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Honeydew feeding increased the longevity of two egg parasitoids of the pine processionary moth

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Abstract

The longevity of a generalist (*Ooencyrtus pityocampae*) and a specialist (*Baryscapus servadeii*) egg parasitoid of the pine processionary moth (*Thaumetopoea pityocampa*) was compared under laboratory feeding conditions including water and honeydew from aphid species growing on maritime pine (*Pinus pinaster*) or pedunculate oak (*Quercus robur*).

The longevity of both parasitoid species increased when specimens were fed with honeydew. This increase was larger for the generalist (3.7, 32.0 and 38.0 days) than for the specialist (3.0, 23.3 and 21.5 days) parasitoid species when fed with water, oak and pine aphid honeydew respectively. The phenology of the specialist species *B. servadeii* is well-adapted with its host availability with or without food supply. The generalist species *O. pityocampae* could overlap its host emergence curve during 14.0–20.0 days when fed with oak and pine aphid honeydew respectively, vs. no overlap when no supplementary food was provided. Analysis of honeydew composition indicated that sugars and amino acids may have distinct effects on parasitoid longevity.

Introduction

Parasitoids play an important role in regulation of insect herbivore populations (Lewis et al. 1997; Stiling and Cornelissen 2005). In particular they can have a notable effect on the density of host species with cyclic dynamics by enhancing the deceleration phase of the cycle (Umbanhowar and Hastings 2002). However, parasitoids may not always have a good reproductive success in natural conditions, particularly because they are lacking carbohydrate resources to sustain their foraging activity (Lewis et al. 1998; Wäckers 2003; Winkler et al. 2006; Jonsson et al. 2008). Many studies (Siekmann et al. 2001; Wäckers 2003), including laboratory rearing trials (Fuchsberg et al. 2007; Hogervorst et al. 2007) have shown that sugar-rich food improves adult parasitoid fitness, via increased reproductive life span, fecundity, flight activity or a combination of these elements (Wäckers 2003). Sugar-rich food would be particularly critical for synovigenic species which are born with immature eggs and need to feed on carbohydrates to sustain egg production during their entire lifetime. However the beneficial effect of sugar-rich food may depend on its composition, e.g. presence of specific sugars and amino acids (Wäckers 2001; Williams and Roane 2007; Faria et al. 2008).

Nectar and honeydew (i.e. carbohydrate-rich faeces of plant sap-feeding Homoptera) are important sources of carbohydrates (Völkl et al. 1999; Wäckers 2003; Vattala et al. 2006). Although nectar is the most common liquid carbohydrate-rich food in natural conditions, it can be scarce in intensively managed ecosystems such as plantation forests, where the diversity and abundance of flowering plants are low (Russell 1989; Jactel et al. 2005). In that context, honeydew may be an interesting alternative to nectar (Winkler et al. 2006; Faria et al. 2008;

Wäckers et al. 2008). For example, *Cinara* spp. can produce 400–700 kg of honeydew/ha/year in coniferous forest [Zoebelein (1954) and Zwöfler (1952) *quoted in* Stadler and Michalzik (1999)]. Mixtures of plant species are more likely to provide a consistent supply of honeydew because different species host different aphids which produce at different times and so complement each other (Zoebelein 1957). Furthermore, honeydew differs in sugar and amino acid composition between aphid species and according to their host species (Hendrix et al. 1992; Völkl et al. 1999), thus providing insect parasitoids with various food supplies.

Complementary food requirements may differ between specialist and generalist parasitoid species. Generalist species can reproduce on different host species; they are often less sensitive to the life cycle of a particular host species and may not have evolved a strict adjustment to the phenology of any of their hosts. On top of improving individual fitness, sugar-rich foods may help generalist species to increase adult longevity and allow a better overlap with their host presence in the field, particularly when they need to alternate on several host species with different periods of occurrence. In specialist species, natural selection is likely to have resulted in an optimal overlap with their specific host phenology. In this case, sugar-rich food supply may not be as important for adult longevity but can still be important for improving fecundity or mobility. Surprisingly, to our knowledge, there is no study that addressed this issue, i.e. which compared the response to sugar-rich diets of a generalist and a specialist parasitoid species of a given insect species.

The pine processionary moth (PPM) *Thaumetopoea pityocampa* (Denis & Schiffer-Müller) (Lepidoptera, Notodontidae) is the most damaging insect defoliator of pine species in southern Europe. Larvae are gregarious, they feed on needles in winter and pupate into the soil in spring where they can realize a diapause spanning from few months to several years (prolonged diapause). Adult moths' emergence occurs in early summer and moths immediately mate and lay eggs during the days following their emergence (Démolin 1969). PPM is the most abundant pest insect in the Landes de Gascogne forest (Abgrall and Bouhot 1990; Samalens 2009), the largest plantation forest in Europe with nearly one million hectares of maritime pine *Pinus pinaster* (Aiton). In this plantation forest landscape, some patches of broadleaved species like pedunculate oak *Quercus robur* (Linnaeus) persist. Parasitism by Hymenoptera is the main mortality factor in the egg stage in PPM (Santos et al. 2008). One of the most abundant egg parasitoids in the Landes de Gascogne is *Ooencyrtus pityocampae* (Mercet) (Hymenoptera, Encyrtidae), a generalist species which emerges 2 months earlier than PPM (Masutti 1964) and is known to parasitize other host insects feeding on broadleaved and conifer tree and shrub species (Masutti 1964; Battisti et al. 1988). These insect herbivores can lay eggs during the time lag between *O. pityocampae* emergence and that of *T. pityocampae*, although Battisti et al. (1988) never found any parasitized eggs before the emergence of PPM. The other main PPM parasitoid species is *Baryscapus servadeii* (Domenichini) (Hymenoptera, Eulophidae), a specialist species with an emergence season well matched with the one of its host (Géri 1980). Both species are synovigenic (Battisti et al. 1990 and pers. comm.).

We carried out an experiment to compare the response of *O. pityocampae* and *B. servadeii* to different trophic resources, i.e. two types of honeydew produced by two aphid species feeding on oak or pine trees. The objectives of our study were to test the following hypotheses: (i) honeydew feeding improves the longevity of PPM egg parasitoids; (ii) both types of honeydew have different effects on parasitoid longevity, because they have different compositions in sugar and amino-acids; (iii) the positive effect of honeydew on longevity is more important for the generalist than for the specialist parasitoid species because the latter is already well-adapted to its host phenology.

Materials and Methods

Insect material

Both *O. pityocampae* and *B. servadeii* are bivoltine. The first generation emerges from the PPM eggs parasitized in the previous year and the second from the newly laid PPM eggs (Masutti 1964). Emergent parasitoids of the first generation parasitize PPM eggs when they are available and then give birth to the second parasitoid generation. Individuals of the second generation are able to parasite PPM eggs directly after emergence, in summer and give birth to individuals which realize a diapause by overwintering inside the PPM eggs that have not produced caterpillars. These parasitoids become the first generation of the following year. Both parasitoid species are usually solitary and display unisexual reproduction. The males are only rarely recorded (Battisti 1989; Battisti et al. 1990).

Females of *O. pityocampae* and *B. servadeii* were obtained from 1242 PPM egg masses collected in summer 2007 in eight different *Pinus nigra* stands of Mont Ventoux (South-East of France). Egg masses containing overwintering parasitoids of the second generation were sent to Pierroton (South-West of France) in autumn 2007 and were stored in outdoor conditions until spring 2008. From May 2008, egg masses were monitored daily for parasitoid emergence. Emerging parasitoids from both species were counted and individually placed into 12 ml plastic vials plugged with cotton. The day of the emergence, individual parasitoids were allocated to each experimental feeding treatment.

Honeydew production

The effect of two types of honeydew on the longevity of the parasitoid individuals was tested. Pedunculate oak *Q. robur* and maritime pine *P. pinaster* aphids, i.e. *Tuberculatus querceus* (Kaltenbach) and *Eulachnus* spp. were respectively chosen for the two 'honeydew' treatments, because these two tree species are the most abundant in the Landes de Gascogne forest (Inventaire Forestier National 1999) and these aphid species can produce honeydew in spring, when PPM parasitoids occur.

Approximately 15 individuals of each aphid species were transferred onto ten 5-year-old host trees in a greenhouse, in order to obtain regular honeydew production under standardized climatic conditions (≈25°C, 85% humidity). Honeydew was collected by gravity on plastic A3 sheets suspended underneath the branches of each tree. After 2 days allowing honeydew droplets to cover the plastic sheets, they were collected and preserved at a temperature of −20°C.

Experimental design

A set of ≈40 females of each parasitoid species was assigned to each of the three different trophic resources namely: water (control), oak and pine aphid honeydew. The final number of monitored individuals in each treatment was not always the same because some insects were lost or damaged in the course of experiments.

We used tap water in the 'water' experimental treatment, soaked up on 1 cm × 1 cm pieces of cotton. Plastic sheets (1 cm × 1 cm) covered with honeydew were used in the two 'honeydew' experimental treatments. Diet material was renewed twice a week in all treatments before complete evaporation of water or drying of honeydew. On the pieces of plastic there was not always the same amount of honeydew but it was anyway more than enough food for one individual parasitoid.

Experimental vials were monitored on a daily basis and the status (alive, dead) of each insect recorded.

Monitoring pine processionary moth phenology

Previously collected data were used to obtain the emergence season of the PPM adults. PPM females lay eggs the day following emergence (Démolin 1969), so that adult emergence can be used as a proxy of egg presence. PPM emergence was monitored with light traps. Traps were automatically activated every night from day 166 to day 258, every year from 1978 to 1984 and in 1990. A 100 l container was placed under a 500 W mercury lamp positioned at ≈1 m height in a firebreak next to maritime pine stand border. An automatic clock switched on the lamp from 9 pm to 6 am the following day. Ethyl-acetate was used as insecticide inside the container. Captured pine processionary moths were removed from the container and counted every week.

Characterization of the oak and pine aphid honeydew composition by ^{1}H-NMR metabolic profiling

Honeydew was collected by scratching with a cutter from the plastic A3 sheets, stored at −20°C in Eppendorf® vials and lyophilized before NMR analysis. Two samples of oak aphid honeydew were obtained on days 155 and 160 and four samples of pine aphid honeydew on days 164, 171, 197 and 198. The honeydew dry matter inside the Eppendorf® vials varied from 4.5 to 16.4 mg for pine aphid honeydew and from 26.4 to 57.4 mg for the one from oak. Each lyophilized honeydew sample was dissolved in 0.6 ml deuterated water (99.9%), vortexed for 1 min, submitted to ultrasonic bath for 5 min and then centrifuged 2 min at 17 746 ***g*** (A14 Jouan, Saint–Herblain, France). The honeydew composition was determined using proton Nuclear Magnetic Resonance spectroscopy (^{1}H-NMR) of diluted sample (appendix S1). In order to get the same concentrations in NMR vials for the whole set of samples, a dilution was carried out to reach 4 mg of lyophilized honeydew in 0.5 ml deuterated water.

The compounds identified in the one-dimensional (1-D) ^{1}H-NMR spectra of honeydew were quantified using the integration mode of TOPSPIN software 1.3 (Bruker BioSpin, Karlsruhe, Germany) and the number of protons of the corresponding resonance, to calculate the concentration in the NMR vial. The relative concentration of NMR unknown compounds (named according to the form of the resonance, S for singlet, M for multiplet and its frequency in ppm) was calculated on the assumption that the measured resonance corresponded to one proton (appendix S1).

Statistical analysis

For each species and each feeding treatment, raw data were used to derive the Kaplan–Meier estimates of the survival functions. We used G^{ρ} family of tests proposed by Harrington and Fleming (1982) to test for significant differences between survival curves stemming from the experimental treatments. G^{ρ} tests were performed using the software R (R Development Core Team 2008).

The available data on PPM emergence were averaged per week to obtain a mean emergence curve of the PPM. The mean gain of longevity brought by each trophic resource was added to the natural emergence season of *O. pityocampae* and *B. servadeii* to estimate the effect of the trophic resources on the overlap between the emergence seasons of the PPM and its egg parasitoids. The surface under each section of the emergence curve of the PPM was calculated. Interpolation was used to obtain a daily proportion of emergent PPM from the raw data. For each trophic resource proposed to the parasitoids, the number of overlapping days between the presence of the parasitoid and its host was calculated. Furthermore, the relative proportion of PPM egg masses potentially parasitized was calculated as the surface of the emergence curve with overlapping divided by the total surface of the emergence curve.

For the study of honeydew composition, principal component analysis (PCA) of ^{1}H-NMR data was used to visualize the global composition of the whole set of samples and search for spectra regions discriminating the two honeydew types. For this multivariate analysis ^{1}H-NMR spectra were reduced into 179 variables (buckets) corresponding to spectral domains and containing integrated regions of equal width of 0.04 ppm scaled to the total intensity of the spectra using AMIX software (Analysis of MIXtures software 3.9, Bruker BioSpin, Karlsruhe, Germany) between 0.7 and 8 ppm. The region of residual unsuppressed water (δ 4.80–4.62) was excluded. PCA was carried out using mean centred data scaled to unit variance for the 179 buckets of the full NMR dataset.

Results

Effect of trophic resources on the PPM egg parasitoids longevity

The descriptive statistics of the longevity of *B. servadeii* and *O. pityocampae* are given in table 1. Figure 1 shows the Kaplan–Meier estimates of the survival functions of both parasitoid species in the different experimental treatments.

Survival curves changed according to the three experimental treatments for each species ($P < 0.001$ in both G^{ρ} tests). The lower average longevity was always observed with the control treatment, i.e. water, with 3.0 and 3.7 days for *B. servadeii* and *O. pityocampae* respectively (table 1). The highest values occurred with honeydew produced by aphids reared either on pine or oak.

Aphid honeydew increased longevity 8–10 folds compared to water control for *B. servadeii* or *O. pityocampae* respectively (table 1, fig. 1). Both honeydew

Table 1 Longevity data (days) of two pine processionary moth (*T. pityocampa*) egg parasitoids (a specialist species: *B. servadeii* and a generalist species: *O. pityocampae*), overlap between their emergence seasons and proportion of the PPM eggs concerned by parasitism, according to different trophic resources supplied to the parasitoid females

Species	Trophic resources	n	Mean	SE	Min	Max	Overlapping period (day)	PPM eggs concerned (%)
Baryscapus servadeii	W.	39	3.0	0.3	1	7	63	100
–	P.H.	40	21.5	1.0	7	38	63	100
–	O.H.	39	23.3	1.5	3	38	63	100
Ooencyrtus pityocampae	W.	39	3.7	0.3	1	7	0	0
–	P.H.	38	38.0	1.8	4	50	20	25.3
–	O.H.	38	32.0	2.6	1	58	14	12.9

W. Water (control); O.H.: Oak aphid honeydew; P.H.: Pine aphid honeydew.

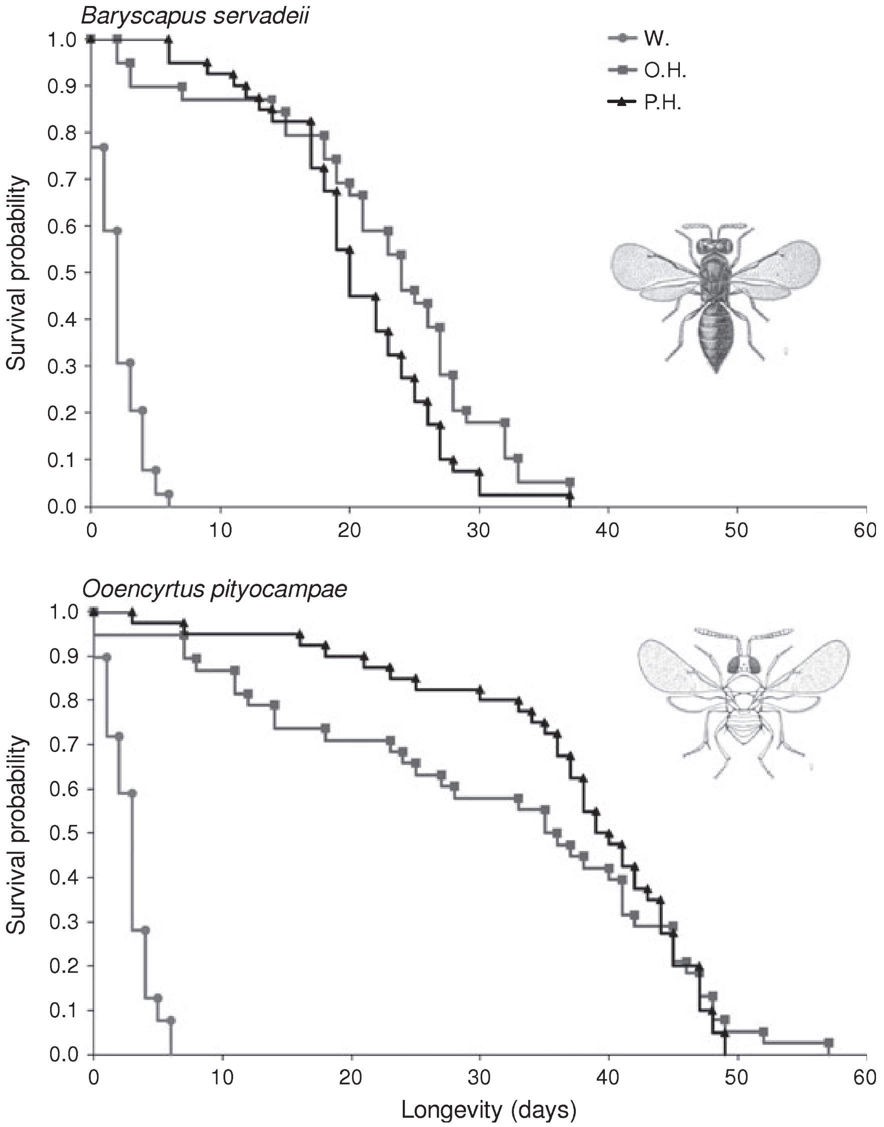

Fig. 1 Kaplan–Meier estimates of the survival functions of the specialist species *B. servadeii* and the generalist species *O. pityocampae* according to trophic resources supplied to the parasitoid females. W.: Water (control); O.H.: Oak aphid honeydew; P.H.: Pine aphid honeydew. Drawings are from PROMOTH (2002).

types increased the longevity of *B. servadeii*, with oak aphid honeydew leading to a slightly longer mean longevity (table 1). Survival curves differed significantly according to the type of honeydew (fig. 1, $P = 0.039$ in G^{ρ} test). However, it had no significant impact on the survival curves of *O. pityocampae* ($P = 0.699$ in G^{ρ} test), even pine aphid honeydew allowed a longer longevity (table 1).

The Kaplan–Meier survival curves, for both types of honeydew, were significantly different between the two parasitoid species ($P < 0.001$ in both G^{ρ} tests). The specialist species *B. servadeii* consistently exhibited shorter longevity than the generalist species *O. pityocampae* (1.4–1.8 times for oak and pine aphid honeydew respectively).

Effect of trophic resources on the overlap between emergence seasons of the PPM and its egg parasitoids

On average, in South-West of France, PPM eggs were laid and thus exposed to parasitism from the beginning of July (day 182) to the beginning of September (day 245), with a peak at the end of July (≈day 210). PPM emergence data were consistent across years, with peak dates varying by ±10 days. It was then possible to compare emergence data of

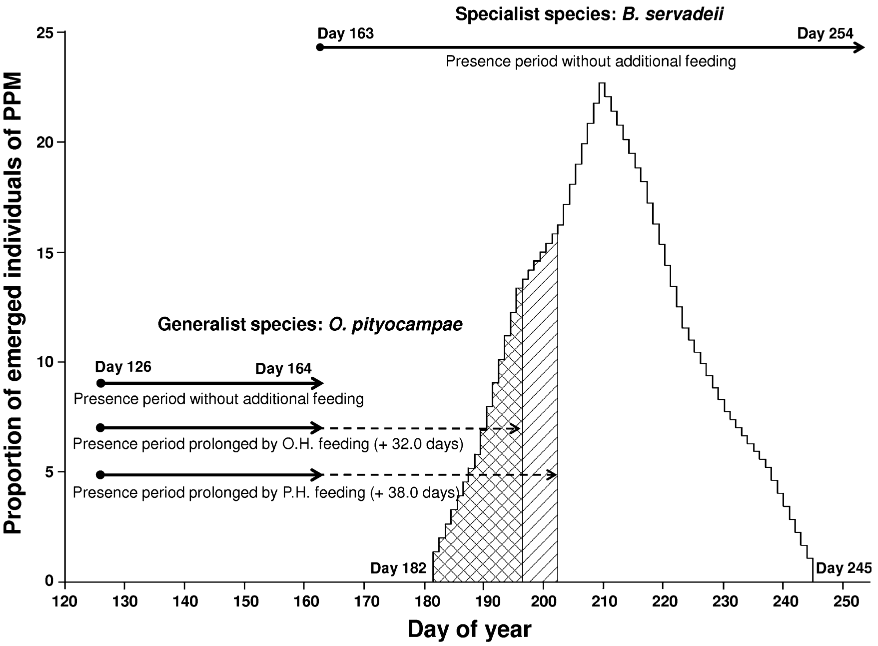

Fig. 2 Mean emergence curve of the pine processionary moth (*Thaumetopoea pityocampa*) and emergence season of its two main egg parasitoids *O. pityocampae* and *B. servadeii* without additional feeding or with honeydew feeding. O.H.: Oak aphid honeydew; P.H.: Pine aphid honeydew. Dotted lines represent the gain of longevity (in days) obtained by honeydew feeding. Hatched areas represent the proportion of PPM eggs concerned by *O. pityocampae* parasitism depending on trophic resources supplied to the parasitoid females.

PPM and its parasitoids from different years. Adults of the specialist egg parasitoid species *B. servadeii* had a flight season well-synchronized with their host (fig. 2), occurring from mid-June (day 163) to mid-July (day 254). On the contrary, the generalist species *O. pityocampae* emerged earlier than the beginning of the egg-laying by PPM for about 2 months. Its emergence season lasted from the beginning of May (day 126) to mid-June (day 164) and is extended to days 196 and 202 when *O. pityocampae* individuals were fed with oak and pine aphid honeydew respectively (fig. 2). Depending on the trophic resource, the emergence season of adult parasitoids would then overlap the one of the PPM for 0–20 days (table 1). These overlaps correspond to proportions of PPM eggs concerned by parasitism ranging from ≈13% (oak aphid honeydew) to ≈25% (pine aphid honeydew) (table 1).

Honeydew composition

The qualitative and quantitative composition of the two types of honeydew was determined using ^{1}H-NMR profiles. Chemical shifts of compounds that were identified from the two types of aphid honeydew spectra are listed in table S1 of appendix S1. Melezitose was only observed in the pine aphid honeydew and two unknowns (unkM1.76 and unkT5.14) were only observed in the oak aphid honeydew (fig. S1 in appendix S1).

The first principal component of the PCA, explaining 48% of the total variability, clearly separated the pine aphid honeydew from the oak aphid honeydew samples (fig. 3). Examination of PC1 loadings suggested that the difference involved sucrose, unkM1.76 on the negative side and glucose, fructose, shikimate, quinate, melezitose, lactate, malate, isoleucine, valine on the positive side.

The compounds identified in the 1-D ^{1}H-NMR spectra of honeydew and highlighted by PCA analysis were quantified. The main identified compounds of oak and pine aphid honeydew are sucrose, fructose and glucose (fig. S2 in appendix S1). Aphid honeydew of oak had higher content of sucrose than the one of pine, but the latter contained more glucose and fructose (fig. S2 in appendix S1).

Discussion

The present study showed that sugar-rich food sources like honeydew do increase the longevity of pine processionary moth egg parasitoids. The longevity was increased by 8–10 times when the specialist *B. servadeii* and the generalist *O. pityocampae* species were

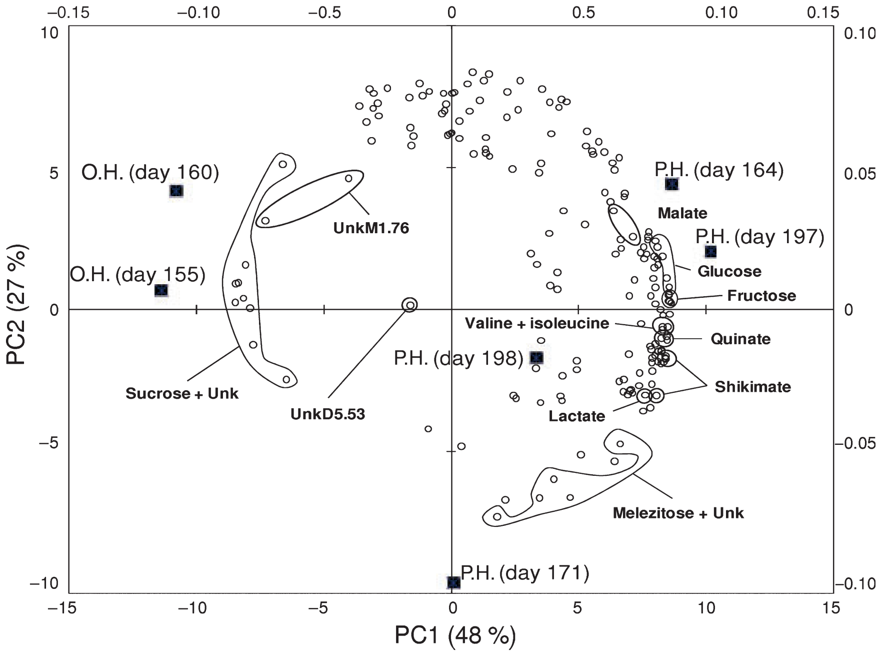

Fig. 3 Principal component analysis (PCA) of ^{1}H-NMR profiling data of the four samples of pine aphid honeydew (P.H.) and the two samples of oak aphid honeydew (O.H.). Plots are based on the two first components, the first and the second axis explaining 75% of total variance. Honeydew samples are identified by a black square and ^{1}H-NMR spectral domains identified by a white circle are annotated with the corresponding metabolite when identified (table S2 in appendix S1). Score plots are varying between −15 and 15 and −10 and 10 on x- and y-axis respectively and loading plots are varying between −0.15 and 0.15 and −0.10 and 0.10 on x- and y-axis, respectively.

respectively fed with honeydew instead of water. Numerous studies have already demonstrated the positive effect of honeydew diet on parasitoids' longevity, fecundity or mobility (Teraoka and Numata 2000; Fadamiro and Chen 2005; Fuchsberg et al. 2007; Faria et al. 2008) and honeydew is known to be directly used by wasps as carbohydrates supply in the field (Casas et al. 2003; Jonsson et al. 2008). Although not tested in this study due to technical difficulties, fecundity is also likely to be improved by honeydew feeding in *B. servadeii* and *O. pityocampae*. Both egg parasitoid species are synovigenic (Battisti et al. 1990) and may then benefit from carbohydrates supply to produce more eggs for a longer time (Wäckers 2003). Honeydew would therefore enhance the biological control of PPM by improving both the longevity and the fecundity of egg parasitoids, thus increasing the chances to find and parasitize a suitable host.

Both parasitoid species exhibited different responses to the type of diet. Survival curves did not differ according to the type of honeydew in the case of the generalist parasitoid *O. pityocampae*, but pine aphid honeydew allowed a longer longevity than oak aphid honeydew for 6 days. This species is known to parasitize eggs of broadleaved herbivorous insects, such as Heteroptera Pentatomidae [*Rhaphigaster nebulosa* (Poda), *Piezodorus lituratus* (Fabricius), *Eurydema oleraceum* (Linnaeus)] or Lepidoptera Notodontidae [*Pheosia tremula* (Clerck)] (Masutti 1964) and also conifer insect herbivores such as Lepidoptera Sphingidae [*Hyloicus pinastri* (Linnaeus)] or Lasiocampidae [*Dendrolimus pini* (Linnaeus)] (Battisti et al. 1988). It can be then assumed that *O. pityocampae* has evolved the ability to feed indiscriminately on diverse types of honeydew found in various forest habitats. On the contrary, the specialist parasitoid *B. servadeii* showed a particular response to the oak aphid honeydew, which slightly improved its mean longevity compared to the pine aphid honeydew. This result is intriguing because specialist parasitoids are more likely to stay in the main habitat of their host, i.e. in pine forest. However in natural conditions, pine forests are most often in admixture with deciduous trees and then even specialist parasitoids of pine herbivores may have evolved the ability to forage on non-pine trees. Further trials, including choice tests, are therefore needed to better investigate the respective effect of honeydew originating from different insect and tree species on a larger sample of PPM egg parasitoids.

The differences in honeydew composition may also explain their different effects on *B. servadeii*.

Pine aphid honeydew contained higher concentrations of glucose and fructose, while oak aphid honeydew contained more sucrose. In most of insect parasitoid feeding experiments, pure solutions of these sugars (Wäckers 2001; Hogervorst et al. 2007, 2009; Williams and Roane 2007; Faria et al. 2008; Lee and Heimpel 2008) provided a longer longevity than honeydew or nectar, without any primacy of one of them. Thus, the lower capacity of pine aphid honeydew to raise the lifespan of *B. servadeii* may not originate in the concentration of these sugars but in the presence of some unfavourable compounds. Some insect-synthesized sugars present in honeydew, such as melezitose, were shown to have a negative effect on parasitoid longevity (Wäckers 2001; Wäckers et al. 2008). This trisaccharide is particularly abundant in conifer aphid honeydew [Maurizio (1985) *quoted in* Wäckers (2000)]. We observed that it was in high proportion in the pine aphid honeydew and under the limit of NMR detection in the oak aphid honeydew which may explain the higher positive effect of the latter (Wäckers 2000). The effect of amino acids on insect parasitoid fitness has been poorly considered although several studies on honeydew composition have been previously published (van Helden 1995; Blüthgen et al. 2004; Faria et al. 2008; Hogervorst et al. 2009). Generally, honeydew contains low levels of amino acids (Wäckers 2003), notably because sap feeding insects tend to excrete carbohydrates and retain nitrogen rich nutrients such as amino acids from ingested phloem sap (Wäckers 2000). Amino acids did not increase the longevity of *Trichogramm* spp. parasitoids (McDougall and Mills 1997), probably because these parasitoids have enough reserves of nitrogen remaining from the larval stage (Wäckers 2003). In our study, we observed that pine aphid honeydew contained more valine and isoleucine than oak aphid honeydew where they were below detection limit. It is then possible that these amino acids were unsuitable for *B. servadeii*, but further studies are required to confirm this assumption. However, some amino acids and other metabolites such as plant secondary compounds might be difficult to detect because of their very low concentration in honeydew, in comparison with sugars with the NMR method. On the other hand some metabolites observed by NMR in honeydew extracts remained unknowns and may correspond to secondary compounds.

As a specialist egg parasitoid, *B. servadeii* exhibited an emergence season that completely matched with the egg laying season of its host. One can thus assume that any supplementary food, such as honeydew, may not be used by adult females to increase their lifespan but to increase their fitness for example through egg maturation in synovigenic species (Hougardy and Grégoire 2000). Prolonged lifespan is particularly important for generalist parasitoids as it increases the likelihood of matching with the time of occurrence of several potential hosts. As confirmed by our study, *O. pityocampae* generally emerges two months before PPM in Southern Europe (Masutti 1964; Battisti et al. 1988; Santos et al. 2008). Even if egg masses have been collected in the South-East of France, they were stocked in South-West of France during winter. Thus, as diapause termination in *Ooencyrtus* spp. is conditioned by environmental parameters (Anderson and Kaya 1975), emergence dates are expected to be the same for the egg masses we used than the ones of the local population in South-West of France, provided that there is no genetic difference between populations. *O. pityocampae* is known to be able to parasitize alternative hosts (Masutti 1964; Battisti et al. 1988). However, Battisti et al. (1988) only recorded parasite eggs of other host species during the flight season of the PPM. These authors had no explanation for that observation. If alternative hosts are missing when *O. pityocampae* emerge in spring, but further research is needed to clarify this point, then the presence of suitable carbohydrate food resources may be of critical value to prolong their lifespan until the occurrence of PPM eggs. We found that the provision of honeydew, which starts to be produced by pine or oak aphids in spring time, would allow parasitizing from 13% to 25% of PPM egg masses. However this is probably overestimated since other mortality factors may constraint the longevity of parasitoids in the field, resulting in shorter overlap. One would therefore expect increased selection pressure on late emerging adults unless the resource of honeydew or other carbohydrates is not limiting.

Our results showed that PPM parasitoid species benefited from honeydew produced by either oak or pine aphids. This trophic resource allowed a marked increase in parasitoid lifespan as compared to water. In particular the increase in longevity of *O. pityocampae*, a generalist species, improves the match between its emergence season and the one of its host. *B. servadeii*, a specialist parasitoid species of PPM, a conifer specialist defoliator, also benefit from oak aphid honeydew to increase its longevity. This finding supports the hypothesis that species-rich tree communities can provide specialist natural enemies with complementary food resources, which may explain why more diverse forests are less prone to pest insect damage (Jactel and Brockerhoff 2007). Our study lacks replication but replicating specialist species was impossible because the parasitoid community under study only featured one species of this type. Furthermore, our results stem from laboratory experiment and further investigations should be carried out in the field to confirm our findings (Casas et al. 2003; Jonsson et al. 2008). On the other hand, our data focused on the honeydew composition but its availability under natural conditions is another important point which deserves more attention.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Representative 1–D ^{1}H–NMR spectra of pine and oak aphid honeydew.

Fig. S2. Relative quantification of compounds identified in the 1–D ^{1}H–NMR spectra of pine and oak honeydew.

Table S1. Compounds identified in the 1–D ^{1}H-NMR spectra of pine and oak honeydew.

Table S2. NMR spectral domains (or buckets) annotated on fig. 3.

Appendix S1. Characterisation of oak and pine aphid honeydew composition by 1H–NMR metabolomic profiling.

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