J. Appl. Ent. 126, 572–576 (2002)

ISSN 0931-2048

Effect of temperature and host stage on performance of *Aphelinus varipes* Förster (Hym., Aphelinidae) parasitizing the cotton aphid, *Aphis gossypii* Glover (Hom., Aphididae)

O. Röhne
The Norwegian Crop Research Institute, Plant Protection Centre, Department of Entomology and Nematology, Ås, Norway

Ms. received: May 9, 2000; accepted: February 26, 2001

Abstract: Development time, mummification, pupal mortality, host feeding and sex ratio of a Norwegian strain of *Aphelinus varipes* Förster parasitizing the cotton aphid, *Aphis gossypii* Glover were studied at 20, 25 and 30°C in controlled climate cabinets. Petri dishes with cucumber (*Cucumis sativus* L) leaves on agar were used as experimental units. Cotton aphids in different larval instars and as adults, reared at the three different temperatures, were presented to *A. varipes* in a 'no-choice' situation for 6 h. These presentations were done at 25°C in each experiment to avoid an influence of temperature on parasitization rate. More first instar aphids were parasitized than third and fourth instars among the aphids reared at 20°C. Pupal mortality of the parasitoid was not influenced by temperature. It was lower in aphids parasitized as adults than in aphids parasitized in second instar. The sex ratio of *A. varipes* was female-biased, and varied between 92% females developed from aphids reared at 25°C and 70% from aphids reared at 20°C. The sex ratio was not significantly influenced by host stage. The development time of *A. varipes* ranged from 17.5 days at 20°C to 9.8 days at 30°C.

1 Introduction

The cotton aphid, *Aphis gossypii* Glover, is an important pest on cucumber in European greenhouses (van Schelt, 1993), and its occurrence in other greenhouse crops is increasing (van Steenis, 1995). As the cotton aphid has developed resistance to many commonly used pesticides (Gubran et al., 1993; Kerns and Gaylor, 1993), it is of great interest to explore natural enemies that can be used in the biological control of this aphid species. The purpose of this study was to determine whether *Aphelinus varipes* Förster could be a supplement to the aphidiine-species (Braconidae) that are used in biological control of the cotton aphid in greenhouses today.

Few aphelinids are known to parasitize the cotton aphid successfully. Only *Aphelinus asychis* Walker (= *A. flavipes* Förster and *A. semiflavus* Howard) has been used for biological control of the cotton aphid (Wyatt, 1969). Van Steenis (1995) collected life history data from a strain of *A. varipes* from Cameroon with the cotton aphid as host and concluded that *A. varipes* could be useful in biological aphid control programmes, especially when used together with *Aphidius colemani* Viereck (Hym., Aphidiidae).

Different strains of one parasitoid species can have different life-history parameters. Due to this intraspecific variability, choosing the right strain is of crucial importance for the success of biological control (Haardt and Höller, 1992). In this study, some important life history traits of a Norwegian strain of *A. varipes* parasitizing the cotton aphid are described. The traits studied were host feeding, mummification, pupal mortality, development time and sex ratio, when cotton aphids in different stages were presented to *A. varipes*. As cotton aphids are large and dark (black-green) at lower temperatures, whereas they are smaller and lighter (yellow) at higher temperatures (Blackman and Eastop, 1984; Aldyhim and Khalil, 1993), the experiment was performed at 20, 25 and 30°C, to find out whether this morphological variation had any influence on the traits studied.

2 Materials and methods

2.1 Host plants

Cucumber plants (*Cucumis sativus* L) (cv. 'Rhinish grape') were grown in a greenhouse at 22 ± 2°C, and moderately fertilized. The plants were 3–5 weeks old when they were used in the experiments.

2.2 Host aphids

Cotton aphids were collected from cucumber greenhouses in Lier, Norway in 1994. They were reared on cucumber under the above-described conditions.

 0931–2048/2002/2610–0572 $ 15.00/0

2.3 Parasitoids

Aphelinus varipes is distributed in Norway (Compton, 1981). This species was collected from a culture of peach aphids, *Myzus persicae* (Sulzer), which it had accidentally infested, at the Agricultural University of Norway at Ås in 1994. In 1995, they were transferred to cotton aphids cultured on cucumber. The experiments were performed 1 year later. The culture was held in cages at 22 ± 2°C and in natural light. The parasitoids used in the experiments were 24–72-h old, mated females. They had some host experience from cotton aphids placed in the emergence vials for host feeding (2–3 aphids per parasitoid) the day before the experiments.

2.4 Experimental units

Petri dishes (Bock, Art. Nr. 41113, 31 mm height; 77 mm diameter) with a lid were used as described by van Schelt (1993). A gauze (50 mm in diameter) was incorporated into the lid for air exchange. One centimetre of water agar was poured into each dish, and just before it solidified, a punched cucumber leaf disc was put upside down on the agar. Van Schelt (1993) recommended that this should be done when the agar has cooled down to 30°C, but 45°C seemed to be a more suitable temperature, because the leaf disc then makes better contact with the agar, and stays green for a longer time. The Petri dishes were placed upside-down on shelves (with ventilation holes) to simulate a more natural situation for the aphids, and to prevent the leaf from being contaminated by honeydew and mould (van Schelt, 1993).

2.5 Experimental procedure

Experiments were carried out with all five host stages, and at three different development temperatures; 20, 25 and 30°C. Three climate cabinets (Heigar Thermaks KPB 6395 FL, Heigar, Oslo, Norway) with 65% relative humidity and 16 h light : 8 h dark were used. Ten adult cotton aphids were placed in each Petri dish for 24 h. When the offspring of these aphids had reached the desired stage, 60 aphids per dish were selected, and the surplus was removed. There were three replicates (dishes) for each of the 15 combinations of host stage and temperature.

Four female parasitoids were allowed to host feed and parasitize in each Petri dish for 6 h at 25°C and 65% relative humidity, before the dishes were placed back at the three different development temperatures. The exposure was carried out at this same temperature in every experiment, to avoid an influence of temperature on parasitization rate. The proportion of aphids mummified was used as a measure of the host acceptance of *A. varipes* and the suitability of the host in a 'no-choice' situation. Host feeding, pupal mortality, sex ratio, development time from egg to mummy (pupa) and from mummy to adult were also measured. The mummies were transferred to glass vials (50 mm height; 20 mm diameter) for eclosure. The development time for the two different sexes of *A. varipes* was only obtained when second instar cotton aphids were parasitized.

2.6 Data analysis

The data were analysed using two-way analysis of variance (anova) and checked for interaction. Data on development time were analysed using the averages for each Petri dish. Since some of the proportional values were less than 0.30 or larger than 0.70, an arcsin square root-transformation was used to stabilize variances. Multiple comparisons were made using an LSD-procedure ($\alpha = 0.05$) with Bonferroni adjustment (Reimer, 1959).

3 Results

In five of the 45 Petri dishes, the aphids died before mummification because the leaves did not live long enough. These five leaves were all slightly thinner and younger than the rest. In these dishes, only the number of host feedings were recorded.

3.1 Host feeding

Aphids killed by host feeding were easily recognized due to a red-brown colour and a shrunken appearance. There were no significant differences in the number of host feedings between the five different host stages or the three different host development temperatures (table 1).

3.2 Mummification

There were no significant differences in percentage of mummified aphids between the temperatures. At 20°C, there were significantly more aphids mummified among those exposed to parasitism in the first instar (68%), than in the third or fourth instar (23 and 32%). At 30°C, there was a non-significant difference between first instar (33%) and fourth instar aphids (12%) ($P < 0.06$) (table 2). These differences between the temperatures on the effect of host stage was indicated by a significant interaction between temperature and host stage ($P < 0.04$).

3.3 Pupal mortality

The overall pupal mortality was 33.6% for *A. varipes*. It was significantly lower when adult aphids were parasitized than when aphids in the second instar were parasitized (table 1). There were no significant differences in pupal mortality between the three different development temperatures ($P > 0.59$).

3.4 Sex ratio

A total of 81.9% of the total hatching of *A. varipes* were females. Significantly more females eclosed from aphids reared at 25°C (92%) than from aphids reared at 20°C (70%) ($P < 0.01$). There were no significant differences in parasitoid sex ratio between the five different aphid stages (table 1).

3.5 Development time

Average development time from egg to mummy was significantly shorter at 25°C (5.1 days) and 30°C (4.9 days) than at 20°C (7.8 days) ($P < 0.001$). At 20°C, the development time from egg to mummy was significantly longer in aphids parasitized in the third instar than in aphids parasitized in any of the other host stages (table 3). At the two other temperatures, there were no significant differences in the development

Table 1. *Life history parameters of* Aphelinus varipes *with cotton aphid (*Aphis gossypii*) as host, parasitized in the five different host stages. Four parasitoids were allowed to host feed and parasitize in each dish for 6 h*

Host stage	1st instar Mean ± SE	*n*	2nd instar Mean ± SE	*n*	3rd instar Mean ± SE	*n*	4th instar Mean ± SE	*n*	Adult Mean ± SE	*n*
Host feeding (%)	10 ± 1^{a}	9	10 ± 2^{a}	9	11 ± 2^{a}	9	10 ± 1^{a}	9	6 ± 1^{a}	9
Pupal mortality (%)	36 ± 7^{ab}	9	44 ± 2^{a}	9	25 ± 3^{ab}	8	47 ± 2^{ab}	8	7 ± 6^{b}	6
Sex ratio (% fem.)	74 ± 5^{a}	9	84 ± 2^{a}	9	79 ± 3^{a}	8	84 ± 2^{a}	8	95 ± 5^{a}	6

Different letters between host stages indicate a significant difference ($P < 0.05$; LSD with Bonferroni after two-way ANOVA); *n* is the number of replicates (dishes with 60 aphids).

Table 2. *Percentage mummified cotton aphids (*Aphis gossypii*) when parasitized by* Aphelinus varipes *in the five different stages and at three different temperatures. Four parasitoids were allowed to host feed and parasitize in each dish for 6 h*

Host stage	1st instar Mean ± SE	*n*	2nd instar Mean ± SE	*n*	3rd instar Mean ± SE	*n*	4th instar Mean ± SE	*n*	Adult Mean ± SE	*n*
20°C	68 ± 9^{a}	3	36 ± 3^{ab}	3	23 ± 11^{b}	3	32 ± 12^{b}	3		
25°C	27 ± 2^{a}	3	39 ± 11^{a}	3	26 ± 7^{a}	3	12 ± 7^{a}	2	32 ± 2^{a}	3
30°C	33 ± 4^{a}	3	24 ± 5^{a}	3	26 ± 11^{a}	2	12 ± 6^{a}	3	23 ± 1^{a}	3

Different letters between host stages indicate a significant difference ($P < 0.05$; LSD with Bonferroni after two-way ANOVA); *n* is the number of replicates (dishes with 60 aphids).

Table 3. *Development time (days) for* Aphelinus varipes *parasitizing the cotton aphid (*Aphis gossypii*) in the five different stages and at three different temperatures*

Host stage	1st instar Mean ± SE	*n*	2nd instar Mean ± SE	*n*	3rd instar Mean ± SE	*n*	4th instar Mean ± SE	*n*	Adult Mean ± SE	*n*
Egg to mummy										
20°C	7.7 ± 0.06^{a}	3	7.7 ± 0.05^{a}	3	8.2 ± 0.06^{b}	3	7.6 ± 0.12^{a}	3		
25°C	5.0 ± 0.01^{a}	3	5.0 ± 0.02^{a}	3	5.2 ± 0.10^{a}	3	5.2 ± 0.12^{a}	2	5.1 ± 0.04^{a}	3
30°C	4.8 ± 0.15^{a}	3	4.9 ± 0.11^{a}	3	5.0 ± 0.22^{a}	2	5.1 ± 0.28^{a}	3	5.1 ± 0.04^{a}	3
Egg to adult										
20°C	16.9 ± 0.12^{ab}	3	16.7 ± 0.19^{a}	3	18.2 ± 0.41^{b}	3	18.1 ± 0.37^{ab}	3		
25°C	10.5 ± 0.08^{a}	3	11.1 ± 0.20^{abc}	3	11.6 ± 0.13^{bc}	3	12.0 ± 0.53^{b}	2	10.7 ± 0.19^{ac}	3
30°C	9.5 ± 0.21^{a}	3	9.7 ± 0.02^{a}	3	10.0 ± 0.28^{a}	2	10.1 ± 0.19^{a}	3	9.6 ± 0.40^{a}	3

Different letters between host stages indicate a significant difference ($P < 0.05$; LSD with Bonferroni after two-way ANOVA); *n* is the number of replicates (dishes with 60 aphids).

time from egg to mummy between parasitoids from aphids parasitized in different stages (table 3).

The total development time of *A. varipes* was 17.5 days at 20°C, 11.1 days at 25°C and 9.8 days at 30°C, and the times differed significantly from each other ($P < 0.001$). At 20°C, the total development time was significantly longer in aphids parasitized in the third instar than in aphids parasitized in the second instar (table 3). At 25°C, the total development time was significantly longer in aphids parasitized in the third or fourth instar than in aphids parasitized in the first instar (table 3). The total development time was also significantly longer in aphids parasitized in the fourth instar than in aphids parasitized as adults (table 3). At 30°C, there were no significant differences in the total development time between parasitoids from aphids parasitized in different stages (table 3).

At 30°C, the development time from egg to mummy was significantly shorter for male *A. varipes* (4.0 days) than for females (4.9 days) ($P < 0.05$; two-way ANOVA). Total development time was also significantly shorter for male *A. varipes* (9.0 days) than for females (9.9 days) at 30°C. At 20 and 25°C, there were no significant differences between females and males in the development time.

4 Discussion

The amount of host feeding observed for *A. varipes* was similar to that observed for other *Aphelinus*-species (Cate et al., 1977; Kuo, 1986; Tang and Yokomi, 1996; Tokumaru and Takada, 1996). The results also indicate that *A. varipes* has a daily number of parasitizations that is comparable to *Aphelinus gossypii* Timberlake (Tokumaru and Takada, 1996).

4.1 Effect of rearing temperature

The variation in size and colour between cotton aphids reared at different temperatures only seemed to have an effect on the sex-ratio allocation of the egg-laying *A. varipes*. The number of aphids killed by host feeding

and parasitization did not vary between the rearing temperatures. The pupal mortality of *A. varipes* did not increase with temperature. Many experiments have shown that the pupal mortality of *Aphelinus*-species is not influenced by temperature up to about 32°C (Force and Messenger, 1964a; Tang and Yokomi, 1995). Aphidiinae-species, on the other hand, have a higher juvenile mortality at higher temperatures (Force and Messenger, 1964b).

4.2 Effects of host instar at parasitization

The experiments indicate that *A. varipes*, like other aphelinid species (Starý, 1988; Tang and Yokomi, 1996) have the highest parasitization rate for the earlier host instars. Many Aphidiinae-species, on the other hand, show a preference for second and third instar aphids (Hågvar and Hofsvang, 1991), or show no clear host-stage preferences (Völkl et al., 1990). The host stage preference and host stage suitability are often correlated for parasitoids (Godfray, 1994). This does not seem be the case for *A. varipes*, in which the pupal mortality was lowest in adult aphids (table 1). It is more likely that the lower rate of parasitization in the later host stages is due to a more effective defence against parasitoids, thus making successful parasitization of older aphids more difficult (Force and Messenger, 1965; Cate et al., 1977; Gerling et al., 1990). In *Aphelinus asychis*, host age did not seem to influence the juvenile mortality of the parasitoid (Cate et al., 1977).

4.3 Strain characteristics

The overall sex ratio of this Norwegian strain of *A. varipes* was found to be more female biased (81.9%) than van Steenis (1995) observed for an African strain of *A. varipes* parasitizing the cotton aphid (55%). The development time of the Norwegian strain was also markedly shorter at 20 and 25°C compared with the results for the African strain (van Steenis, 1995) (fig. 1). Different strains of *Aphelinus abdominalis* Dalman could most easily be separated by looking at the development time of the female (Haardt and Höller, 1992). This also seem to be the case for *A. varipes*.

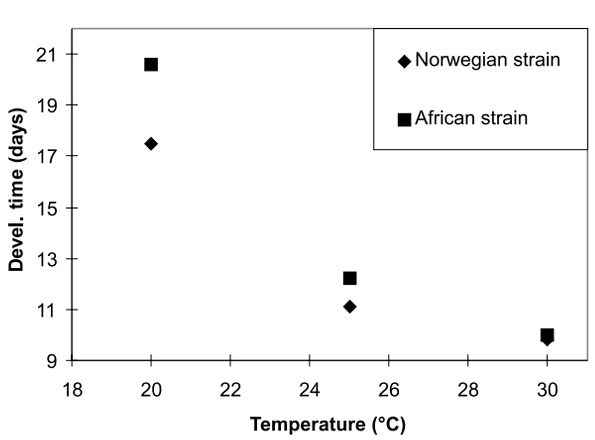

Fig. 1. *Total development time for* Aphelinus varipes *parasitizing the cotton aphid (*Aphis gossypii*) at three different temperatures. The results from the Norwegian strain of* A. varipes *are compared with an African strain of* A. varipes *(van Steenis, 1995). All values have SE ± 0.25 days or less (not shown on the figure)*

4.4 Conclusion

A. varipes could be a useful supplement to the aphidiine-species that are used in biological control of the cotton aphid in greenhouses today, as *A. varipes*, unlike aphidiines, host feeds on aphids in addition to parasitization, and is less affected by high temperatures. *A. varipes* also has a preference for earlier aphid stages, and this may reduce potential competition between the parasitoid species if used together. The short development time at 20 and 25°C, and the high proportion of females are properties that make the Norwegian strain of *A. varipes* an interesting alternative for the biological control of the cotton aphid in greenhouses.

Acknowledgements

I thank Professor Trond Hofsvang at the Norwegian Crop Research Institute and Per Kristian Larsen for their valuable comments on the manuscript.

References

Aldyhim, Y. N.; Khalil, A. F., 1993: Influence of temperature and daylength on population development of *Aphis gossypii* on *Cucurbita pepo*. Entomol. Exp. Appl. **67,** 167–172.

Blackman, R. L.; Eastop, V. F., 1984: Aphids on the World's Crops: an Identification and Information Guide. New York, Chichester: John Wiley and Sons,

Cate, R. H.; Eikenbary, R. D.; Morrison, R. D., 1977: Preference for and effect of greenbug parasitism and feeding by *Aphelinus asychis*. Environ. Entomol. **6,** 547–550.

Compton, S., 1981: The Chalcid fauna of the Jostedalen (Hym. Chalcidoidea). Fauna Norv. Series B **28,** 83–89.

Force, D. C.; Messenger, P. S., 1964a: Duration of development, generation time, and longevity of three hymenopterous parasites of *Therioaphis maculata*, reared at various constant temperatures. Ann. Entomol. Soc. Am. **57,** 405–413.

Force, D. C.; Messenger, P. S., 1964b: Fecundity, reproductive rates, and innate capacity for increase of three parasites of *Therioaphis maculata* (Buckton). Ecology **45,** 707–715.

Force, D. C.; Messenger, P. S., 1965: Laboratory studies on competition among three parasites of the spotted alfalfa aphid *Therioaphis maculata* (Buckton). Ecology **46,** 853–859.

Gerling, D.; Roitberg, B. D.; Mackauer, M., 1990: Instar-specific defense of the pea aphid, *Acyrthosiphon pisum*: influence on oviposition success of the parasite *Aphelinus asychis* (Hymenoptera: Aphelinidae). Jour. Insect Behav. **3,** 501–514.

Godfray, H. C. J., 1994: Parasitoids: Behavioral and Evolutionary Ecology. Princeton, NJ: Princeton University Press.

Gubran, E. M. E.; Delorme, R.; Auge, D.; Moreau, J. P., 1993: Pyrethroids and organochlorines resistance in

cotton aphid *Aphis gossypii* (Glov.) (Homoptera: Aphididae) in the Sudan Gezira. Int. J. Pest Manage. **39,** 197–200.

HAARDT, H.; HÖLLER, C., 1992: Differences in life history traits between isofemale lines of the aphid parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). Bull. Entomol. Res. **82,** 479–484.

HÅGVAR, E. B.; HOFSVANG, T., 1991: Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. Biocontr. News Inform. **12,** 13–41.

KERNS, D. L.; GAYLOR, M. J., 1993: Biotic control of cotton aphids (Homoptera: Aphididae) in cotton influenced by two insecticides. J. Econ. Entomol. **86,** 1824–1834.

KUO, H. L., 1986: Resistance of oats to cereal aphids: effects on parasitism by *Aphelinus asychis* (Walker). In: Interactions of Plant Resistance and Parasitoids and Predators of Insects. Ed. by BOETHEL, D. J.; EIKENBARY, R. D. New York, Chichester: Ellis Horwood Ltd, 125–137.

REIMER, C., 1959: Statistical analysis of percentages based on unequal numbers, with examples from entomological research. Can. Entomol. **91,** 88–92.

VAN SCHELT, J., 1993: A fecundity test for the aphid parasite Aphidius sp. In: Proceedings of the Seventh Workshop of the Global IOBC Working Group 'Quality Control. of Mass Reared Arthropods'. Ed. by NICOLI, G.; BENUZZI, M.; LEPPLA, N. C. September, Rimini, Italy. 191–199.

STARÝ, P., 1988: Aphelinidae. In: Aphids: Their Biology, Natural Enemies and Control. Ed. by MINKS, A. K.; HARREWJIN, P. Vol. 2B. Amsterdam: Elsevier, 185–188.

VAN STEENIS, M. J., 1995: Evaluation and application of parasitoids for biological control of *Aphis gossypii* in glasshouse cucumber crops. PhD Thesis, Wageningen, The Netherlands: Wageningen Agricultural University.

TANG, Y. Q.; YOKOMI, R. K., 1995: Temperature-dependent development of three hymenopterous parasitoids of aphids (Homoptera: Aphididae) attacking citrus. Environ. Entomol. **24,** 1736–1740.

TANG, Y. Q.; YOKOMI, R. K., 1996: Biology of *Aphelinus spiraecolae* (Hymenoptera: Aphelinidae), a parasitoid of the spirea aphid (Homoptera: Aphididae). Environ. Entomol. **25,** 519–523.

TOKUMARU, S.; TAKADA, H., 1996: Numbers of eggs deposited and host feeding in *Aphelinus gossypii* Timberlake (Hymenoptera: Aphelinidae), a parasitoid of *Aphis gossypii* Glover (Homoptera: Aphididae). Jpn. J. Appl. Entomol. Zool. **40,** 242–244.

VÖLKL, W.; STECHMANN, D. H.; STARÝ, P., 1990: Suitability of five species of Aphidiidae (Hymenoptera) for the biological control of the banana aphid *Pentalonia nigronervosa* Coq. (Homoptera, Aphididae) in the South Pacific. Trop. Pest Manage. **36,** 249–257.

WYATT, I. J., 1969: Parasite control of *Aphis gossypii* on cucumbers. Glasshouse Crops Res. Inst. Littlehampton. Annu. Report **1968,** 86–87.

Author's address: OLAF RÖHNE, Norwegian Crop Reseach Institute, Plant Protection Centre, Department of Entomology and Nematology, Høgskoleveien 7, N- 1432 Ås, Norway. E-mail: olav.rohne@c2i.net