Thermal activity thresholds of parasitoids *Aphidius avenae* and *Aphidius gifuensis* (Hymenoptera: Braconidae): implications for their efficacy as biological control agents in the same location

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Abstract

Aphid parasitoids are important components in biological control of aphid populations in a variety of crop systems. *Aphidius avenae* Haliday and *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae) are key biological control agents for *Sitobion avenae* (F.) (Hemiptera: Aphididae), which causes serious damage to wheat worldwide. This study aimed to evaluate their parasitic potentials focusing on their thermal biological characteristics in a temperate area. Based on supercooling temperature recording techniques, the freezing point, minimum temperature for walking, and the temperature for activity recovery after chill coma of *A. gifuensis* females (-19.8, -5.1, and 5.9 °C, respectively) were significantly lower than those of *A. avenae* (-18.1, -3.3, and 7.8 °C, respectively). The results showed that *A. gifuensis* had lower temperature tolerance compared with *A. avenae*, illuminating that it could take precedence in suppressing population growth of *S. avenae* when aphid colonies were still small in early spring.

Key Words: thermal tolerance; biocontrol potential; aphid

Resumen

Los parasitoides de áfidos son componentes importantes en el control biológico de las poblaciones de áfidos en variedades de sistemas de cultivo. *Aphidius avenae* Haliday y *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae) son agentes clave de control biológico de *Sitobion avenae* (F.) (Hemiptera: Aphididae), que causa daños graves al trigo en todo el mundo. Este estudio tuvo como objetivo evaluar sus potenciales como parásitos enfocandose en sus características biológicas térmicas en una área templada. Sobre la base de la técnica de grabación del sobre-enfriamiento de temperatura, el punto de congelación, la temperatura mínima para caminar y la temperatura para la recuperación de la actividad después del efecto decoma por frío de hembras de *A. gifuensis* (-19.8, -5.1 y 5.9 ° C, respectivamente) fueron significativamente inferiores a los de *A. avenae* (-18,1, -3,3, y 7,8 ° C, respectivamente). Los resultados mostraron que *A. gifuensis* tenía más baja tolerancia a la temperatura en comparación con *A. avenae*, revelando que podría prevalecer en la supresión del crecimiento de la población de *S. avenae* cuando las colonias de áfidos fueron todavía pequeñas en al principio de la primavera.

Palabras Clave: tolerancia térmica; control biológico potencial; áfido

Aphids are considered as one of the most important pests of wheat worldwide, as they can cause heavy economic damage to wheat both as a phloem feeders and as vectors of plant viruses (van Emden & Harrington 2007; Bragard et al. 2013; Harris & Maramorosch 2014). The extensive use of traditional insecticide has negative effects on the environment, natural enemies, and food safety. Hence, there is an increasing interest in the use of biological control agents to replace chemical pesticides in aphid control programs (Cui et al. 2012; Raymond et al. 2015). Aphid parasitoids are important components in biological control of aphid populations in a variety of crop systems both in greenhouses and field situations (Boivin et al. 2012). Aphidius avenae Haliday and Aphidius gifuensis Ashmead (Hymenoptera: Braconidae), which accounted for about 48% of the total natural enemies in wintergrowing wheat fields in China, are the 2 dominant parasitoid species in suppressing Sitobion avenae (F.) (Hemiptera: Aphididae) population growth (Wang et al. 2008, 2009; Liu et al. 2009; Wang et al. 2011). In a previous survey, we found that *A. gifuensis* occurred earlier than *A. avenae*, and it was the major parasitoid species in winter-wheat fields in early spring (Dong 2012). It is well known that an early appearance of parasitoids in the field will facilitate the build-up of a high ratio of parasitoids to aphids early in the crop growing season, which may help to slow down the initial growth rate of the aphid population, and later suppress the aphid population expansion (Powell 1986; Kindlmann & Dixon 2010; Dong et al. 2012).

Environmental factors, especially temperature, play a critical role in the distribution and occurrence of most ectotherm species (Hughes et al. 2011). Studying the thermal limits of insect activities is the best way to understand how extreme climatic events could affect insect fitness and population dynamics (Terblanche et al. 2007). The occurrence of extreme temperatures, which could directly lead to the mortality of insects such as parasitoids, may become more common in an era of climate change (Hance et al. 2007). In addition, other traits of parasit-

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oids are related to temperature, such as the flight and walking behavior (Jerbi-Elayed et al. 2015), the attack rates on aphids (Romo & Tylianakis 2013), the antennal segmentation and sensilla morphogenesis (Sun et al. 2014), and the percentage of parasitized host eggs (Spínola-Filho et al. 2014; Jerbi-Elayed et al. 2015). In temperate regions, sublethal temperatures can leave an insect incapable of activity or development and indirectly lead to mortality. Therefore, tolerance to extreme temperature and to temperature fluctuation in the early spring season, and the initial time of occurrence are critical for *A. avenae* and *A. gifuensis* to suppress *S. avenae* populations in winter-wheat fields.

The thermal activity threshold is an important component in the examination of the thermal biology of insects (Hughes et al. 2010a, 2010b; Hazell & Bale 2011; MacMillan & Sinclair 2011; Rodrigues et al. 2013). A number of factors can affect the activity thresholds of parasitoids. The lower surface-to-volume ratio of A. avenae could make this species more resistant to high temperatures than Aphidius rhopalosiphi De Stefani-Perez, which has a similar ecology (Le Lann et al. 2011). Fluctuating thermal storage of the mummies of Schizaphis graminum (Rondani) (Hemiptera: Aphididae) significantly enhanced the cold tolerance of A. gifuensis adults compared with that at constant temperature (Song et al. 2012). Dong (2012) found that the A. gifuensis females that emerged from the mummies collected from wheat fields in the higher latitude region of Langfang, China, were more tolerant to low temperatures than those from the lower latitude of Taian, China, indicating that the same species of wasps' thermal tolerance in different distribution areas may be different due to climate diversity.

The supercooling point (SCP) and freezing point (FP) can be used to evaluate parasitoids' tolerance to low temperature related to the range of temperature experienced (Lee 1991; Jones et al. 2008). Other traits investigated include non-lethal measures of heat coma, chill coma, and chill coma recovery (Ayrinhac et al. 2004; Castaneda et al. 2005; Hughes et al. 2011). These traits could be used to determine the efficacy of *A. avenae* and *A. gifuensis* during the aphid damaging periods. Focusing on the thermal activity threshold of *A. avenae* and *A. gifuensis*, the objective of this study was to compare the parasitic potential between 2 parasitoid species that have a similar ecology. Knowledge of potential differences in thermotolerance between *A. avenae* and *A. gifuensis* could be useful to the development of large-scale production and release technologies for these 2 natural enemies and enhance the efficacy of biological control practice for aphids.

Materials and Methods

REARING OF APHIDIUS AVENAE AND APHIDIUS GIFUENSIS

Colonies of A. avenae and A. gifuensis were initiated from individual mummies collected from wheat plants at the experimental station of Shandong Agricultural University, China (36.15°N, 117.15°E), and reared at a 16:8 h L:D photoperiod and 22 °C on wheat 'Lumai 2' seedlings infested with S. avenae. Parasitoids were allowed to complete at least 1 generation under these conditions before use in the experiments. To obtain adult parasitoids, synchronized 1-d-old mummies were collected. All mummies were placed in glass vials (10 per vial), closed with plastic lids that were modified to allow for the movement of air through a small hole (1 cm diameter) covered by 75 µm mesh muslin. In each vial, a cotton wool wick saturated in 10% honey and water solution served as food and moisture sources for emerging parasitoids. Each day, newly emerged wasps were collected into clean glass tubes $(2.5 \times 20 \text{ cm})$ with access to both water and honey solution. Parasitoids were used in experiments less than 24 h after emergence. Experimental individuals of each species were measured to make sure they were of the same size for each species.

SUPERCOOLING POINT AND FREEZING POINT

The SCP and FP were determined by attaching a copper–constantan thermocouple (0.125 mm diameter) with a small amount of Vaseline grease to the upper surface of each adult within a polypropylene tube. The tube was sealed with cotton wool and inserted in a larger polypropylene tube placed in the chamber of a controlled rate freezer (DCW23506, Ningbo, China). Changes in temperature were continuously monitored with an electronic recorder. Prior to immersion in a refrigerated alcohol bath, the tubes were enclosed in an aluminum cylinder that acted as a heat sink and provided temperature stabilization during cooling. The temperature of the bath was lowered from 10 °C to -30 °C at a rate of approximately 1 °C/min. The lowest temperature reached before an exothermic event occurred due to release of latent heat was taken as the SCP of the individual. The burst of heat during the formation of ice crystals would raise the temperature of the individual to its FP.

MINIMUM TEMPERATURE FOR WALKING AND CHILL COMA

Temperature was decreased from 22 °C (the rearing or acclimation temperature) to -15 °C at a rate of 0.2 °C/min, representative of rates experienced in nature, to avoid other responses being induced in the small subject specimens, such as rapid cold hardening (Kelty & Lee 1999). The minimum temperatures were selected on the basis of preliminary experiments, in which they resulted in 100% entry into chill coma. The temperatures at which each individual lost the ability to walk (minimum temperature for walking, WTmin) and entered chill coma were recorded. In the experiments, chill coma was defined as the last movement of an appendage (i.e., leg or antenna) as described by Hazell et al. (2008).

CHILL COMA RECOVERY AND ACTIVITY RECOVERY

Chill coma recovery and activity recovery were observed in a fresh sample of parasitoids. Temperature was decreased from 22 to -15 °C at a rate of 0.2 °C/min, thereby reaching temperatures that ensured 100% of the parasitoids were in chill coma. Specimens were held at these temperatures for 5 min before being warmed to their rearing or acclimation temperature at a rate of 0.2 °C/min. The temperatures at which each individual regained the ability to move their appendages (chill coma recovery) and then walk (activity recovery) were recorded.

MAXIMUM TEMPERATURE FOR WALKING AND HEAT COMA

Temperature was increased at a rate of 0.2 °C/min from 22 to 50 °C. This maximum temperature was selected on the basis of preliminary experiments, in which it induced heat coma in all individuals. The temperatures at which an individual was not able to walk (maximum temperature for walking, WTmax) and entered heat coma (last movement of an appendage) were recorded.

It was not possible to record the activity recovery temperature after WTmax and heat coma exposure because these 2 temperatures were typically lethal.

STATISTICAL ANALYSES

Differences in SCPs and FPs of male or female adults of *A. avenae* and *A. gifuensis* were analyzed using 1-way analysis of variance (ANO-VA), and means were separated using Tukey's honestly significant difference (HSD) test. For thermal activity thresholds, individual comparisons were made using the Bonferroni 95% confidence intervals. SPSS 16.0 for Windows was used for calculations.

Results

SUPERCOOLING POINT AND FREEZING POINT

There were no differences in both SCP and FP between the male and female wasps of *A. gifuensis* (SCP: *P* = 0.328, FP: *P* = 0.545) or *A. avenae* (SCP: *P* = 0.732, FP: *P* = 1.000) (Table 1). The mean SCP of *A. gifuensis* females was lower than that of *A. avenae* males (*P* = 0.031). A difference was observed in FP between female wasps of *A. gifuensis* (-19.8 ± 0.4 °C) and females of *A. avenae* (-18.1 ± 0.3 °C) (*P* = 0.007) or males of *A. avenae* (-18.0 ± 0.4 °C) (*P* = 0.01).

MINIMUM TEMPERATURES FOR WALKING, CHILL COMA, CHILL COMA RECOVERY, AND ACTIVITY RECOVERY

With decreasing temperature, the wasps lost the ability to move their appendages and then were unable to walk. The temperatures at which these thermal thresholds, i.e., WTmin, chill coma, chill coma recovery, and activity recovery, occurred are shown in Table 2.

Differences in WTmin between the females or the males of the 2 parasitoid species, and between the females and males of *A. gifuensis* were significant (F = 9.9; df = 3; P < 0.001). Females of *A. gifuensis* had the lowest WTmin (-5.1 ± 0.3 °C). No difference was observed in the temperature at which the parasitoids entered chill coma (F = 1.3; df = 3; P = 0.284).

A difference was observed in the temperature at which the parasitoids began to recover after chill coma (F = 6.3; df = 3; P = 0.001). The males of *A. avenae* had the highest temperature (5.9 ± 0.3 °C) at which they began to recover after chill coma, followed by the females of *A. avenae* (5.1 ± 0.2 °C), males of *A. gifuensis* (4.6 ± 0.1 °C), and the females of *A. gifuensis* (4.1 ± 0.4 °C). The females of *A. gifuensis* had a lower temperature (5.9 ± 0.3 °C) for activity recovery after chill coma compared with the females of *A. avenae* (7.8 ± 0.9 °C) (P = 0.034).

MAXIMUM TEMPERATURE FOR WALKING AND HEAT COMA

There was no difference (F = 1.3; df = 3; P = 0.292) in WTmax between the males and females or between the 2 species (Table 3). A difference was observed for the temperature at which the parasitoids entered heat coma between females of *A. avenae* (43.0 ± 0.3 °C) and males of *A. gifuensis* (41.3 ± 0.7 °C) (P = 0.024).

Discussion

Parasitoids are considered efficient candidates for biological control agents, and their utilization is an important component of integrated pest management (Liu et al. 2015). In seasonal temperate environments, there will often be a threshold temperature above which insect activity, breeding, and diapause occur (Jeffs & Lewis 2013). Quantifying parasitoids' thermal activity thresholds may allow us to make the best use of them in wheat aphid and other aphid control programs both in greenhouses and in open fields.

THERMAL TOLERANCES MATCH SEASONAL OCCURRENCE OF THE TWO PARASITOID SPECIES

Although there was no significant difference in SCP between the male or female wasps of the 2 species, the mean FP of A. gifuensis females was significantly lower than that of A. avenae females (Table 1). Moreover, WTmin and the temperature at which the females of A. gifuensis regained the ability to walk after chill coma were significantly lower than those of A. avenae females (Table 2). Females of A. gifuensis had lower thermal activity thresholds than females of A. avenae, which might account for their smaller mean body size compared with the females of A. avenae, because WTmin was previously shown to correlate with surface-area-to-volume ratio (SVR) in other parasitoids, where species with higher SVR can maintain activity at lower temperatures than those with lower SVR (Renault et al. 2003; Le Lann et al. 2011). This trait could lead to the occurrence of A. gifuensis in large quantity in the early spring season, when Dong (2012) found its populations reaching peak numbers about 10 d earlier than populations of A. avenae in wheat fields.

Once the parasitoids survive the winter, a gradually increasing temperature in spring would then allow significant movement of individuals from overwintering sites, both as adult parasitoids and as eggs or larvae within aphids (Starý 1988). Both A. gifuensis and A. avenae have been occurring from late Mar to early Jun (early spring to early summer) in the main cultivating area of wheat in China. The females of A. avenue had significantly higher heat coma temperatures than the males of A. gifuensis (Table 3). The variation in content and composition of heat shock protein in these 2 parasitoid species may be the key factor that caused the different high-temperature tolerance capacity. In addition, a previous study found that Wolbachia-infected parasitoids were more tolerant to high-temperature stress than uninfected individuals (Wu et al. 2015). As temperatures rise, the enhanced tolerance of A. avenae to high-temperature stress would allow this species to produce more eggs to develop into adults locating the increased number of host aphids, and become the major parasitoid in the late grain filling and milk ripe stages (Dong 2012). The sympatric species A. gifuensis and A. avenae from the same ecological guild have different thermal tolerances, which may allow them to occur in the same wheat field habitat, and protect them from severe competition within the same niche of S. avenae, especially when the aphid number is low in early spring.

DEVELOPMENT OF RELEASE TECHNIQUES

In Taian, Shandong Province, the mean minimum temperature in late Mar was 2 °C, and the extreme low temperature was –8 °C in the past 10 yr. The parasitoid *A. gifuensis* was the most dominant species from wheat jointing to early grain filling stages (Dong 2012). Therefore, large numbers of *A. gifuensis* could be reared and released in the early spring season to control aphids in the fields when the temperature is above 5.9 °C. This recommendation may guide the farmers to develop an efficient aphid control practice based on conservation and augmentation biological control techniques in the fields. Furthermore,

Table 1. The supercooling points (SCP) and freezing points (FP) of Aphidius gifuensis and Aphidius avenae.

	A. gif	uensis	A. avenae	
Variable	Female (<i>n</i> = 28)	Male (<i>n</i> = 30)	Female (<i>n</i> = 31)	Male (<i>n</i> = 26)
SCP (°C)	-21.52 ± 0.44b	-20.59 ± 0.40ab	–20.56 ± 0.37ab	-19.98 ± 0.30a
FP (°C)	-19.82 ± 0.41b	-19.08 ± 0.43ab	-18.08 ± 0.34a	$-18.04 \pm 0.39a$

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Values (mean ± SE) labeled with different letters in each row are significantly different at the 5% level by Tukey's HSD test after 1-way ANOVA.

	Table 2. Temperatures (°C) of minimum temperature for	or walking, chill coma, chill coma recovery	v. and activity recovery in A	<i>phidius avenae</i> and <i>Aphidius aifuensis</i> .
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Parasitoid		Minimum temperature for walking	Chill coma	Chill coma recovery	Activity recovery
A. gifuensis	Female	-5.1 ± 0.3c	−6.8 ± 0.2a	4.1 ± 0.4c	5.9 ± 0.3b
	(<i>n</i> = 35)	(-5.6 to -4.6)	(−7.2 to −6.3)	(3.3–5.0)	(5.3–6.6)
	Male	-4.0 ± 0.2b	-6.4 ± 0.2a	4.6 ± 0.1bc	6.3 ± 0.4ab
	(<i>n</i> = 35)	(-4.4 to -3.7)	(-6.9 to -6.0)	(4.3–4.9)	(5.5–7.1)
A. avenae	Female	-3.3 ± 0.4ab	–6.4 ± 0.1a	5.1 ± 0.2bc	7.8 ± 0.9a
	(<i>n</i> = 32)	(-4.1 to -2.4)	(–6.7 to –6.2)	(4.6–5.6)	(5.8–9.7)
	Male	-2.9 ± 0.3a	-6.3 ± 0.2a	5.9 ± 0.3a	7.9 ± 0.7a
	(<i>n</i> = 31)	(-3.6 to -2.2)	(-6.7 to -6.0)	(5.3–6.5)	(6.5–9.2)

Values (mean ± SE) within columns followed by the same letters are not significantly different (comparisons made using Bonferroni 95% confidence intervals). Values in parentheses are the range from minimum to maximum.

 Table 3. Temperatures (°C) of maximum temperature for walking and heat coma of Aphidius avenae and Aphidius gifuensis

Parasitoid		Maximum temperature for walking	Heat coma
A. gifuensis	Female	40.2 ± 0.8a	$42.7 \pm 0.4ab$
	(<i>n</i> = 35)	(38.7-41.7)	(41.8-43.6)
	Male	38.9 ± 0.8a	$41.3 \pm 0.7b$
	(<i>n</i> = 35)	(37.3-40.6)	(39.9-42.8)
A. avenae	Female	40.6 ± 0.4a	43.0 ± 0.3a
	(<i>n</i> = 32)	(39.8–41.4)	(42.4–43.6)
	Male	39.5 ± 0.6a	42.0 ± 0.5ab
	(<i>n</i> = 31)	(38.3–40.6)	(41.0–43.0)

Values (mean \pm SE) within columns followed by the same letters are not significantly different (comparisons made using Bonferroni 95% confidence intervals). Values in parentheses are the range from minimum to maximum.

our results indicate that *A. avenae* was more suitable than *A. gifuensis* for the biological control of aphids in high-temperature areas or greenhouse environments. To develop and release the 2 parasitoid species in fields or greenhouses under optimum conditions, we suggested that the temperature fluctuation could be from 5.9 to 40.2 °C for *A. gifuensis* and from 7.8 to 40.6 °C for *A. avenae*.

In combination with promising field practices such as intercropping, semiochemical release, and intercropping combined with semiochemical release, the increasing numbers of parasitoids *A. avenae* and *A. gifuensis* were important in suppressing wheat aphid populations in the field (Liu et al. 2009; Wang et al. 2009; Wang et al. 2011; Dong et al. 2012). To efficiently develop parasitoid conservation strategies and to formulate more efficient biological control programs, further studies should be conducted. More detailed work is needed on whether other factors such as fecundity, long-distance migration, and hyperparasitism could influence the occurrence and density of the parasitoids.

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