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Morphological and physiological differences between mummy colour morphs of *Aphidius rhopalosiphi* (Hymenoptera : Braconidae) : an adaptation to overwintering?

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ABSTRACT. Colours of individuals are one of the more current morphological clues linked to overwintering adaptations. We studied morphological and physiological differences between colour morphs of *Aphidius rhopalosiphi* mummies in relation to possible adaptation to winter survival. Aphids *Sitobion avenae* were parasitised and placed in a controlled temperature room at 10˚C, under a photoperiod of L9 :D15. Mummies were sorted by colour. Volume, fresh mass, water content, development time and cocoon thickness were recorded individually. Differences in development time were observed between dark and white forms, cocoon thickness and water content. We could not relate these difference to diapause status. However, these features are very likely linked to distinct cold resistance patterns and may be indications of a quiescence state.

KEY WORDS : *Aphidius rhopalosiphi*, morph colour, water content, development time, cocoon thickness, diapause, quiescence, cold resistance.

INTRODUCTION

In temperate climates, many insects spend an important part of their lives in an overwintering stage. These insects show a large range of adaptive responses to the detrimental winter conditions. Moreover, within populations different strategies may be present and individual variation reinforces the adaptive range. For instance, several species exhibit a large variability in the colour morphs present within winter populations (LEATHER et al., 1993). Colour could play an important role in thermoregulation. According to SCHLINGER & HALL (1959) the white-coloured cocoon of *Trioxys utilis* Muesebeck, 1956 (Hymenoptera : Braconidae) is more common in warmer weather – white colour acting as heat reflectant – whereas dark brown cocoons are mostly encountered under cold weather conditions where the colour contributes to more efficient heat absorption. Dark colour is also an advantage to those insects that overwinter as immobile, concealed forms, as cryptic colour offers some protection against predators that depend on vision (LEATHER et al., 1993). Some authors have stated that, for parasitoids, colour of mummies may be an efficient and practical tool for distinguishing between diapausing and non-diapausing individuals. SCHLINGER & HALL (1960) have indeed shown that diapausing individuals of *T. utilis* exhibited a dark brown colour and a much thicker cocoon. Previous works suggested that diapausing and non-diapausing individuals of *A. rhopalosiphi* (De Stefani-Peres) can be safely recognized by the colours of their mummies (KRESPI et al., 1994; LANGER & HANCE, 2000; RIGAUX et al., 2000). KRESPI et al. (1994) noted that diapausing last instars of *A. rhopalosiphi* formed darker mummies and thicker cocoons than those of non-diapausing parasitoids. Similar differences have been observed in a number of Aphidiids by STÀRY (1970).

During a three generations experiment, LANGER & HANCE (2000) induced diapause in *A. rhopalosiphi* individuals. In accordance with the literature, they assigned black mummies to diapausing ones and stated that, after three generations, some of the mummies remained white and thus non-diapausing. As mild winters are quite frequent in Belgium, they concluded that *Aphidius rhopalosiphi* (De Stefani-Peres) (Hymenoptera : Braconidae : Aphidiinae) may overwinter using two different strategies : diapause and quiescence of the last instar larvae within the aphid mummies. However, in nature and in laboratory experiments, we observed a large range of *A. rhopalosiphi* mummy colours whatever the conditions. Our aim was thus to study morphological and physiological differences between colour morphs of *A. rhopalosiphi* mummies, formed under the same constrained climatic conditions, in order to better understand the role of colour variations for this species.

MATERIAL AND METHODS

Laboratory cultures of *A. rhopalosiphi* and its host *Sitobion avenae* Fabricius 1775 (Homoptera : Aphididae) were started from individuals collected in winter wheat fields during summer 1999 at Louvain-la-Neuve, Belgium (50.3˚N latitude). Aphids and parasitoids were reared on

winter wheat seedlings, *Triticum aestivum* (L.), at 20˚C under a photoperiodic regime of L16 :D8. Experiments were carried out from March 2000 until March 2001. A batch of *ca*. 5000 second and third instars of *S. avenae* was exposed during 48 hours to 50 pairs of parasitoids *A. rhopalosiphi*. Aphids were then placed in a controlled temperature room at 10˚C under a photoperiod of L9 :D15, corresponding to autumnal conditions in Belgium (RIGAUX et al., 2000). Once mummies were formed at 10˚C, *i.e.* 25 days after exposure, they were collected and separated according to colour. To do that, we removed individuals from the control room at the same time, the first day the first mummies were noticed. Then, they were classified into three groups of *ca*. 100 individuals each, using a universal RAL colour chart. We considered that colours RAL 1001, RAL 1002, RAL 1013, RAL 1014, RAL 1015 and RAL 7044 represented white mummies (n=101), whereas brown mummies (n=95) were characterized by RAL 8008, RAL 8011, RAL 8024, RAL 8025 and RAL 8028. For this study, we did not take into account the intermediate colours RAL 1004, RAL 1011, RAL 1019, RAL 7006 and RAL 7008. For each specimen, we recorded volume, dry mass, water mass, water content, development time and cocoon thickness. A colour video module connected to a monitor and a micrometric scale was used to determine the size of each mummy, total length (L) and width (Wi). Volume was calculated using the formula (πWi*Wi*L)*(4/3). Fresh mass was recorded for each individual with an electro balance with a precision of 1 µg (Mettler Me22). Some of these mummies were used to measure individual dry mass and to calculate water mass and water content. The other mummies were left in the same conditions until emergence in order to record the time to emergence and cocoon thickness.

Development time and thickness of the cocoon

Thirty-six dark and thirty-eight white mummies were reared until emergence at 20˚C, L9 :D15. The development time is the total duration between egg laying and adult emergence recorded individually at 12-hour intervals. Development time on a day-degree (˚D) basis was computed as : $^{\circ}D = DT\ (T\text{-}T_0)$ for $T > T_0$, where T is the temperature in ˚C, DT is the observed developmental period in days and T_0 the lower thermal threshold. For *A. rhopalosiphi* we used 6˚C for the thermal threshold (CAMPBELL et al., 1974; RUGGLE & HOLST, 1994; SIGSGAARD, 2000). The thickness of the cocoon wall was measured for 12 dark mummies (three repetitions per mummy) and 10 white mummies (three repetitions per mummy) by scanning with an electron microscope (JSM 6301F).

Dry mass, water mass and water content

The dry masses of 63 white and 59 dark mummies were obtained after drying for three days in an oven at 60˚C (VERNON & VANNIER, 1996; WORLAND et al., 1998). A desiccator was used to transfer each mummy from the oven to the balance. Mummies remained out of the dry air of the desiccator for less than five minutes and each specimen was placed into an aluminum curl paper to prevent damage. Water mass (Wm) was individually calculated as the difference between the fresh mass (Fm) and dry mass (Dm). The fresh water content, "Wf" is the total water mass expressed as a percentage of the fresh mass. The dry water content "Wd" is the total water mass expressed as a percentage of the dry mass (CHAUVIN & VANNIER 1997).

Statistical analysis

Normality of the data was assessed for thickness of the cocoon, water mass expressed as a percentage of fresh mass and as a percentage of dry mass. Logarithmic transformation (ln) and normality were assessed for volume, fresh mass, dry mass and water mass. Development time data were non-normally distributed. Analysis of variance (ANOVA) and Kruskal-Wallis test were used to determine the significance of observed differences between the two colour groups. The analyses were performed using Statistical Analysis System (SAS Institute, 1990).

RESULTS

All results (mean ± SEM) are summarized in Table 1. No differences were found between dark and white forms mass of *A. rhopalosiphi* mummies in volume, fresh mass, dry mass or water ($p > 0.05$).

In our experiment, the time between egg and mummy stage (at 10˚C) was 25 days. The elapsed time between the mummy stage and adult emergence was 3.5 days for the first emergence and 10 days for the last. Mean development time of dark mummies was longer than that observed for white ones (mean difference of 13.39 ˚D, $p < 0.05$, Table 1). This difference is obviously too low to

TABLE 1

Comparison between dark and white mummies for each criterion and the level of significance between colours. Time before emergence was non-normally distributed and the non-parametric Kruskal-Wallis test has been used in that case.

Variable	N		Means ± SEM		Analysis
	Dark	White	Dark	White	
Volume (mm^3)	95	101	8.71 ± 0.26	8.64 ± 0.27	F=0.18 P=0.6741 NS
Fresh mass (mg)	95	101	0.43 ± 0.01	0.41 ± 0.01	F=0.34 P=0.5632 NS
Dry mass (mg)	59	63	0.16 ± 0.01	0.16 ± 0.01	F=0.00 P=0.9614 NS
Water mass (mg)	59	63	0.26 ± 0.01	0.29 ± 0.01	F= 2.91 P=0.0908 NS
Water content/Dry mass (%)	59	63	165.08 ± 2.87	182.27 ± 2.84	F=18.09 P<0.0001 ***
Water content/Fresh mass (%)	59	63	62.02 ± 0.42	64.35 ± 0.37	F=17.71 P<0.0001 ***
Mummies thickness (µm)	12	10	16.47 ± 1.45	12.10 ± 1.28	F=4.88 P=0.0390 *
Time before emergence (° days)	36	38	182.44 ± 4.46 (156-240)	169.05 ± 2.76 (149-212)	$\chi 2$=3.86 P=0.0493 *

conclude that brown mummies were in diapause. Values for white mummies ranged between 149 and 212˚D, whereas for dark ones they ranged between 156 and 240˚D. A great overlap between colour morphs was thus obvious (Fig. 1), and both dark and white mummies could have a short development time.

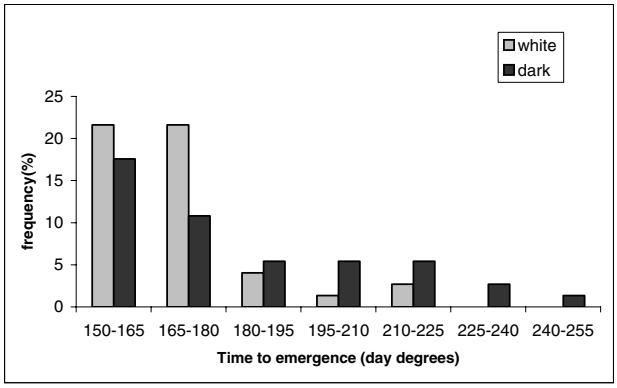


Fig. 1. – Frequency distribution for dark and white mummies, in relation to time before emergence.

Concerning the thickness of the cocoon, dark mummies were very significantly thicker than white ones ($p < 0.0001$), even though values overlapped (Fig. 2). The thinnest dark mummy had a wall of 10.00 μm, while the minimum for white mummies was 2.78 μm. The thickest mummy was 26.15 μm for dark mummies and 20.51 μm for white ones.

A highly significant difference between the two colour morphs was recorded for water content expressed both as a percentage of the dry mass or as a percentage of the fresh mass ($p < 0{,}0001$). In both cases, the mean water content was lower for dark mummies than for white ones (Table 1). Figure 3 shows frequency distribution of water content (Wd) as a function of the colour morph. Even though dark mummies exhibited lower water contents than white ones, the distribution of water contents overlapped widely.

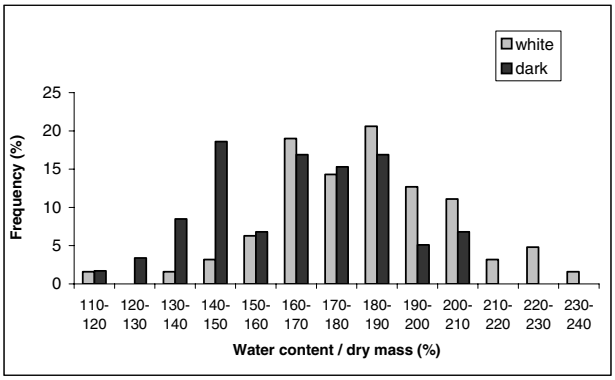
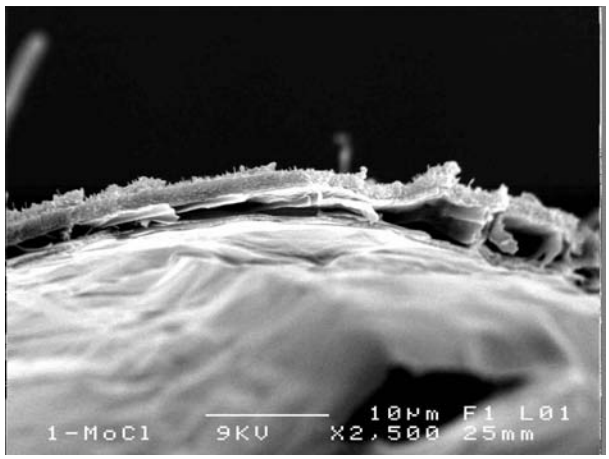


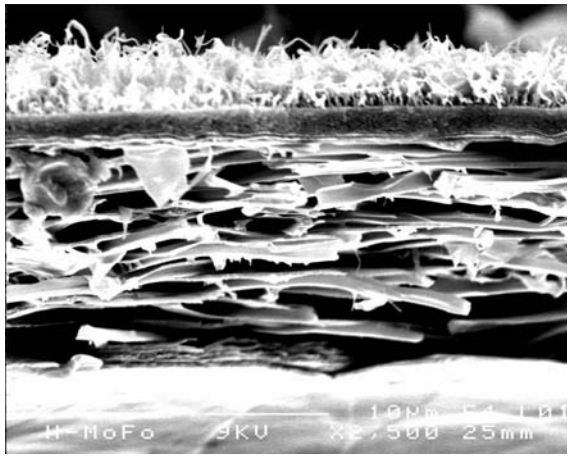
Fig. 3. – Frequency distribution of water content, in relation to dry mass, for dark and white mummies.

DISCUSSION AND CONCLUSIONS

RUGGLE & HOLST (1994) considered that the current development time for non-diapausing *A. rhopalosiphi*



1) White mummy : 3.69 μm



2) Dark mummy : 20.77 μm

Fig. 2. – Cross-section through the mummy (cocoon of the parasitoid and aphid cuticle) of one white and one dark mummies (pictures 1 and 2).

individuals is 194˚D and SIGSGAARD (2000) considered it to be 205˚D. KRESPI et al. (1997) observed that most adults of *A. rhopalosiphi* emerged within a month following oviposition and required 200-360˚D to complete development. However, in their case, 0.7% of parasitoids needed 480-560 ˚D and 1.5% required 1140 to 1580˚D. Regarding these high values, KRESPI et al. (1997) underlined that these individuals were probably in diapause. In our experiment none of the mummies took such a long time for development, indicating that even the dark mummies we obtained were not in diapause. Indeed, the longest development time recorded was only 240˚D, well below the value given by KRESPI et al. (1997) for diapausing individuals. It is still possible that some dark mummies with a longer development time were in quiescence. Indeed, this type of dormancy is probably a phenomenon confined to early winter or to winter-active insects, and only results in growth retardation (LEATHER et al., 1993).

Considering the cocoon thickness, KRESPI et al. (1997) observed similar values to us with thickness less than 10 μm for non-diapausing individuals of *A. rhopalosiphi* and between 20 and 40 μm for diapausing ones. In their case as in ours, only yellowish and brownish mummies, and not intermediate colours, were taken into account. In our

experiment, some of the dark brownish mummies also had a thick cocoon, but were not diapausing. In a study on *Aphidius smithi* (Sharma et Subba Rae) (Hymenoptera : Braconidae), WIACKOWSKI (1962) observed three kinds of cocoon : white-brown and thin, dark-brown and thin, and dark-brown very thick, the last one corresponding to a typical diapause. We must further investigate the possibility of similar trends in *A. rhopalosiphi* mummies. One principal role of the silken cocoon is probably to protect the parasitoid from desiccation. In some parasitoids the overwintering cocoons (low ambient humidity) are more robust than summer ones (high ambient humidity) (TAGAWA, 1996). Within our data, it is possible that dark morphs were cold adapted forms showing a thick cocoon (*ca.* 25 μm).

LANGER & HANCE (2000), under the same experimental conditions (10˚C; L9 :D15), observed that the supercooling point, a common comparative cold tolerance measure (*e.g.* SALT, 1961; LEE, 1991), of white mummies (-26.0 ± 0.3˚C) was on average 1.1˚C higher than the value they obtained for dark mummies (-27.1 ± 0.7˚C). This significant difference may partly be explained by our results as it corresponds also to a significant difference of water content (Wf) between white and dark mummies. It is commonly assumed (*e.g.* SALT, 1961; CANNON & BLOCK, 1988; RING & DANKS, 1994) that insects reduce their water content before winter, while cryoprotectant contents generally increase, enhancing cold-hardiness significantly. Water content reduction is often a prerequisite for survival during exposure to extremes of cold.

According to SALT (1961) "cold-hardiness and diapause are separate phenomena, the relation between the two, arising from their co-occurrent timing". ADEDOKUN & DENLINGER (1984) demonstrated also that cold-hardiness and diapause are both commonly associated with overwintering, but that the relationship between the two is often obscure. This conclusion is also shared by VERNON & VANNIER (2002). Generally, insects show a complex and often interactive range of reactions to the deteriorating environmental conditions that occur as winter approaches. In temperate regions, where mild winters are quite frequent, a part of the *A. rhopalosiphi* population undergoes quiescence. The other part overwinters under a diapause state (LANGER & HANCE, 2000). In our experiment, the environmental conditions of temperature and photoperiod corresponded to autumnal conditions in Belgium. These conditions seemed not favorable to the induction of diapause. However, it is possible that in response to the deteriorating environmental conditions, the mummies with a longer development time were in quiescence representing a first step to diapause. The overlapping of development time, water content and cocoon thickness between colour morphs that we observed probably indicates different capacities to survive winter.

We presume that the colour of the mummy reflects the colour of the cocoon but also the colour of the cuticle of the dead aphid. The colour of the mummy is not only related to the diapause status but also to the aphid morph and its physiological status. A complementary possibility is that colour differences will be related to atmospheric humidity at the time of mummy formation (NOWBAHARI & THIBOUT, 1990). In a new experiment, we are currently attempting to identify the possible links between development time (diapausing criterion) and cocoon thickness (cold resistance criterion). We are also trying to relate these two criteria to other physiological or morphological characteristics using multivariate analyses.

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