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Cascading effects of caffeine intake by primary consumers to the upper trophic level

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Research Paper

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Abstract

Secondary metabolites are central to understanding the evolution of plant–animal interactions. Direct effects on phytophagous animals are well-known, but how secondary consumers adjust their behavioural and physiological responses to the herbivore's diet remains more scarcely explored for some metabolites. Caffeine is a neuroactive compound that affects both the behaviour and physiology of several animal species, from humans to insects. It is an alkaloid present in nectar, leaves and even sap of numerous species of plants where it plays a role in chemical defences against herbivores and pathogens. Caffeine effects have been overlooked in generalist herbivores that are not specialized in coffee or tea plants. Using a host–parasitoid system, we show that caffeine intake at a relatively low dose affects longevity and fecundity of the primary consumer, but also indirectly of the secondary one, suggesting that this alkaloid and/or its effects can be transmitted through trophic levels and persist in the food chain. Parasitism success was lowered by ≈16% on hosts fed with caffeine, and parasitoids of the next generation that have developed in hosts fed on caffeine showed a reduced longevity, but no differences in mass and size were found. This study helps at better understanding how plant secondary metabolites, such as caffeine involved in plant–animal interactions, could affect primary consumers, could have knock-on effects on upper trophic levels over generations, and could modify interspecific interactions in multitrophic systems.

Introduction

Plants produce a wide variety of secondary metabolic compounds such as alkaloids that mediate plant–animal interactions (Schoonhoven *et al*., 2005). These phytochemicals are found in nectar and in plant tissues at different concentrations and, although the dose-dependent role of some of them in attracting and rewarding pollinators has been demonstrated (Wright *et al*., 2013; Stevenson *et al*., 2017), they are generally regarded as defence mechanisms to reduce damage from non-adapted phytophagous animals, because they are bitter tasting and toxic (Szentesi and Wink, 1991; Adler, 2000; Thomson *et al*., 2015; Muñoz *et al*., 2020; Mustard, 2020). In the context of evolutionary arms race, the complex mechanisms of secondary metabolite production evolved by plant species to resist herbivory have, in return, made herbivores adapt a wide range of mitigation processes of their negative effects (Dearing *et al*., 2005). For example, some generalist herbivore species, such as the aphid *Myzus persicae* (Hemiptera: Aphididae), show strain-specific adaptations to plant defences, conferring them resistance to lupanine or to nicotine (Cardoza *et al*., 2006; Ramsey *et al*., 2014).

Evidence is accumulating that plant secondary metabolites can also mediate multitrophic interactions, well beyond plant–herbivore two-way interactions (Gols, 2014; Harvey and Gols, 2018; Ode, 2019; Sedio, 2019). Herbivore-induced plant volatiles (HIPVs) are known to impact the structure of species interactions in food-webs, because herbivores, pollinators, their natural enemies and competitors all respond to HIPVs (Vet and Dicke, 1992; Dicke and Baldwin, 2010). Concerning secondary metabolites that are ingested from the plant tissues, some herbivorous insects have evolved mechanisms of sequestration in a way that compounds can be transferred to higher trophic levels (Duffey, 1980; Szentesi and Wink, 1991; Schmidt *et al*., 2000; Erb and Robert, 2016). Predators and parasitoids usually suffer from reduced fitness when consuming herbivores sequestering secondary metabolites, or even just feeding on plants that produce them (Barbosa *et al*., 1986; Gols, 2014). For example, the caterpillar *Manduca sexta* (Lepidoptera: Sphingidae) can sequester nicotine, creating toxic conditions for the development of the endoparasite *Apanteles congregatus* [syn. *Cotesia congregata*] (Hymenoptera: Braconidae) (Thurston and Fox, 1972). As a textbook example, Campbell and Duffey (1979) demonstrated that the corn earworm *Heliothis zea* (Lepidoptera: Noctuidae) fed on artificial diet containing α-tomatine was toxic to its endoparasitoid *Hyposoter exiguae* (Hymenoptera: Ichneumonidae). Secondary consumer had lower pupal eclosion rates and was smaller in size, with potential detrimental effects on biological control strategies against this pest of tomatoes (Campbell and Duffey, 1979).

Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid present in various families of plants such as coffee (Gentianales: Rubiaceae) and tea plants (Theales: Theaceae), mostly in fruits

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and seeds, pollen, leaves, but also in the phloem and xylem sap (Mazzafera and Gonçalves, 1999; Gonthier *et al.*, 2011; van Breda *et al.*, 2013; Mustard, 2020). It has been detected in the floral nectar and pollen of *Citrus* (Sapindales: Rutaceae) and other plants from sub-tropical regions (Kretschmar and Baumann, 1999; Sano *et al.*, 2013; Wright *et al.*, 2013), but also in the yaupon *Ilex vomitoria* (Celastrales: Aquifoliacae) (Power and Chesnut, 1919) and the linden *Tilia* spp. (Malvales: Tiliaceae) (Naef *et al.*, 2004) in temperate regions. Caffeine effects are well documented on the animal brain and body, including on arthropods (Mustard, 2014; Thomson *et al.*, 2015). Its role in protecting plants against herbivores and pathogens (Ashihara *et al.*, 2008; Sano *et al.*, 2013), and in influencing herbivore and pollinator behaviours is well understood (Wright *et al.*, 2013; Couvillon *et al.*, 2015; Prado *et al.*, 2021). In *Drosophila* fruit flies (Diptera: Drosophilidae), for example, caffeine reduces sleep duration and increases locomotor activities at both day and night (Hendricks *et al.*, 2000; Nall *et al.*, 2016; Keebaugh *et al.*, 2017). At high doses, caffeine is toxic for phytophagous insects, it can shorten life span, and deter pollinators from visiting plants and reduce their memory (Ashihara *et al.*, 2008; Nikitin *et al.*, 2008; Mustard, 2014). At low doses in laboratory studies, caffeine enhances pollinator memory of reward and increases pollination (Wright *et al.*, 2013; Thomson *et al.*, 2015).

Studying the effects of caffeine on insects could help deepen our understanding of how such molecule mediates plant–insect interactions, through direct effects on the biology of primary consumer, but also how it could have knock-on effects on higher trophic levels and could modify interspecific interactions. Species interactions in crop systems with high concentrations of caffeine are mostly governed by caffeine resistance evolved by the different protagonists (Damon, 2000), but what happens in trophic systems with low-caffeine plants with regards to the effect of the alkaloid remains to be studied. Ecological aspects of caffeine–insect interactions are also relevant in applied ecology; it has potential for manipulating the foraging behaviour of natural enemies of insect pests, by providing them with caffeinated food sources, but also for developing plants resistant to phytophagous animals (Cardoso *et al.*, 2014). For example, transgenic chrysanthemum (Asterales: Asteraceae) have been produced to express the caffeine biosynthetic pathway and produce caffeine in tissues, which helps the plants resisting aphids (Kim *et al.*, 2011). However, unlike some other secondary metabolites, caffeine has not received much attention with regard to its role in host–parasitoid interactions, even when focusing on specialized phytophagous insects on coffee plants (Damon, 2000; Jaramillo *et al.*, 2006; Vega *et al.*, 2009; Green *et al.*, 2015).

Within both this fundamental and applied context, we used aphids and parasitoids as a model system to explore potential cascading effects of caffeine intake across trophic levels. Parasitoids are essential components of most terrestrial ecosystems and provide important ecosystem services, such as the regulation of herbivorous pests. Endoparasitoids are interesting models because they feed on the entire body of their hosts during development (Godfray, 1994). Parasitoids may thus directly encounter and ingest toxic metabolites in the haemolymph or tissues of their hosts, and ingestion of plant toxins by the herbivore may reduce the suitability of the host or alter its sensitivity to parasitism (Gols, 2014; Ode, 2019). In some cases, plant quality may reduce the host's immune response (e.g., encapsulation), and benefit the parasitoid (Bukovinszky *et al.*, 2009). Finally, parasitoids have developed many ways to assess host quality which modifies host handling behaviour and foraging decisions (Godfray, 1994; Wajnberg, 2006). Optimal choices of hosts and nutritional resources have to be made to increase offspring quality and survival rates. In particular, we questioned how parasitoids could adapt their behavioural response to the host's diet, and how the oviposition decision on hosts fed on caffeine diet could ultimately influence the parasitism success, and the fitness of the offspring generation. We hypothesized that if caffeine negatively affected the primary consumers' fitness, it would also affect the secondary consumer's host handling behaviour, and would cascade to the next parasitoid generation by negatively affecting their survival and life-history trait values.

Materials and methods

Biological material

We used the generalist and major pest aphid *M. persicae* as a model for the primary consumer compartment, and the generalist parasitoid *Aphidius matricariae* (Hymenoptera: Braconidae) as a model for the secondary consumer. For a fundamental study, this is a convenient system to work with because interactions between *M. persicae* and *A. matricariae* are well explored, because artificial diets are already developed for the aphid, and because *M. persicae* is already known to develop alkaloid-resistant strains when shifting host plants (Cardoza *et al.*, 2006; Ramsey *et al.*, 2014). Studying the effects of alkaloids in generalist herbivores such as *M. persicae* is important because they feed on diverse families of plants and are thus likely to be exposed to a great variety of secondary metabolites. *Aphidius matricariae* is a generalist aphid parasitoid species that is widely used for the biological control of aphid pests (Hance *et al.*, 2017). Parasitoids and aphids were purchased from the Viridaxis SA company (Charleroi, Belgium), and no reported resistance or tolerance to alkaloids exists in these strains. Experiments were done in the laboratory at 20 ± 2°C, 60 ± 10% relative humidity and 16:8 h light:dark photoperiod.

Effect of caffeine on primary consumers

Two treatments were applied: artificial diet alone, provided by Viridaxis SA and specifically developed for *M. persicae* (e.g., on other aphid species: Cambier *et al.*, 2001; van Emden and Wild, 2020), and artificial diet with caffeine at 0.1 mg ml^{-1} (0.5 mM). Note that during preliminary assays, aphids exposed to a 1 mg ml^{-1} (5 mM) caffeine treatment all died before reaching the reproductive period (i.e., null fecundity), and before turning into third-instar larvae (mean longevity 2.2 ± 0.2 days). Comparisons were thus only done between the control and the caffeine 0.1 mg ml^{-1} treatments. Because the concentration of caffeine in the sap of most plants on which this aphid feeds is unknown, these concentrations were chosen according to preliminary results on aphid parasitoids (Tougeron *et al.*, unpublished data), and to previous work on *Drosophila melanogaster* (Nikitin *et al.*, 2008; Keebaugh *et al.*, 2017) that are comparable in size with large aphids and aphid parasitoids. To feed aphids, 600 µl of the solution were deposited on the surface of a small sterilized plastic petri dish (Ø 5 cm) and it was immediately covered with a piece of stretched parafilm to create a thin membrane through which aphids could feed (Pirotte *et al.*, 2018; van Emden and Wild, 2020). Thirty adult female aphids were taken from the cultures and placed on a fresh *Brassica rapa* (Brassicales: Brassicaceae) leaf until they deposited larvae. Among newborn

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aphids, 20 of them per treatment were placed individually on artificial feeders and monitored daily for their longevity, and fecundity (daily mean offspring produced by adult female and total offspring number). Newborn larvae were removed from the artificial diet feeder as they were counted. Aphids were gently moved to new feeders every 3 days to ensure good quality of the artificial diet.

Cascading effects on secondary consumers

Mated parasitoid females (<48 h old, at their egg-load peak) and fed with a 50% honey dilution were exposed to 15 third-instar aphids, which is the favourite host stage of this species (Rezaei *et al.*, 2019), and that had been allowed to settle for 20 min on a *B. rapa* leaf placed in a glass petri dish (Ø 10 cm) on a 1.5% agar substrate, to avoid any potential effect of the artificial diet feeder on parasitoid behaviour. This experiment was repeated ten times (with $N = 10$ parasitoid females per treatment) for aphids exposed to the control treatment and the 0.1 mg ml^{-1} caffeine treatment since they were born (i.e., for around 4 days). No visible effect of caffeine was detected on the aphid development rate up to the third larval instar.

For each arena, we monitored by direct observation through binocular microscope the proportion of contacted aphids each parasitoid actually oviposited in (acceptance rate), and the time spent from host encounter (usually followed by antennal evaluation) to leaving the aphid with or without sting (mean handling time) (Gerling *et al.*, 1990). The mean number of kicks by aphids (defence strategy) was recorded for each contacted aphid. The experience ended after 15 min or after the parasitoid had made antennal contact with all 15 aphids at least one time. Aphids were kept by groups of 15 on *B. rapa* leaves and the parasitism rate was estimated as being the number of aphids that turned into mummies (dead aphid containing a parasitoid pupa)/the total number of aphids in which oviposition occurred. Emergence rate was evaluated for each clutch as the number of mummies from which a parasitoid emerged/the total number of mummies. After the emergence of the following generation, parasitoids were sexed and individually kept in plastic tubes (1.5 ml) with honey and water, and their longevity was monitored. The day they died, we measured parasitoid fresh mass and the size of the left hind leg tibia, as standard proxy traits of parasitoid fitness (Godfray, 1994).

Finally, we used a dissection experiment to complete data on parasitism success and try to detect whether parasitoid mortality occurs mainly during the early immature stages or during pupation. Four additional batches of 15 parasitized aphids, fed on either treatment (caffeine or control), were each exposed to one different parasitoid female. The same protocol as for the main experiment was used, and each aphid in which oviposition occurred was placed on a fresh *B. rapa* leaf, until we obtained 15 parasitized aphids per assay. Dissections were done under stereo microscope on a total of 40 surviving parasitized aphids (ten per batch) on the seventh day of their development; before the potential formation of the mummy, but late enough in the parasitoid development cycle to observe if late instar larvae were alive (fresh and moving) or dead (dry, dark-coloured and not moving).

Statistical analyses

Generalized Linear Models (GLMs) were fit to the data to analyse the effect of the caffeine treatment on total and daily offspring produced, assuming quasi-Poisson distribution, and a Cox model was used to analyse aphid longevity data. Generalized Linear Mixed Models (GLMMs) were used to compare the handling time of aphids by parasitoids between treatments assuming a Gaussian distribution, and to compare the number of kicks, assuming a Poisson error distribution, using the identity of the parasitoid female as a random effect. Acceptance rates, parasitism rates and emergence rates were compared between treatments using GLMMs assuming binomial error distributions. Fresh mass and tibia size of the new generation were compared between treatments and sexes using GLMMs assuming Gaussian error distributions, longevity was compared using a Cox survival model fitted with the package *coxme* (Therneau, 2015), and using the mother identity as a random effect for both types of models. Models were tested using type II ANOVA from the package *car* (Fox and Weisberg, 2011). All statistical analyses were carried out using the R software (R Core Team, 2020).

Results

Effect of caffeine on primary consumers

Aphids had a lower longevity and produced fewer nymphs when exposed to caffeine, but the caffeine treatment did not affect the mean daily offspring number produced by aphid females (table 1).

Cascading effects on secondary consumers

There were no differences in host handling time by parasitoids, nor in kicking occurrence by aphids between the two feeding treatments. Acceptance rate and emergence rate were similar between treatments. Parasitism rate (i.e., successful mummy formation) was 16% lower for parasitoids exposed to aphids fed with caffeine compared to parasitoids exposed to control aphids (table 2). After dissection at day 7 (before mummy formation), we found 60% survival in parasitoids in control aphids ($N = 15$), and 46% survival for those parasitizing aphids with caffeine diet ($N = 13$).

Sex ratio (♂:♀) was (1:1.5) for control treatment and (1:1.3) for the caffeine treatment. Parasitoid longevity (Cox model, $z = -0.67$, DF = 1, $P = 0.50$), fresh mass (GLMM, $\chi^2 = 2.18$, DF = 1, $P = 0.14$) and tibia size (GLMM, $\chi^2 = 0.04$, DF = 1, $P = 0.83$) did not differ between sexes so data were pooled for the rest of the analyses. There was a marginally significant effect of the aphid diet treatment origin on parasitoid longevity ($\chi^2 = 3.4$, DF = 1, $P = 0.048$). Parasitoids that have developed on aphids fed on control diet lived on average 4.5 days longer than on aphids fed with 0.1 mg ml^{-1} caffeine. There were no differences in parasitoid fresh mass ($z = -2.13$, DF = 1, $P < 0.05$) and parasitoid tibia length ($\chi^2 = 2.8$, DF = 1, $P = 0.09$) between treatments (fig. 1, control: $N = 35$, and caffeine: $N = 23$ parasitoids of the next generation).

Discussion

Our results confirm the historical observations that alkaloids and other secondary metabolites can be involved in plant–herbivore–secondary consumer tritrophic interactions, in addition to directly affecting plant–herbivore and plant–pollinator relationships (Campbell and Duffey, 1979; Ode, 2019). On this point however, previous work has focused on a limited set of secondary plant metabolites, mostly acutely toxic compounds of specific plant families, and some molecules such as caffeine have been understudied within the context of host–parasitoid interactions. We report a caffeine effect in a cosmopolitan generalist herbivore

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Table 1. Life-parameter table and statistical results of Cox model (longevity) or GLMs (total and daily offspring), for *Myzus persicae* fed on either artificial diet alone (control) or added to 0.1 mg ml^{-1} caffeine

Parameter (mean ± SEM)	Control	Caffeine (0.1 mg ml^{-1})	*t*-value or *z*-value	*P*-value
Longevity (days)	18.8 ± 1.9	13.2 ± 1.7	2.1	<0.05
Total offspring	27.8 ± 4.4	16.1 ± 3.9	1.9	<0.05
Daily offspring	1.9 ± 0.2	1.6 ± 0.3	1.1	0.28

Mean daily offspring number is provided for the reproductive period. $N = 20$ female aphids per treatment. DF = 1 for all tests.

and parasitoid species, in which no particular resistance, detoxification or sequestration mechanisms to caffeine have been reported. *Myzus persicae* feeds on many plant families, and *A. matricariae* attacks many aphid genera (Hullé *et al.*, 2020), so they both could be exposed to caffeine, although at relatively low concentrations because these species are not related to tea or coffee crops. This study model opens interesting perspectives to understand both how caffeine affects host–parasitoid interactions, and how this molecule may be used in the future for the manipulation of insects in biological control strategies.

We observed a diminution of aphid longevity, therefore reducing their reproductive period and total progeny, but not their fecundity, as mean daily offspring production was not affected. Aphid defence behaviour (kicking) was not affected by caffeine intake, suggesting that they keep responding to environmental stimuli such as visual cues of a parasitoid approaching, or conspecific alarm pheromones. In parasitoids, we initially hypothesized that both the behaviour of the parental generation, and the survival and traits of the offspring generation would be influenced by caffeine intake by the host. However, we observed that parasitoid behaviour (host handling time and aphid acceptance rate) was not affected by the caffeine treatment, at the tested dose. It is possible that aphids feeding on even higher concentrations of caffeine would be rejected by parasitoids after antennal or ovipositor contact (allowing detection of chemical cues). However, it would require the parasitoid to be able to detect caffeine within the host prior to oviposition, for which we have no evidence.

Nevertheless, the decision made by the parental generation to oviposit in a host fed on caffeine diet affected the following generation of parasitoids. The lower parasitism rates recorded in aphids exposed to caffeine intake, but similar emergence rates, as also supported by dissection results, are indicative of lower success and higher mortality primarily of early parasitoid developmental stages (egg to prepupa, before mummy formation), which may be due to caffeine toxicity (Mustard, 2020). Of course, the caffeinated diet of the host may still affect parasitoid pupa formation to some extent, and additional deaths may occur in parasitoids at this critical stage of metamorphosis. We finally report an effect of aphid diet on parasitoid adult longevity, a fitness indicator, either because they parasitize low-quality aphids, or because they are directly affected by caffeine when they feed on aphid tissues. Parasitoids are likely at risk of toxicity transfer from their host, as shown, for example, for the snowdrop lectin entomotoxin transferred from the diet of *M. persicae* to tissues of *Aphidius ervi* (Hymenoptera: Braconidae) endoparasitoid pupae (Couty *et al.*, 2001). The next steps of the work will be to perform LC-MS or HPLC analyses (e.g., Kim *et al.*, 2011; Mendes *et al.*, 2019) to try detecting if caffeine – or its main metabolites – has been transferred from aphid to parasitoid tissues. More generally, host quality (size, age, nutritional content, immune defences) is crucial for proper parasitoid development and survival (Harvey, 2000), but can be altered by the plant quality (Harvey and Gols, 2018). When feeding caffeine directly to parasitoids in their nectar diet, we observed that their longevity decreased (Tougeron *et al.*, unpublished data). It is thus likely that what we observed in the present study can arise from direct effects on the parasitoid larvae being surrounded by toxic compounds in the host, and not only due to aphids being less suitable hosts, having been poisoned by the alkaloid intake (Gols, 2014).

Although the aim of our study was not to mimic the presence of caffeine as it occurs in natural environments, defining a concentration to test in the lab was not straightforward. Indeed, caffeine concentrations vary a lot among species of the same genera and among varieties of the same species (Wright *et al.*, 2013; Pham *et al.*, 2019), and each plant organ has variable concentrations (Kretschmar and Baumann, 1999; Dado *et al.*, 2019). Alkaloid levels in the xylem or phloem sap of most plant species have rarely been quantified (Mazzafera and Gonçalves, 1999), and as concentrations may differ between leaf tissues and sap, it is possible that their effects could vary between leaf consumers and sap-feeding animals (Mazzafera and Gonçalves, 1999; Gonthier *et al.*, 2011; van Breda *et al.*, 2013). More generally, studying caffeine – and other alkaloids – concentrations in more plant species and in various organs is needed. Caffeine has been detected at the concentrations of ~0.25 and 0.10 mM in floral nectar of *Coffea canefora* and *Coffea liberica*, respectively, and the concentration was 0.02 mM in *Citrus paradisi* (Wright *et al.*, 2013). Therefore, the tested concentrations of caffeine in our study (0.1 mg ml^{-1}, 0.5 mM) were higher than those typically found in nature, but we tested within the millimolar range in which most experimental studies have found caffeine effects on both vertebrates and invertebrates (Nikitin *et al.*, 2008; Mustard, 2014; Thomson *et al.*, 2015).

Myzus persicae is a generalist herbivore and some strains have expanded their host range to include the lupine, *Lupinus angustifolius* (Fabales: Fabaceae), and have become more resistant to the lupine-specific alkaloid lupanine than non-adapted lineages (Cardoza *et al.*, 2006). Lineages that have expanded their host range to include tobacco also often have elevated nicotine tolerance (Zepeda-Paulo *et al.*, 2010), due to the production of detoxifying enzymes (Ramsey *et al.*, 2014). *Myzus persicae* is active on *Citrus spp.* trees (Ghosh *et al.*, 2015) and on other caffeine-producing plant species, but the extent to which they are actually exposed to caffeine on these plants, and how strains could adapt resistances to caffeine remains to be studied. Ramsey *et al.* (2014) reported that a low concentration (0.01 mM) of nicotine stimulated the fecundity of the nicotine-tolerant *M. persicae* strain, showing hormetic effects that were also reported in bees (Cutler and Rix, 2015). In our study on caffeine, the 5 mM dose was lethal for the strain of *M. persicae* that we used, while 0.5 mM allowed aphids to survive, although with fitness costs. Lower doses of caffeine might be necessary to unravel any hormesis effect on this aphid species. In any case, further studies should focus on assessing the resistance threshold of herbivorous insects and their natural enemies to different types of alkaloids, and also determining variations in resistance between insect species and lineages. In the context of host–parasitoid evolutionary arming race, it is crucial to understand the relative costs and benefits of exposure to plant-secondary metabolites, for each of the interacting species (Gols, 2014), which could depend on the concentration and on the nature of the secondary metabolite.

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Table 2. Behavioural parameter table and statistical results of GLMMs, for ten *Aphidius matricariae* females per treatment, each exposed to 15 *Myzus persicae* fed either on artificial diet alone (control) or added to 0.1 mg ml^{-1} caffeine

Parameter (mean ± SEM)	Control	Caffeine (0.1 mg ml^{-1})	*t* or *z*-value	*P*-value	*N*
Acceptance rate (%)	73 ± 4	68 ± 5	0.63	0.53	207
Parasitism rate (%)	54 ± 7	38 ± 4	1.75	<0.05	144
Emergence rate (%)	84 ± 10	80 ± 10	0.48	0.63	64
Handling time (s)	29.6 ± 3.0	25.1 ± 2.4	0.55	0.58	207
Kicking occurrence	1.3 ± 0.2	1.1 ± 0.2	0.70	0.48	207

DF = 1 for all tests. *N* represents the total number of aphids on which each parameter was evaluated.

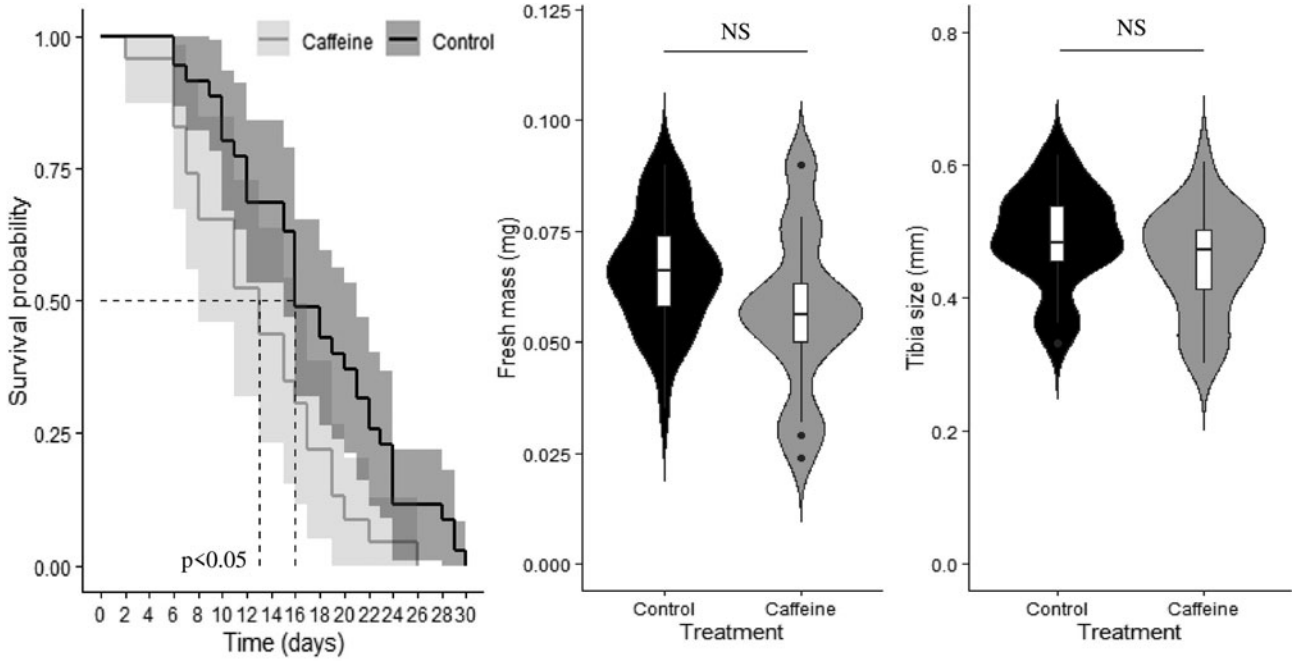

Figure 1. Survival curves (±95% CI), fresh mass and mean tibia size of *Aphidius matricariae* parasitoids of the next generation which developed on aphids that had either fed on control artificial diet (black) or on artificial diet with 0.1 mg ml^{-1} caffeine (grey). Dotted lines represent 50% parasitoid survival. *N* = 35 (control) and 23 (caffeine). NS, not significant.

To conclude, using a host-parasitoid insect model, we described how caffeine, a plant secondary metabolite overlooked in non-coffee or tea plant systems, has detrimental effects on two trophic levels. It highlights the importance of such molecules not only regarding plant–animal interactions, but also regarding food-web and ecosystem functioning. The effects of caffeine on insect circadian activities, behaviour and physiology have been extensively studied (Damon, 2000; Mustard, 2014; Keebaugh *et al.*, 2017), but how they translate to species interaction and community-level ecological effects, for example, via sleep disruption, remains overlooked (Tougeron and Abram, 2017). Of course, the study of host–parasitoid interactions in coffee production systems is also interesting, because caffeine concentrations are high and herbivores have developed detoxification mechanisms (Infante, 2018), and because not all parasitoids have proven successful to control these phytophagous insects (Jaramillo *et al.*, 2006). Manipulation of caffeine-producing plants for pollination, or for pest control by boosting the predatory activity of natural enemies or by increasing plant resistance are interesting perspectives (Kim *et al.*, 2011; Cardoso *et al.*, 2014; Thomson *et al.*, 2015), but one first needs to assess potential trade-offs with the survival of upper trophic levels and other organisms in the interacting network.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485321000687

Data. Raw data can be found in the Supplementary material file.

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Author contributions. KT conceived the study, designed the experiment, collected and analysed data and wrote the manuscript. TH secured funding and revised the article.

Conflict of interest. None.

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