on 19th September, 2005. Because of the aforementioned reasons, these values can also be established to be too high compared with the real parasitism rate of 38% in 2004 and 33% in 2005, which were yearly averages. Summing up these results, it is suggested that a whole year be studied in order to get an overall picture of the interrelationship between *R. theobaldi* and *A. epicharmus*.

It can also be stated that observation of population dynamics of the studied species under natural conditions is possible only in the case of raspberry cultivars that split as early as May so that adults of the first generation of *R. theobaldi* can oviposit under the bark, and have a readily peeling outer cortex where large numbers of larvae of the pest are able to develop during the vegetation period (e.g. Autumn Bliss). Wet weather and the use of fertilizers are also important as they encourage primocanes to grow and increase in girth very rapidly, and this results in increased numbers of splits. As far as chemical plant protection methods are concerned, one spraying is not enough to control *R. theobaldi*, and insecticides should be applied carefully in order not to kill parasitoid adults. According to the present observations and those of Stoyanov (1963), the peaks of emergence of adult wasps were at the time when a large number of mature midge larvae were present under the bark of stems. These peaks were found in early June, July and August under our continental climatic conditions. On the basis of these results, application of chemicals is not recommended during the periods mentioned above. Taking into consideration the need of saving natural enemies, the best combination of integrated pest management technologies against raspberry cane midge and the midge blight complex are the growing of late and hardly splitting cultivars and the avoidance of using chemical insecticides entirely or at least during the main emergence periods of *A. epicharmus*, the principal parasitoid of *R. theobaldi*. It is possible that the rate of parasitism of the raspberry cane midge larvae may be increased from the beginning of the vegetation period by leaving the primocanes cut the previous autumn in the plantations instead of eliminating them. Hence, by providing a large number of overwintering sites for the larvae of *A. epicharmus* on the spot, more parasitoid adults could be given the chance to find their host as early as they emerge. Applying this technology, raspberry cane midge damage and the consequent disease problems could be controlled more adequately.

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