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Influence of temperature on patch residence time in parasitoids: Physiological and behavioral mechanisms.

Running title: Influence of temperature on patch residence time

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ABSTRACT.

1. Patch time allocation has received much attention in the context of optimal foraging theory, including the effect of environmental variables. We investigated the direct role of temperature on patch time allocation by parasitoids through physiological and behavioral mechanisms and its indirect role via behavioral defences of the hosts.

2. We compared the influence of foraging temperature on patch residence time between an egg parasitoid, *Trichogramma euproctidis*, and an aphid parasitoid, *Aphidius ervi* that attacks hosts able to actively defend themselves.

3. Patch residence time decreased with an increase in temperature in both species. The increased activity levels with warming, as evidenced by the increase in walking speed, partly explained these variations but other mechanisms were involved. In *T. euproctidis*, the ability to externally discriminate parasitized hosts decreased at low temperature, resulting in a longer patch residence time. For *A. ervi*, we observed that aphids frequently escaped at intermediate temperature and defended themselves aggressively at high temperature but displayed few defence mechanisms at low temperature. These defensive behaviors resulted in a decreased patch residence time for the parasitoid, and partly explained the fact that *A. ervi* remained for a shorter time at the intermediate and high temperatures than at the lowest temperature.

Keywords: patch time allocation, host-parasitoid interaction, climate, discrimination ability, sex allocation, anti-predator behaviors.

INTRODUCTION

Resources such as food, mate or hosts are usually heterogeneously distributed in the environment and their exploitation requires time. One of the main decisions an organism faces when foraging is how to manage residence time between visited patches in order to maximize fitness yield (Wajnberg, 2006). Patch time optimization received much attention in the context of optimal foraging, and was first addressed by Charnov (1976) in his Marginal Value Theorem (MVT). The main assumption of the MVT is that a forager should leave a depleted patch when the current rate of gain falls below the average rate it would come across in the environment, thus maximizing the long term rate of fitness gain. This model is one of the most influential in behavioral ecology but it has been subjected to criticism as it assumes that foragers are omniscient, and does not take into account proximate behavioral mechanisms involved in patch time allocation (van Alphen & Bernstein, 2008).

Insect parasitoids have been widely used as model organisms to study patch time allocation as their hosts are often patchily distributed and their foraging behavior directly influences progeny production. Over the years, many authors investigated behavioral mechanisms involved in patch time allocation (e.g., Waage, 1979; Driessen *et al.*, 1995) and have shown that parasitoids can adapt patch residence time to a wide variety of environmental variables (for a review, see Wajnberg, 2006), among them biotic factors such as presence of competitors (Wajnberg *et al.* 2004; Goubault *et al.*, 2005; Le Lann *et al.*, 2010) and host kairomones (Li *et al.*, 1997; Louâpre & Pierre, 2012) or abiotic factors such as photoperiod during development (Roitberg *et al.*, 1992) and barometric pressure (Roitberg *et al.*, 1993).

Despite the considerable influence of temperature on life history, physiological and behavioral traits of insects (Huey & Kingsolver, 1989; Nylin & Gotthard, 1998; Angilletta *et al.*, 2004), its influence on patch time allocation has rarely been addressed. Temperature may directly affect patch residence time through physiological or behavioral mechanisms. Firstly, walking speed, search rate, handling time and related behaviors are known to change with temperature in ectotherms because of its influence on metabolic rate (see the Metabolic Theory of Ecology, Brown *et al.*, 2004; Moiroux *et al.*, 2012). Usually, walking speed and search rate follow a hump-shaped relationship with temperature in insects (Sentis *et al.*, 2012). It is thus likely that patch residence time would vary with temperature as a direct consequence of its influence on the speed of movements and behaviors. Secondly, behavioral mechanisms may have been selected in response to temperature. To our knowledge, only Amat *et al.* (2006) have convincingly addressed this issue in a parasitoid. They observed that a drop in temperature before foraging increased patch residence time and that this environmental cue was more important than foraging temperature itself. The authors proposed that thermal variations may be perceived as a signal of unfavourable weather conditions and that searching for a new patch would increase mortality risk or physiological costs. Finally, multi-objective behavioral mechanisms related to patch time allocation may be adopted by animals in response to temperature. Wajnberg (2012) clearly showed that sex allocation influenced patch time allocation in the egg parasitoid *Trichogramma chilonis* as laying a son did not change its patch residence time while laying a daughter decreased its patch time allocation. Considering that haplodiploid parasitoids can adapt sex allocation to their foraging temperature (Moiroux *et al.*, 2014), we may thus expect that females would also change their patch time allocation according to sex allocation.

Temperature may also indirectly influence patch time allocation via its effect on the nature and frequency of host defences (e.g., Miyatake *et al.*, 2008; Ma & Ma, 2012). For example, when exposed to alarm pheromone, pea aphids *Acyrthosiphon pisum* are less likely to drop from the feeding site to the ground when the environment is hot and dry than when it is more benign (Dill *et al.*, 1990). Temperature may thus affect patch time allocation through its influence on host defences since parasitoids can change patch time allocation as a response to host defence behaviors. This was for example observed for the ichneumonid *Diadegma semiclausum*, which remains longer on a patch when attacked hosts temporally leave (Wang & Keller, 2003).

The aim of this study was to investigate both the direct and indirect effects of temperature on patch time allocation in parasitoids. We compared the influence of temperature on patch residence time of two different parasitoid species exploiting hosts that exhibit or not defensive behaviors: (i) the parasitoid *Trichogramma euproctidis* Girault (Hymenoptera: Trichogrammatidae), which attacks eggs of the lepidopteran species *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and for which we were able to distinguish male egg deposition from female egg deposition by observation (Moiroux *et al.* 2014) to study influence of sex allocation, and (ii) the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Aphidiinae) which attacks potato aphids, *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae), that are able to actively defend themselves. For this parasitoid species, we were unable to measure sex allocation. Both parasitoid species were maintained at their optimal temperature and placed at low and high temperatures close to their thermal limits before foraging. Their patch residence time and foraging behaviors were then recorded. We also measured thermal variations in walking speed for *T. euproctidis* as a proxy of physiological changes, while data were already available from Gilchrist (1996) for *A. ervi*. The nature and frequency of defensive behaviors displayed by aphids attacked by *A. ervi* was also measured. We expected that patch time allocation should be directly influenced by temperature for both species as a consequence of changes in walking speed, and indirectly via its effect on sex allocation in *T. euproctidis* (Moiroux *et al.* 2014) and via its effects on behavioral defences of hosts in *A. ervi*.

MATERIALS & METHODS

Trichogramma euproctidis

Trichogrammatidae wasps are small endoparasitoids of eggs of several lepidopteran species. The *T. euproctidis* strain used in this study originates from individuals sampled in Egypt and was maintained at 24 ±1˚C, 50% RH, and LD 16:8 h on cold-killed eggs of *Ephestia kuehniella*.

Patch residence time. Parasitized eggs were taken from the *T. euproctidis* colony and isolated in 300µL Beem® polyethylene capsules with a drop of honey. The next morning, newly emerged females were mated once with a virgin male, stored at 24˚C, and transferred to a climate room at either 14, 24 or 34˚C one hour before observation. The first and last temperatures are close to the species thermal limits but do not cause any deleterious effects on walking activity (see results on walking activity). After this acclimatization period, females were offered, at the same temperature, 20 cold-killed *E. kuehniella* eggs, placed side by side in 4 lines of 5 eggs on a filter paper disc. The experimental arena was delimited by the plastic end piece of a camera (Ø20 mm) covered with fluon to prevent females from climbing up the side. The number and duration of the following behaviors were recorded for 30 females per temperature with a camera (Dino-lite Pro AM413ZT, x 60) and analyzed using The Observer XT software (Noldus, Wageningen, The Netherlands): entering the patch, leaving the patch, walking, host feeding, drumming the host with antennae, drilling, egg male deposition, egg female deposition, host rejection, and resting. Egg deposition was distinguished from drilling by positioning of the abdomen, which was perpendicular to the host when drilling and parallel during egg deposition. Egg female deposition was distinguished from egg male deposition by the presence of a pause during oviposition sequence (Suzuki *et al.* 1984). Host rejection consisted of drilling not followed by egg deposition. Walking was recorded when a female was moving and did not mark a pause while drumming hosts with antennae was noted when a female was on an egg and touched it repeatedly with its antennae. We did not consider host rejection with antennae, as it was sometimes very fast, especially at high temperature, and difficult to distinguish from walking on eggs. Patch residence time was measured from the time the female entered the patch until she remained inactive for more than 30 minutes or walked off the patch for more than 3 minutes at 34˚C, 4min45s at 24˚C and 7min30s at 14˚C. These durations are proportional to speed variation due to temperature measured in the experiment on walking speed described below. Additional analyses were performed considering a similar threshold, i.e. females walked off the patch for more than 3 minutes for the three temperatures, and the results led to the same conclusion in both cases.

Walking speed. Newly emerged (<24 h), unfed females were placed individually in an empty Petri dish (Ø92 mm) on a light table at 10, 15, 20, 25, 30 or 35±1˚C. The movement of 15 females per temperature was video-recorded (Camera Panasonic WV-BD400) for 300 seconds and then analyzed with a program developed by Vigneault *et al.* (1997). The contrast between individuals and the light table was used to track females and the program calculated the mean walking speed (mm/sec), deduced from the distance traveled in 300 seconds minus duration of stops.

Aphidius ervi

Insect colonies. *Aphidius ervi* is a solitary koinobiont parasitoid of several aphid species. A few weeks prior to the tests, an *A. ervi* colony was established with individuals bought from BioBest Canada (Leamington, Canada). Parasitoids were reared in cages on the potato aphid *M. euphorbiae* at 20±1°C, 60±10% RH, 16L: 8D photoperiod. The aphid colony was initiated with individuals collected from potato fields in the vicinity of Québec City, Canada. This colony was maintained on potato plants, *Solanum tuberum* Linnaeus (Solanales: Solanacae), cultivar "Norland" under the same conditions.

Patch residence time.

Parasitized aphids were reared at 20±1°C on potato plants and isolated in gelatine capsules once they mummified. After emergence, each female parasitoid was kept with a male at 20±1°C for 24 hours in a Petri dish containing moistened cotton and honey. This period allowed females to mature eggs (Le Lann *et al.*, 2010). The next day, their foraging behavior was observed in climate chambers at 12, 20 or 28°C. The first and last temperatures are close to *A. ervi* thermal extremes but do not induce any lethal effects or deleterious effects on walking activity (according to Gilchrist, 1996). One hour before the observation, a potato leaf (ø 4cm approximately) was taped on a piece of paper and 60 apterous aphids were placed on it, 15 from each larval instar (L1 to L4). A range of different instars were exposed to *A. ervi* females because their defensive behaviors change with age (Barrette *et al.*, 2010). Prior to the test, parasitoid females were acclimatized to the experimental temperature for one hour. Females were then introduced in the experimental arena (ø 26cm, h 8.5 cm) and the following behaviors were recorded with a camera (Panasonic WV-BD400) on 25 females per temperature and analyzed with The Observer XT software (Noldus, Wageningen, The Netherlands): entering the patch, leaving the patch, walking, successful oviposition, failed oviposition, host rejection and resting. A successful oviposition was recorded when the parasitoid abdomen came into contact with the aphid while a failed oviposition was recorded when a parasitoid initiated an attack but failed to touch the aphid with its abdomen because of aphid behavioral defences. Host rejection was recorded when a female parasitoid came into contact with a motionless aphid and did not attack it. Both the number and duration of these behaviors were recorded, taking into account host instar which was evaluated visually by aphid body size. Patch residence time was measured from the time the female entered the patch until she left it for more than one minute at 28°C, 2 minutes at 20°C and 6 minutes at 12°C or stopped any activity for more than 15 minutes. These durations are proportional to speed variations with temperature measured by Gilchrist (1996). Additional analyses were performed considering a similar threshold, i.e. females left the patch for more than 1 minute for the three temperatures, and the results led to the same conclusion in both cases.

During this test, we also recorded aphid anti-predator behaviors, i.e. kicking, body jerking, or escaping from the patch. Kicking was recorded when an aphid extended one of its leg, usually a hind leg, and shook it up and down. Body jerking consisted of spasmodic horizontal movements of the abdomen. Escaping the patch was recorded when an aphid walked away after an attack and left the potato leaf. These behaviors were only recorded when aphids were attacked by the parasitoid.

For this parasitoid species, we were not able to study the influence of sex allocation on patch residence time. In *Aphidius* parasitoids, sex allocation can not be determined by observation. In his paper on multi-objective behavioural mechanisms, Wajnberg (2012) observed the sex of emerging parasitoids to assess sex allocation during oviposition. However the temperature is known to affect sex ratio via physiological constraints on egg fertilization in haplodiploid parasitoids (Moiroux *et al.* 2014), resulting in differences between sex allocation and realized sex ratio. Observation of the sex of emerging parasitoids was thus not reliable to evaluate sex allocation in our experiment.

Statistical analyses.

Patch residence time was analyzed using a Cox proportional hazard model (Cox, 1972; Tenhumberg *et al.*, 2001). Such model is classically used to analyse censored data such as patch duration (Wajnberg *et al.*, 1999). It determines a patch-leaving tendency for a foraging parasitoid, *i.e.* the probability per unit of time that a female leaves the patch, providing that she is still on it. It is formulated in terms of hazard ratio; a hazard ratio above one indicates an increase in the female's patch-leaving tendency and a hazard ratio below one indicates a decrease in the patch-leaving tendency. For *T. euproctidis*, temperature was considered as fixed factor, while the number of male ovipositions, number of female ovipositions, number of host rejections, number of host feeding, resting periods and temporary exit from the patch were included as time-dependant covariates. The interaction between temperature and the number of male and female ovipositions or with host rejections were also considered. For *A. ervi*, temperature, considered as a fixed factor, total number of ovipositions, number of ovipositions per host larval instar, number of failed ovipositions, number of host rejections, number of aggressive behaviors (i.e., kicking + jerking) and of escaping aphids, number of temporary exits from the patch were included in the model, as were interactions between temperature and the number of successful or failed ovipositions. In both Cox models, the adequacy of the model was assessed by plotting deviance residuals (Therneau *et al.*, 1990). The assumption that hazard rates were proportional for different values of covariates was also checked following Collett (1994) and Wajnberg *et al.* (1999).

In addition, the influence of temperature on the total number of ovipositions, host rejections, host feeding, resting and grooming periods for *T. euproctidis* were analysed using Generalized Linear Models (GLM) with a Poisson error distribution. Data on sex allocation have been previously published in Moiroux *et al.* (2014). The influence of temperature on the proportion of time devoted to each of these behaviors and time spent walking and drumming hosts with antennae was analyzed with a GLM with a Binomial error distribution. For *A. ervi*, the influence of temperature on the number and duration of these behaviors is described in Moiroux *et al.* (2015). The number of kicking, jerking and escaping aphids was compared using a GLM with a Poisson error distribution.

Finally, we fitted an exponential curve to the data to investigate the effect of temperature on *T. euproctidis* walking speed.

All statistical analyses were carried out using R software version 2.14.1 (R Development Core Team, 2011).

RESULTS

Trichogramma euproctidis

Patch residence time. Temperature, the number of male and female ovipositions, and the interaction between temperature and the number of hosts rejection significantly influenced the patch residence time of *T. euproctidis* (Table 1) while host rejection, host feeding, the number of temporary exits and resting periods did not. The tendency to leave the patch increased with temperature (Figure 1) and following a male and female oviposition (i.e. hazard ratio > 1, Table 1). It should be noted that laying a son or of daughter increased patch leaving tendency by very close factors (respectively 0.558 ± 0.166 vs 0.576 ± 0.077). Host rejection increased the tendency to leave the patch at intermediate and high temperature but it decreased this tendency at low temperature (Figure 2). We did not observe any significant influence of the interaction between temperature and the number of male (Temperature(24): $\beta = -0.243 \pm 0.263$, $p = 0.666$; Temperature(34): $\beta = -0.864 \pm 0.473$, $p = 0.167$) or female ovipositions (Temperature(24): $\beta = -0.047 \pm 0.217$, $p = 0.827$; Temperature(34): $\beta = -0.058 \pm 0.220$, $p = 0.791$) on patch leaving tendency.

Temperature did not influence the total number of ovipositions while the number of host rejections was significantly higher at 14˚C than at 24 or 34˚C, and females host-fed more at 34˚C (Table 2). Parasitoid females laid more sons at high temperature than at other temperatures while no difference in sex allocation was observed between low and intermediate temperatures (Moiroux *et al.*, 2014). Females foraging at 14°C spent proportionally less time laying eggs (df = , $p < 0.001$), walking (df = , $p = 0.021$) and feeding on hosts (df = , $p = 0.014$) than females tested at 24 and 34˚C, but spent significantly more time drumming host eggs with their antennae (df = , $p = 0.003$) and rejecting hosts (df = , $p < 0.001$) (Figure 3).

Walking speed. Walking speed of *T. euproctidis* increased significantly with increasing temperature (F = 118.7, df = 1, 87, $p < 0.001$; Figure 4).

Aphidius ervi

Patch residence time. Patch residence time was significantly influenced by temperature (Figure 1B), the number of total successful ovipositions, the number of escaping aphids and the number of aggressive aphids, but not by other time-dependent covariates or the interactions between temperature and any of the time-dependent covariates (Table 3). Females remained longer in the patch after an oviposition and left earlier with increasing temperature and the number of aggressive or escaping aphids.

Aphids. The number of kicks performed by *M. euphorbiae* (t = 7.718, df = 73, $p < 0.001$) and the number of aphids jerking (t = 3.146, p = 0.002) were significantly influenced by the foraging temperature. Aggressive defensive behaviors were more frequent at 28°C and less frequent at 12°C (Figure 5). Foraging temperature significantly influenced the number of aphids escaping from the patch when attacked (t = 3.914, $p < 0.001$). Escape behavior was the most frequent in aphids attacked at 20°C (Figure 5).

DISCUSSION

We investigated the influence of temperature on patch time allocation in two parasitoid species, considering its direct effects through physiological or behavioral mechanisms and its indirect effects via behavioral defences of hosts. Our main results are that: (1) patch residence time decreased as temperature increased, together with an increase in walking speed for both *T. euproctidis* and *A. ervi*, (2) patch time allocation was influenced by an interaction between temperature and the number of host rejections in *T. euproctidis*, and (3) the nature and number of aphid behavioral defences changed with temperature and influenced patch residence time in *A. ervi*.

Patch time allocation. *Trichogramma euproctidis* females used a decremental mechanism when exploiting host patches as each oviposition increased the patch leaving tendency. This decremental mechanism is consistent with the results of a comparative analysis conducted on 17 species of *Trichogramma* by Wajnberg *et al.* (2003). An increased tendency to leave a patch after oviposition is expected when resources are uniformly distributed over patches (Iwasa *et al.*, 1981). However, this hypothesis cannot be confirmed as no reliable distribution data are available for hosts of *Trichogramma* parasitoids (Wajnberg *et al.*, 2003).

On the contrary, patch time allocation increased due to an oviposition in *Aphidius ervi*. This incremental mechanism (Waage, 1979) has been observed in many parasitoid species (for a review, see Wajnberg 2006) including a close and sympatric species, *Aphidius rhopalosiphi* (Outreman *et al.*, 2005). Such behavior is expected when organisms face a large variance in patch quality (Iwasa *et al.*, 1981), as observed for aphid colonies in the field (e.g., Ward *et al.*, 1986).

Patch time allocation and temperature. In both parasitoid species, patch residence time decreased sharply when temperature increased. This result contrasts with a study by van Roermund *et al.* (1994) who did not observe any influence of temperature on patch time allocation in the whitefly parasitoid *Encarsia formosa*. It should be noted that in this last paper, parasitic wasps were tested on patches without hosts. These unnatural conditions likely explain the absence of parasitoid response to temperature.

An increase in female parasitoid activity with warming, as evidenced by the increase in walking speed in *T. euproctidis* (this study) and *A. ervi* (Gilchrist, 1996), would likely modulate patch residence time. The higher the temperature, the faster females perform the component behaviors that make up foraging up to an optimum, as predicted in the context of the Metabolic Theory of Ecology (Brown *et al.*, 2004). In *T. euproctidis* and *A. ervi*, walking speed increased on average 1.7 times between 24 and 34°C and 20 and 28°C, respectively, while patch residence time and duration of the different behaviors recorded (antennation, drilling, egg deposition) decreased 1.7 times. This result suggests a simple physiological change associated with temperature. However, while the walking speed increased 1.6 times from 14 ˚C to 24 ˚C and 2.5 times from 14 ˚C to 34 ˚C in *T. euproctidis*, patch residence time decreased 2.6 and 8.7 times, respectively. In *A. ervi*, walking speed increased 4.1 times from 12 to 20°C and 6.9 times from 14 to 34°C while patch residence time decreased 5 and 8.6 times, respectively. These differences suggest that in both species walking speed alone does not explain the increase in patch time allocation at the lowest temperature. From our results, different mechanisms may be involved for each species: a decrease in external discrimination ability in *T. euproctidis* and variations in nature and frequency of host defensive behaviors in *A. ervi*. These mechanisms were observed in parasitoids and hosts taken from the lab but field populations living under changing environment may respond differently than these strains.

At the lowest temperature, the number of host rejections (i.e. a drilling not followed by egg deposition) by *T. euproctidis* females was higher than at other temperatures. Parasitized eggs were thus mainly rejected after perforation of the chorion by the ovipositor but females were unable to reject them while drumming with their antennae, unlike at 24 or 34˚C. These results indicate that a low temperature during oviposition affected the external discrimination ability of *T. euproctidis* females, which probably contributed to the increased patch residence time recorded at 14°C. Overall, the same number of eggs was parasitized by females across tested temperatures, but it took more time to exploit the patch at low temperature. Several non-exclusive hypotheses can explain the decrease in external discrimination ability. First, the amount or composition of the marking pheromone produced by a female and left on the host after oviposition (Roitberg & Mangel, 1988) may be changed at low temperature, as observed for the marking pheromone produced by beewolf males, *Philanthus triangulum*, to mark their territories (Roeser-Mueller *et al.*, 2010). Secondly, the receptivity of antennae to chemical signals or their integration in the olfactory lobe may also be affected, as suggested for European corn borer males, *Ostrinia nubilalis*, exposed to female sex pheromone at different temperatures (Hilker & McNeil, 2008). An effect of low temperature on the nervous system, especially on learning ability, was also proposed by van Baaren *et al.* (2005) to account for the absence of external discrimination ability in cold-stored females of the mymarid *Anaphes victus*.

This negative effect of low temperature on external discrimination ability probably explains that we observed a significant influence of the interaction between temperature and host rejection on patch residence time in *T. euproctidis*. At intermediate and high temperature, *Trichogramma* females left earlier the patch after host rejection. This decremental mechanism is assumed to be adaptive and has been commonly reported in parasitoids (Wajnberg, 2006) including species from the *Trichogramma* genus (Wajnberg *et al.*, 2003). At low temperature, we observed an incremental mechanism associated with host rejection. This behavioral mechanism has only been reported in one parasitoid species (Nelson & Roitberg, 1995) and is probably the consequence of deleterious effect of temperature on sensory structures in our experiment. *A. ervi* did not suffer as much as *T. euproctidis* from low temperature. It is likely that interspecific variations or local adaptations of reproductive behaviors at low temperature exist since oviposition does not seem to be negatively affected by temperatures close to 0°C in some parasitoids (Meiners *et al.*, 2006). Size differences between the species might for example explain these differences. Due to their small size and thermoregulatory constrains, egg parasitoids might be physiologically more affected by changes in temperature than the larger aphid parasitoids.

We hypothesised that temperature may influence patch time allocation via its effect on sex allocation (Wajnberg, 2012) since in *T. euproctidis*, Moiroux *et al.* (2014) observed that the number of sons laid by a female was greater at high temperature than at low and intermediate temperatures. Laying a son or of daughter increased patch leaving tendency by very close factors. This result contrasts with the paper of Wajnberg (2012) who observed in *T. chilonis* that laying a son did not influence patch residence time while it decreased with oviposition of a daughter. Our result, as well as the absence of interaction between male or female egg deposition and temperature, imply that differences in patch residence time with temperature could not be explained by differences in sex allocation.

In *A. ervi*, the nature and frequency of defensive behaviors displayed by aphids changed with temperature. They frequently defended themselves by kicking and jerking at the highest temperature while they tended to escape at the intermediate temperature. This observation is consistent with results on pea aphids, *Acyrthosiphon pisum,* which are less likely to drop from their feeding site when the environment is hot and dry than when it is more benign (Dill *et al.*, 1990). Aphids may be less prone to leave a leaf when temperature is high as they need more resources to compensate for the increased metabolic rate (Brown *et al.*, 2004) and should actively defend against parasitoids instead of escaping. Aphid aggressive behaviors resulted in an increase in patch leaving tendency for the parasitoid *A. ervi*. This effect may explain why the patch residence time of aphid parasitoids was shorter at 28°C than expected from the change in walking speed when compared to the lowest temperature, at which aphids rarely defended. Since aggressive host behavioral defences increases injury risk for parasitoids, it is likely that the optimal behavior would be to search for another patch with less aggressive hosts. To our knowledge, this is the first experimental evidence that such aggressive behavioral defences influence patch time allocation in insect natural enemies. We also observed that patch residence time decreased with the number of aphids leaving the patch when exposed to a parasitoid. This last result contrasts with observation on the ichneumonid parasitoid *Diadegma semiclausum* that remained longer on a patch when hosts temporally left after attack (Wang & Keller, 2003), waiting for the discovered host to climb back to the leaf (Wang & Keller, 2002). *Aphidius ervi* instead left the patch earlier, probably because females perceived a decrease in patch quality (e.g., Boivin *et al.*, 2004; Outreman *et al.*, 2001). This result may explain why *A. ervi* remained shorter than expected at the intermediate temperature compared to the lowest temperature, since aphids escaped more frequently at intermediate temperature.

Conclusion. Patch time allocation is influenced by several environmental variables, including temperature. We present evidence that foraging temperatures directly influences patch time allocation in parasitoids, mainly through physiological mechanisms, and indirectly through changes in the nature and frequency of host behavioral defences. In the next decades, temperature variance and the frequency of extreme climatic events are expected to increase

(IPCC 2007). From our results, we could predict that some species will be more affected than others, as a change in temperature modified the influence of an host rejection on patch leaving tendency in *T. euproctidis* but not in *A. ervi*. In parasitoid species attacking hosts that actively defend themselves, the influence of the predicted climate change on patch time allocation might be more closely related to the induced changes in host behavioral defences than the direct effect of temperature on the parasitoid itself. This result suggests that both parasitoids and hosts should be considered when investigating the role of climate on patch residence time.

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Table 1. Estimated regression coefficients (β) and their standard errors, and hazard ratios (exp (β)) obtained for covariates of a Cox proportional hazard model relating patch residence time and behaviors observed in *T. euproctidis*.

	β ± s.e.	**exp (β)**	**z**	**p-value**
Temperature(24)	2.189 ± 0.765	8.933	2.862	**0.004**
Temperature(34)	2.654 ± 0.949	14.208	2.797	**0.005**
Male oviposition	0.558 ± 0.166	1.747	3.496	**< 0.001**
Female oviposition	0.576 ± 0.077	1.779	7.407	**< 0.001**
Host rejection	-0.059 ± 0.035	0.942	-1.686	0.091
Temperature(24) : rejection [a]	0.136 ± 0.062	1.146	2.201	**0.027**
Temperature(34) : rejection [a]	0.754 ± 0.184	2.126	4.088	**< .001**

[a] Interaction

615

Table 2. Total number of ovipositions, host rejections, host feeding and resting periods measured on *T. euproctidis* females allowed to parasitize 20 cold-killed *E. kuehniella* eggs at three temperatures. Host rejection refers to a drilling not followed by egg deposition. Mean ± SE.

Temperature (˚C)	**Oviposition**	**Host rejection**	**Host feeding**	**Resting period**
14	18.60 ± 0.23	15.27 ± 1.02	1.62 ± 0.25	0.80 ± 0.19
24	18.63 ± 0.33	3.45 ± 0.14	1.83 ± 0.23	0.40 ± 0.15
34	18.57 ± 0.33	2.17 ± 0.4	2.77 ± 0.50	0.53 ± 0.19
t value (df = 89)	-0.095	-10.660	2.966	-1.255
p-value	0.924	**<0.001**	**0.003**	0.213

Table 3. Estimated regression coefficients (β) and their standard errors, and hazard ratios (exp (β)) for significant covariates of a Cox proportional hazard model relating patch residence time, temperature and behaviors of *A. ervi* females allowed to attack four stages of *M. euphorbiae*.

	β ± s.e.	**exp (β)**	**z**	**p-value**
Temperature(20)	1,817 ± 0.528	6,153	2,823	**0.024**
Temperature(28)	2,344 ± 0.712	10,425	3,046	**0.009**
Oviposition	-0,348 ± 0.104	0,706	-2,370	**0.035**
Aggressive aphids	0,333 ± 0.092	1,395	2,972	**0.016**
Escaping aphids	0,289 ± 0.084	1,335	2,838	**0.031**

FIGURE CAPTIONS

Figure 1. Probability that (A) a female *T. euproctidis* remains on a patch with 20 hosts and (B) a female *A. ervi* remains on a patch with 60 hosts as a function of the time elapsed since it entered the patch at three temperatures.

Figure 2. Graphical representation of the interaction between temperature and the effect of each host rejection in *T. euproctidis*. Hazard ratios (±s.e.) are computed according to the explanation provided by Wajnberg *et al.* (1999)

Figure 3. Proportion of time spent expressing each of the behaviors observed in *T. euproctidis* females allowed to parasitize 20 *E. kuehniella* eggs at three temperatures.

Figure 4. Effect of temperature on the walking speed of *Trichogramma euproctidis* females

Figure 5. Mean number of kicking (A), jerking (B) and escaping (C) aphids attacked by the parasitoid *A. ervi* at three temperatures. Mean ± S.E. Different letters indicate significant differences between temperature for each behavior.

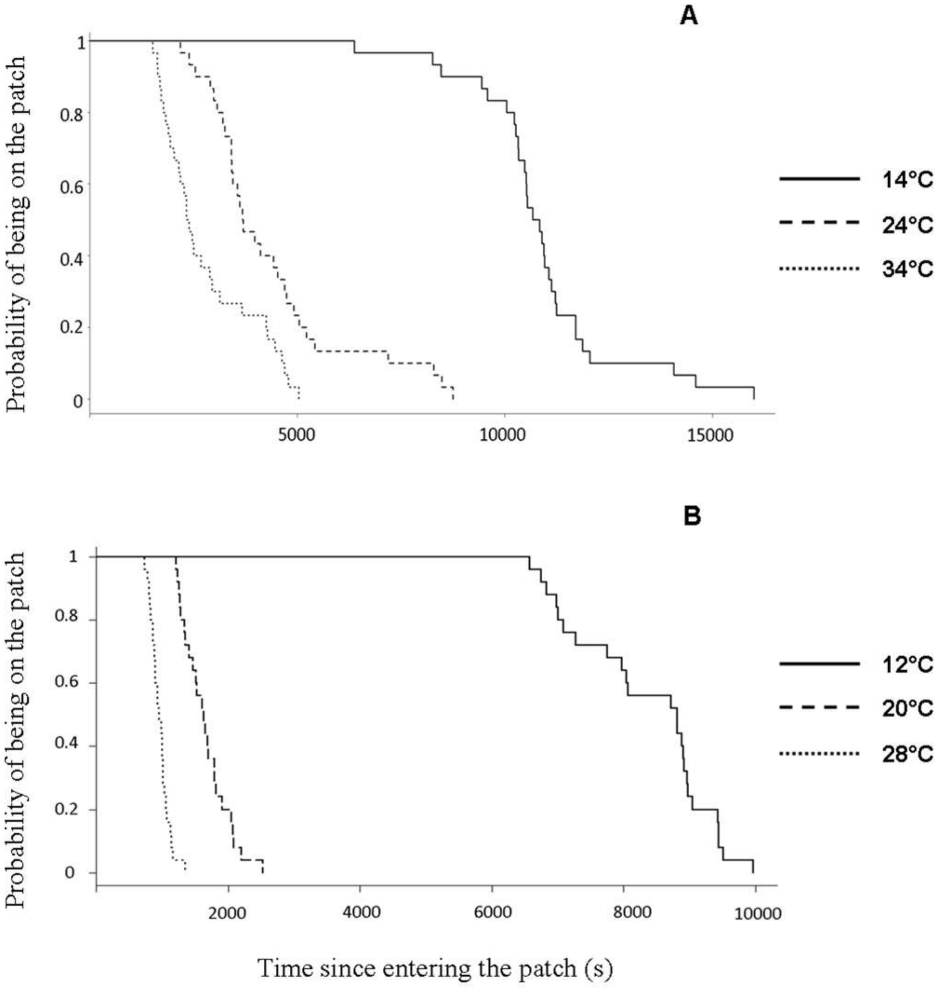
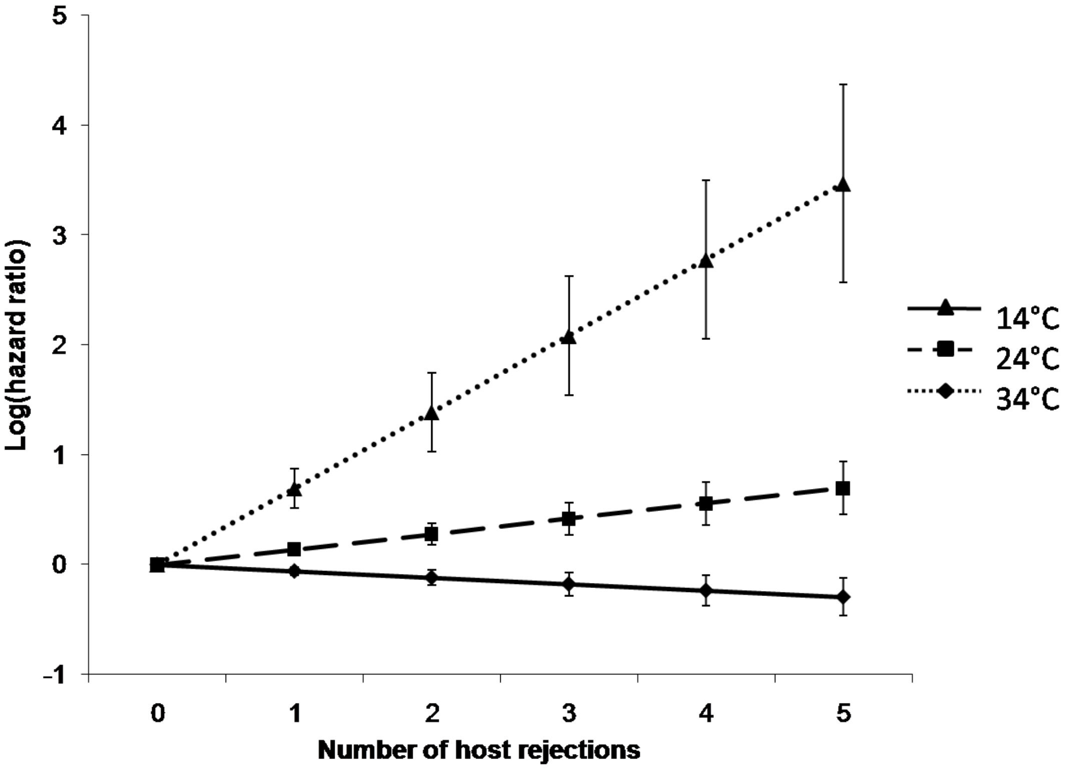

A
Probability of being on the patch
1
0.8
0.6
0.4
0.2
0
5000
10000
15000
14°C
24°C
34°C
B
Probability of being on the patch
1
0.8
0.6
0.4
0.2
0
2000
4000
6000
8000
10000
12°C
20°C
28°C
Time since entering the patch (s)

Figure 1

637



Log(hazard ratio)
5
4
3
2
1
0
-1
0
1
2
3
4
5
Number of host rejections
14°C
24°C
34°C

Figure 2

638

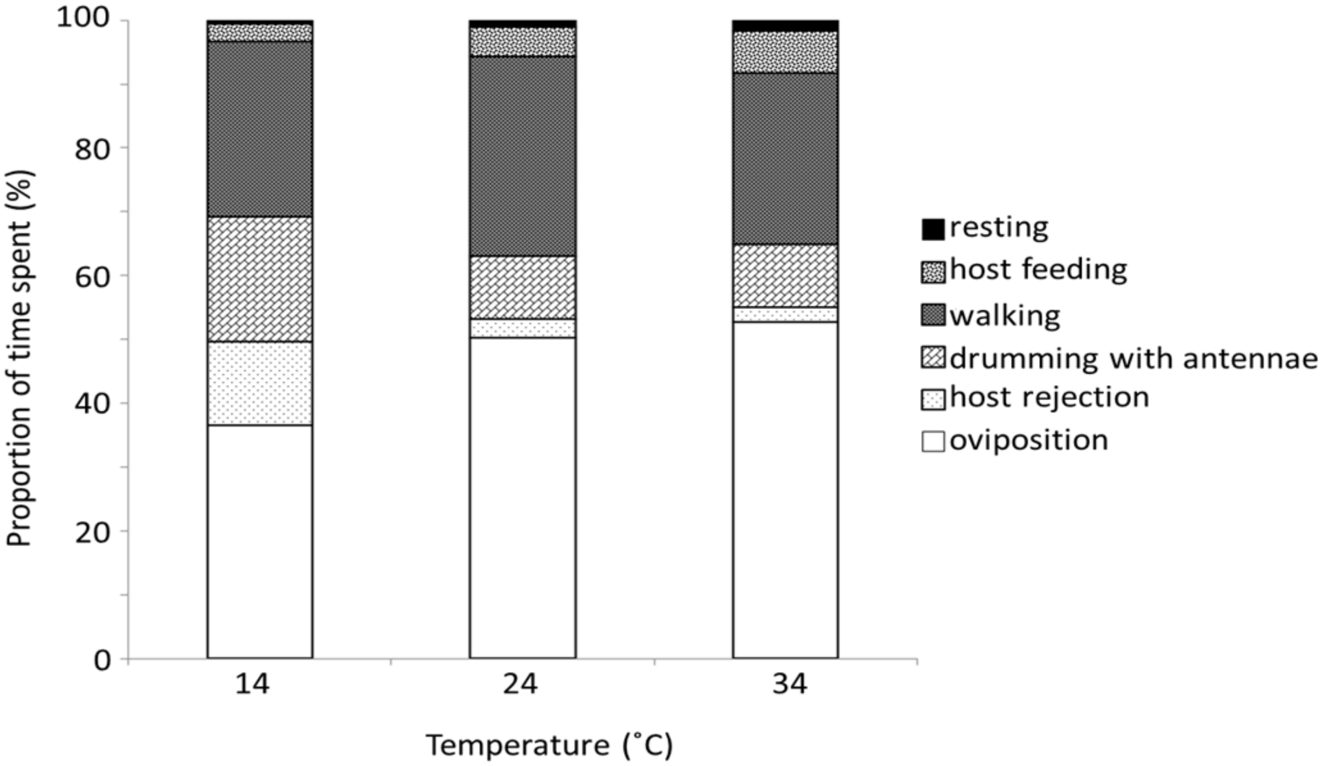

100
80
60
40
20
0
Proportion of time spent (%)
14
24
34
Temperature (˚C)
resting
host feeding
walking
drumming with antennae
host rejection
oviposition

Figure 3

639

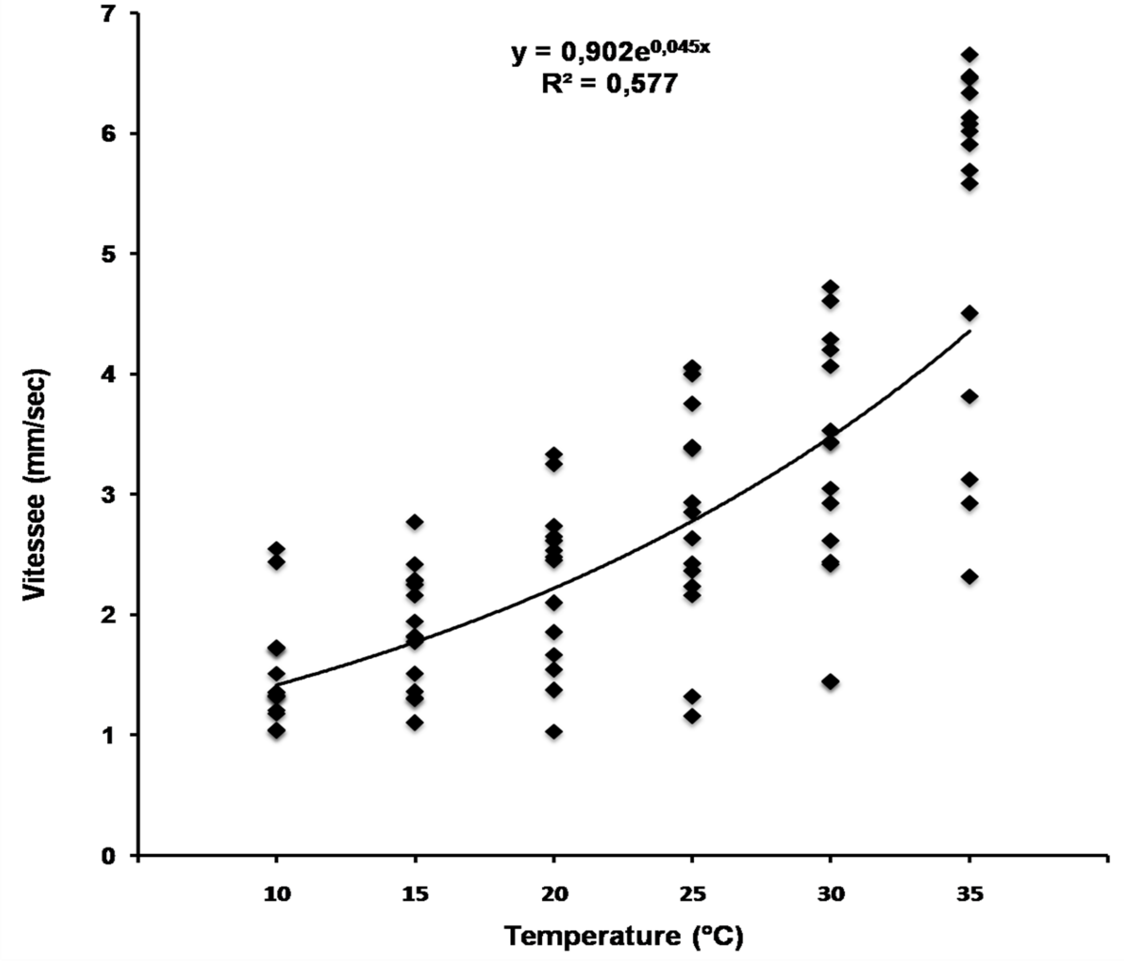

y = 0,902e^0,045x
R² = 0,577
Vitessee (mm/sec)
0
1
2
3
4
5
6
7
10
15
20
25
30
35
Temperature (°C)

Figure 4

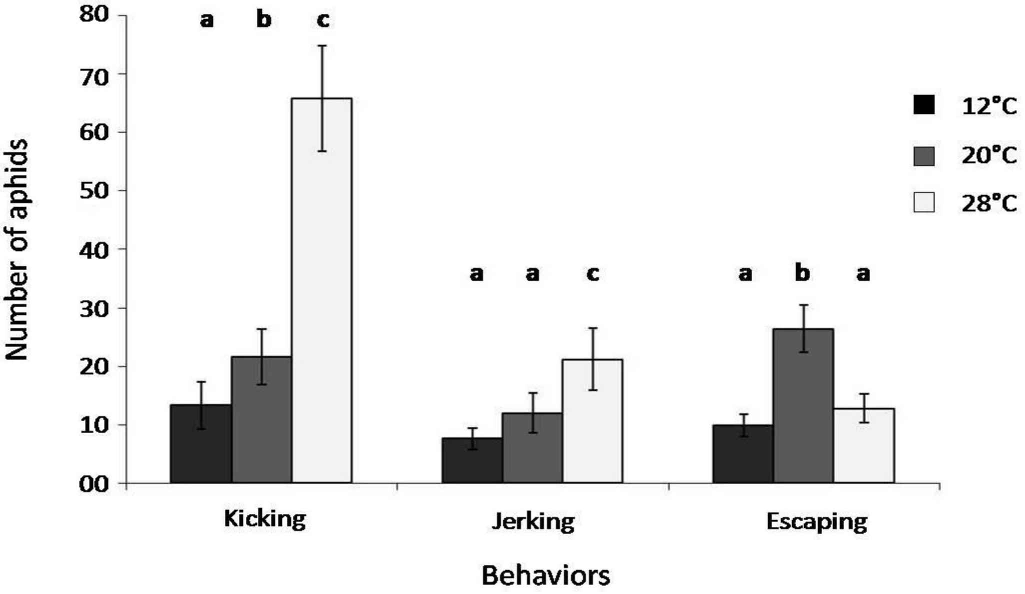

80
70
60
50
40
30
20
10
00
Number of aphids
a
b
c
a
a
c
a
b
a
12°C
20°C
28°C
Kicking
Jerking
Escaping
Behaviors

Figure 5