

Effect of Constant Temperature on Development and Reproduction of the Cotton Aphid (*Aphis gossypii*) (Glover) (Hemiptera: Aphididae) on *Gossypium hirsutum* in Laboratory Conditions

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Abstract

Temperature-dependent development, survivorship and reproduction of the cotton aphid, *Aphis gossypii* on *Gossypium hirsutum* were evaluated at six constant temperatures (10^o, 15^o, 20^o, 25^o, 30^o and 35^oC). The clean statistical breaks between developmental durations of all immature stages of aphids held at 10^o and 15^oC indicate that at lower temperatures, all immature aphid stages (1st to 4th instars) are affected similarly by temperature changes. In viewing the survivorship curves (L_x) for aphids held at the six constant temperatures, a general trend is evident. As temperature increased, survivorship progressively fell more precipitously, indicating increasing aphid mortality at a younger age. At 10^o, 15^o, 20^o, 25^o, 30^o and 35^oC, aphid survivorship dropped below 80% at 39, 29, 33, 21, 20, and 14 days of life, respectively. Gross reproductive rate (GRR) an indicator of lifetime fecundity, ranged from 9.8 for aphids held at 10^oC to 58.9 for aphids held at 30^oC. A general rise in GRR was observed with rising temperature to 30^oC. The optimum temperature is closer to 25^oC. The results of this study agree with previous studies that the optimum aphid development temperature (held constant) in a laboratory study is 25^o to 30^oC.

1. Introduction

The cotton aphid is a polyphagous pest infesting various crops throughout the world. The development, survivorship, and reproduction of the cotton aphid are strongly influenced by temperature and quality of food. The speed of physiological development and rate of reproduction of the aphid increase as the temperature increases to an upper limit. At extreme low and high temperatures, developmental rate and reproduction rate of the cotton aphid are reduced. Report of the developmental rate for different stages of *Aphis gossypii* increased linearly as temperature increased within the range of 15^oC to 30^oC Kersting et al. (1999). Both food quality and temperature play a distinct role in cotton aphid population increase. Developmental rates and fecundity of apterous *Aphis gossypii* on cotton seedlings at different temperatures and a photoperiod of 18:6 (L:D) were studied by Akey and Butler (1989). Many studies have been conducted examining the relationship between different constant temperatures and aphid development including the effects of temperature and humidity on the biology of *Therioaphis maculata* (Buckton) (Graham 1959); the influence of rhythmically fluctuating temperatures on the

development and reproduction of the spotted alfalfa aphid (Messenger 1964); temperature requirements of some aphids and their parasites (Campbell et al. 1974); estimation of thermal thresholds and age-specific life table parameters for the walnut aphid under field conditions (Nowierski 1983); and influence of temperature and daylength on population development of *Aphis gossypii* (Aldyhim and Khalil 1993). The effect of temperature on population growth of the cotton aphid on *Gossypium hirsutum* L. was studied by Kersting et al. (1999) in Turkey and Xia et al. (1999) in China, stated that environmental conditions, especially temperature, affect the population growth of the aphid. Wang and Tsai (2000) studied the effect of temperature on the biology of *A. spiraeicola* in Florida. Similar studies on other aphids include: Ma et al. (2004) (rose grain aphid), Weathersbee et al. (2004) (broom citrus aphid), and Morgan et al. (2001).

Developmental time of aphids decreases as temperature increases up to an upper limit. Above this limit, development and fecundity slow down. Insects are poikilotherms and cannot regulate their body temperature. The development and

reproduction of aphids depend on the temperature of the surrounding environment. Wang and Tsai (2000) stated that pest population prediction systems and management strategies depend on a broad understanding of the pest's biology and population dynamics. The objective of this study was to generate comprehensive life history parameters across a temperature range (10°C-35°C) in a standard rearing environment in the laboratory.

2. Materials and Methods

Cotton plants used in laboratory studies were grown to the eight true-leaf stage in a greenhouse. During this period the plants were in the greenhouse, the temperature was maintained at 26.7°C. Evaporative cooling, gas heating, and an automated shade cloth system maintained a relatively constant temperature during the periods of germination and growth. Seeds of the commercial cotton cultivar Rasi 134 (RCH 134 B) were planted at weekly intervals for eight weeks. Three seeds were planted in each of 125 plastic pots (10.16 cm X 10.16 cm x 10.16 cm) in beginning of June 2009. The growing medium was a standard farming soil. After germination, seedlings were thinned to one dominant seedling per pot. All pots were manually watered daily. Fertilizer was applied (Rashtriya Chemicals and Fertilizers Ltd) once weekly for three consecutive

weeks. Table 1 presents the timeline of cotton planting, fertilizer applications, and plant transfer to growth chambers for the life history study. At the 8 true-leaf stage, plants were brought to the laboratory. Twelve plants were placed into each of six *growth* chambers (ACMAS Technocracy Pvt. Ltd. India, Model ACM 780945) those were set to maintain six constant temperatures: 10, 15, 20, 25, 30 and 35°C. The photoperiods in all growth chambers were held constant at 14:10 (L:D)hrs. The growth chambers had a capacity of 0.5m³ (interior dimensions 51 cm x 69 cm x 145 cm), and a temperature control range of $\pm 0.1^\circ\text{C}$. One day prior to transferring the cotton plants to the growth chamber, adult cotton aphids were placed on the undersurface of detached fourth and fifth true cotton leaves and placed in Petri dishes (15cm diameter x 2.5cm depth) to harvest newly emerged nymphs for the study. Moist filter papers were provided in each Petri dish to prevent the leaves from drying. Twelve cotton plants were transferred to each of the six growth chambers. Newly emerged aphid nymphs (<24hrs old) were transferred individually to the underside of the 4th and 5th main stem leaves (from the top of the plant) of each cotton plant in the growth chamber using a camel's hair brush, with a total of 24 aphids per temperature-treatment.

Table 1 Calendar of cotton planting, fertilization and transport of potted cotton plants to growth chambers for the cotton aphid life history study in 2009

Batch No.	Planting	Fertilization			Plants Transferred to Growth Chamber
		1	2	3	
1	05-28	05-28	06-11	06-18	07-10
2	06-04	06-11	06-18	06-25	07-16
3	06-11	06-18	06-25	07-02	07-24
4	06-18	06-25	07-02	07-09	07-31
5	06-25	07-02	07-09	07-15	08-09
6	07-02	07-09	07-15	07-22	08-16
7	07-09	07-15	07-22	07-29	08-24
8	07-15	07-22	07-29	08-05	08-31

For each plant, fertilizer applications were made at weekly intervals; the plants were transferred to the growth chambers at about six weeks (8 true-leaf stage)

Aphid transfer was done with great care to minimize the potential injury to the young aphids. Individual aphids were confined to a section of leaf using clear plastic, hinged boxes as cages, immediately after they were placed on the leaf. Spring-loaded stainless steel hair clips, were reshaped to fit over the clear plastic cages and were used to hold cages tightly closed and securely attached to leaves, a hole was drilled (1.25cm diameter) in the bottom of each plastic cage and perforated muslin cloth was hot-glued over the hole to provide ventilation to the cage interior while preventing escape of aphids. Rearing plants

were replaced once a week to standardize the host plant quality across temperature treatments.

Individual nymphs were observed at every 24hrs, and nymph duration and mortality were recorded until they reached adulthood. Adult aphids were observed daily and newly born offspring were counted and removed until the last individual from each treatment died.

Data analysis

The following life history parameters of cotton aphids held at constant temperatures of 10, 15, 20, 25, 30 and 35°C were quantified; instar-specific and

total nymphal durations, age-specific survivorship, age-specific fecundity, gross reproductive rate, net reproductive rate, finite rate of increase, doubling time, and intrinsic rate of population increase. The life history parameters were calculated by using the methods described by Andrewartha and Birch (1954) [13]. In this analysis, the first day of the test aphid's life was set as the first pivotal age with age divided into increments of one day. The intrinsic rate of increase (r) was determined by iteratively solving the Euler equation.

$$\sum e^{-rx} l_x m_x = 1$$

Where x is the age in days (including immature stages), r is the intrinsic rate of increase, l_x is the proportion of individuals alive at time x of an original cohort (including immature mortality). The variable m_x is the mean number of offspring produced per surviving aphid during the age interval x (1day). The life table parameters, gross reproduction rate ($GRR = \sum m_x$), net reproductive rate ($R_0 = \sum l_x m_x$), finite rate of increase ($\lambda = e^r$), mean generation time ($GT = \ln R_0 / r$), and doubling time ($DT = \ln 2 / r$) were calculated using the methods described by Andrewartha and Birch (1954) [13].

After computing the intrinsic rate of increase (r) for the original data (r_{all}), the Jackknife method (Meyer et al. 1986) [14] was used to estimate the standard error of the calculated life table statistics. Life history parameters were analyzed using a general linear model with temperature as a source of variation. Least squares means were compared using Fisher's Protected LSD. Developmental threshold temperatures were calculated using regression analyses of development rate (1/development duration) on temperature for each instar and for total nymphal development.

3. Results and Discussion

Instar-specific and total nymphal developmental durations for aphids at the six constant temperatures are presented in Table 2. For 1st, 2nd, 3rd and 4th instars, the developmental duration ranged from 5.58 to 1.11, 6.32 to 1.00, 6.47 to 1.13, and 6.74 to 1.10 days, respectively, for aphids held at 10, 15, 20, 25, 30 and 35°C. Total nymphal duration to reach adulthood ranged from 25.11 d (10°C) to 4.38 d (30°C). With the exception of 1st instar nymphs, the shortest instar-specific developmental durations were exhibited by aphids at 25°C (1.10d, 4th instar) or 30°C (1.00d, 2nd instar, and 1.13d, 3rd instar). The shortest total nymphal duration was observed at 30°C (4.38d). A general rise in developmental duration was observed from 30° to 35°C for total nymphal duration, and for all immature stages except 1st instar, where the difference was numerically very small (0.02). None

of the differences in developmental durations between 30° and 35°C were statistically significant.

As with previous studies, general aphid developmental time shortened with rising temperature, to an upper limit after which the developmental duration increased. Results of this study indicated that the developmental duration for all immature stages is shortest at 25° or 30°C, and total nymphal duration is shortest at 30°C. These results are in agreement with those of Xia et al. (1999) [8] and Kersting et al. (1999) [8]. At 35°C, a numerical rise in developmental duration was observed for all immature stages (except first instar) as compared to 30°C. These differences were not statistically significant, but the fact that they were observed for 2nd, 3rd and 4th instars and for the total nymphal duration indicates that the upper temperature limit above which aphid development will slow may occur between 30° and 35°C. Developmental duration of aphids (all instars and in the total) held at 10°C and 15°C were significantly different from each other and from that of the aphids held at the higher temperatures (Table 2). With the exception of second instars, at higher temperatures of 20°C to 35°C, a general pattern was evident that the number of significant differences between developmental durations of aphids held at the different constant temperatures became fewer as the aphid moved from one instar to the next. For 1st instars, developmental durations at 30° and 35°C were not significantly different, while 3rd and 4th instars had no significant differences in developmental durations between 25°, 30° and 35°, and 20°, 25°, 30° and 35°C, respectively.

The clean statistical breaks between developmental durations of all immature stages of aphids held at 10° and 15°C (Table 2) indicate that at lower temperatures, all immature aphid stages (1st to 4th instars) are affected similarly by temperature changes. However, at higher temperatures (20° to 35°C), a general progressive lack of significant differences in developmental durations as aphids molted to later instars indicates a smaller influence of increasing temperature on larger aphid immature. Developmental threshold temperatures were calculated to be 6.3°, 6.7°, 5.9°, 5.9°, and 6.26°C for 1st, 2nd, 3rd and 4th instars and for total nymphal development, respectively.

Survivorship and fecundity of test aphids held at the six constant temperatures are presented in this study. In viewing the survivorship curves (l_x) for aphids held at the six constant temperatures, a general trend is evident. As temperature increased, survivorship progressively fell more precipitously, indicating increasing aphid mortality at a younger age. At 10°, 15°, 20°, 25°, 30° and 35°C, aphid survivorship dropped below 80% at 39, 29, 33, 21, 20, and 14 days of life, respectively. At the same

temperatures, aphid survivorship dropped below 20% at 52, 51, 47, 44, 38, and 17 days of life, also respectively. The drop in survivorship was particularly sharp at 35°C, dropping from 94% on the 12th day of life to 17% on the 17th day of life, a period of five days. Fecundity curves (Figures 2.1-

2.2) also showed an obvious pattern. Number of days elapsed until first deposition of progeny decreased progressively and sharply at temperatures 10^o (26d) to 15^o (14d) to 20^o C (8d), then stabilized at five days for 25^o, 30^o and 35^oC.

Table 2 Instar-specific and total nymphal development duration days (\pm SEM) of *Aphis gossypii* at six constant temperatures in laboratory

Stage	10°C	15°C	20°C	25°C	30°C	35°C	F	P
Instar I	5.58a (\pm 0.14)	3.56b (\pm 0.14)	2.28c (\pm 0.10)	1.60d (\pm 0.11)	1.13e (\pm 0.07)	1.11e (\pm 0.08)	253.6	<0.001
Instar II	6.32a (\pm 0.19)	3.55b (\pm 0.17)	1.50c (\pm 0.12)	1.40c (\pm 0.11)	1.00d (\pm 0.00)	1.21cd (\pm 0.10)	273.6	<0.001
Instar III	6.47a (\pm 0.22)	3.15b (\pm 0.11)	1.61c (\pm 0.12)	1.15d (\pm 0.11)	1.13d (\pm 0.07)	1.18d (\pm 0.10)	275.8	<0.001
Instar IV	6.74a (\pm 0.49)	3.25b (\pm 0.25)	1.50c (\pm 0.12)	1.10c (\pm 0.07)	1.13c (\pm 0.07)	1.28c (\pm 0.16)	87.8	<0.001
Total duration	25.11a (\pm 0.37)	13.50b (\pm 0.36)	6.89c (\pm 0.38)	5.25d (\pm 0.36)	4.38d (\pm 0.32)	4.53d (\pm 0.37)	515.4	<0.001

Means within a row, followed by the same letter, are not significantly different ($P < 0.05$; LSD)

Table 3 Life history parameters (\pm SEM) of *Aphis gossypii* at six constant temperatures in laboratory

Stage	10°C	15°C	20°C	25°C	30°C	35°C	F	P
Pre-reproductive Period (d)	2.84a (\pm 0.34)	1.79b (\pm 0.18)	1.11c (\pm 0.08)	0.65c (\pm 0.11)	0.83c (\pm 0.08)	0.78c (\pm 0.13)	23.9	<0.001
Age of adult at first reproduction	29.69a (\pm 0.33)	16.56b (\pm 0.31)	9.00c (\pm 0.31)	6.90d (\pm 0.30)	6.20d (\pm 0.27)	6.65d (\pm 0.32)	829.9	<0.001
Lifetime fecundity per female	7.89d (\pm 2.54)	28.50c (\pm 2.47)	43.94b (\pm 2.61)	44.75b (\pm 2.47)	53.08a (\pm 2.26)	13.94d (\pm 2.61)	54.79	<0.001
Longevity (d)	43.32a (\pm 2.54)	40.80a (\pm 2.22)	39.39a (\pm 2.34)	29.65b (\pm 2.22)	28.21b (\pm 2.03)	14.22c (\pm 2.34)	22.49	<0.001
Sample size (n)	19	20	18	20	24	18		

Means within a row, followed by the same letter, are not significantly different ($P < 0.05$; LSD)

Table 4 Life history characteristic (\pm SEM) of *Aphis gossypii* at six constant temperatures in laboratory

Parameters	Temperature in °C						F	P
	10	15	20	25	30	35		
GRR	9.76f (\pm 0.114)	35.95d (\pm 0.111)	46.32c (\pm 0.117)	50.51b (\pm 0.111)	58.93a (\pm 0.102)	17.32e (\pm 0.117)	30273.8	<0.0001
R_0	7.89f (\pm 0.124)	28.50d (\pm 0.114)	43.94c (\pm 0.127)	44.75b (\pm 0.121)	53.08a (\pm 0.111)	13.94e (\pm 0.127)	22611.0	<0.0001
R_m	0.06144e (\pm 0.008)	0.1496d (\pm 0.008)	0.27704c (\pm 0.008)	0.374031 (\pm 0.008)	0.31218a (\pm 0.007)	0.32363b (\pm 0.008)	203.3	<0.0001
λ	1.06350e (\pm 0.011)	1.16176d (\pm 0.011)	1.31957 (\pm 0.011)	1.45516a (\pm 0.011)	1.36771b (\pm 0.010)	1.38394b (\pm 0.011)	166.7	<0.0001
DT	12.36a (\pm 0.409)	4.78b (\pm 0.400)	2.52c (\pm 0.420)	1.88c (\pm 0.400)	2.26c (\pm 0.366)	2.20c (\pm 0.420)	99.6	<0.0001
n	19	20	18	20	24	18		

GRR = Gross Reproductive Rate; R_0 = Net Reproductive Rate; R_m = Intrinsic Rate of increase; DT = Doubling Time;

λ = Finite Rate of increase; n = number of Aphids examined at each temperature

Means within a row followed by the same letter are not significantly different ($P < 0.05$; LSD)

Selected life table parameters are presented in Tables 2.3 and 2.4. The average ages reached by female aphids before production of first progeny decreased sharply as temperature increased from

10^o (29.69d) to 15^o (16.56d) to 20^oC (9.00d), before stabilizing between six and seven days for temperatures 25^o, 30^o, and 35^oC (Table 3).

The pre-reproductive rate of aphids (time between reaching adulthood and producing progeny) ranged from 2.84d at 10°C to 0.78d at 35°C with a low of 0.65d at 25°C. Average longevity of test aphids also declined as temperature increased, with a sharp decline observed at 35°C. Average lifetime fecundity of females rose from a low of 7.9 progeny at 10°C to a peak of 53.1 progeny at 30°C, and then declined sharply to 13.9 progeny at 35°C (Table 3).

Gross reproductive rate (GRR) an indicator of lifetime fecundity, ranged from 9.8 for aphids held at 10°C to 58.9 for aphids held at 30°C (Table 4). A general rise in GRR was observed with rising temperature to 30°C, but GRR declined sharply from 30°C to 35°C (-41.6) and was significantly lower than the GRRs of aphids held at all other temperatures except 10°C (Table 4). The same pattern was observed for net reproductive rate (R_0), which incorporates fecundity and survivorship. Finite rate of growth (λ), which combines fecundity, survivorship and longevity that indicates whether a population is increasing or decreasing (<1=decreasing, >1=increasing, 1=stable), also showed a general increase with increasing temperature, but peaked at 25°C (1.45516). Interestingly, λ was higher at 35°C than 30°C, but this difference was numerically very small and not significant. Doubling time (DT) exhibited much the same pattern as λ as far as advancement of aphid development (unlike λ , a lower DT result in faster aphid population development), with the best DT of 1.88 at 25°C. The DTs at the highest temperatures of 30°C (2.26d) and 35°C (2.20d) were relatively low, particularly when compared to 10°C and 15°C (Table 4).

Life history parameters calculated for this study that measure the onset and level of fecundity of cotton aphids rose with rising temperature to an upper limit. At lower temperatures, the onset of reproduction was delayed and the level of lifetime fecundity was lower. This upper temperature limit appears to be between 30°C and 35°C. At 35°C temperature onset of reproduction was not delayed, but lifetime fecundity was significantly lower than at the more moderate temperatures. The finite rate of growth (λ) of aphids held at 35°C was relatively high and the doubling time (DT) was relatively low when compared to the aphids held at the lower temperatures (Table 4). However, this was offset by the higher mortality of aphids held at the higher temperature.

Results of this study indicate that 25°C to 30°C is the optimum temperature range for cotton aphid development, reproduction and population increase. At 30°C the lifetime fecundity of female aphids was the highest of all temperatures tested. However, at 30°C, the survivorship curve had a steeper decline

from the 15th to the 45th day of life indicating higher mortality in this time period. At 25°C, the finite rate of growth (λ) was highest and the doubling time (DT) was shortest of all the temperatures tested (Table 4). The lower mortality suffered by aphids at 25°C (compared to 30°C) very likely is the cause of the rise in these parameters. Finite rate of growth (λ) and doubling time (DT) are the more important parameters describing population increase.

As a result, the optimum temperature is closer to 25°C. The results of this study agree with previous studies that the optimum aphid development temperature (held constant) in a laboratory study is 25°C to 30°C, and provide affirmation for the previous research.

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