Influence of temperature on the biological parameters of the anholocyclic species *Cinara tujafilina* (Hemiptera: Aphidoidea)

Roma Durak¹*, Beata Borowiak-Sobkowiak²

¹University of Rzeszow, Department of Invertebrate Zoology, 35-601 Rzeszów, Poland
²University of Life Sciences, Department of Entomology and Environmental Protection, 60-594 Poznań, Poland

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Abstract: Aphids are a good model to study insect reaction to habitat change. Temperature is one of the main factors that influences insects. This paper examines the influence of temperature on developmental stages, fecundity, survival rate and demographic parameters of *Cinara tujafilina* (Hemiptera: Aphidoidea, Lachnidae), connected with decorative plants of the Cupressaceae family. *C. tujafilina* was reared in a laboratory on *T. orientalis* at five constant temperatures of 10, 15, 20, 25 and 28°C, 70% humidity and 14L:10D. The pre-reproduction stage varied from 7 at 25°C to 19 days at 10°C. Developmental threshold was assigned at 3.5°C. The longest reproduction stage for the aphids developing was recorded at 25°C, namely 33 days, while the shortest, at the temperature of 10°C, lasted 8 days. At 25°C this species is characterised by the shortest pre-reproduction stage, the highest fecundity, the highest survival rate and the highest demographic parameters, particularly $r_m$ (0.17). The results suggest that the optimal temperature for the species is 25°C, and indicate that climatic change will favourably influence its development and increase its role as a pest of decorative plants.

Keywords: Lachnidae • *Cinara* sp. • Development • Life table • Temperature • Ecology • Climatic change

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1. Introduction

Climatic change phenomena lead to faunistic changes [1]. Aphids have been indicated as a good study model due to their flexibility and telescopic development, which enables them to react very quickly to changing habitat conditions [2,3]. The main factor influencing insects is temperature, apart from photoperiod and the quality of the host plant. Climate changes influence the following: species diversity and the terms of spring aphid flights [3-9], the geographical range of thermophilous species [10], life cycles (an increased share of anholocyclic clones, i.e. those which develop parthenogenetically throughout the year in comparison with holocyclic aphids, i.e. those producing sexual morphs) [11-14], as well as biology and development [15]. Climate warming most strongly influences the development and the number of aphid generations in comparison with other insects [4,7]. Yamamura & Kiritari [16] and Harrington *et al.*,[4] claim that as a consequence of 2°C temperature rise during the year aphids can produce up to 5 more generations.

Climate changes particularly favour fauna feeding on plants introduced in European countries, also including ornamental plants. Decorative plants of the Cupressaceae family, such as *Chamaecyparia* sp., *Cupressus* sp., *Juniperus* sp, *Thuja* sp, are now more and more often grown in parks and gardens. Thanks to climate warming the insects connected with those plants can expand their ranges and adapt their life cycles to local conditions.

*Cinara tujafilina* (del Guercio, 1909) (Hemiptera: Aphidoidea, Lachnidae) has been recently recorded in Poland as a permanent species, however, it is recorded more frequently every year [17]. It is an anholocyclic species, which means it always develops
parthenogenetically. Its original range covered the warm regions of Asia [18]. As a result of warming, the species expanded its range onto Europe, e.g. Italy [19] and Poland [17]. C. tujafilina host plants belong to the Cupressaceae family, with the main Thuja orientalis [18]. Other species of the Cinara sp. genus also infest the Cupressaceae, particularly Cinara cupressi, and are considered very serious pests of those plants [20,21]. Little is known about C. tujafilina biology. Some studies of yearly dynamics of this species have been conducted in Japan [17,22]. As an anholocyclic species it has significant potential to develop on host plants almost year round, completely depending on the ambient temperature. Thus the question arises as to how the temperature influences its development and fecundity and how its demographic parameters change depending on temperature and whether they enable it to become a serious pest.

These previous studies indicate relationships between temperature and developmental stages length, development rate, survival, fecundity and demographic parameters of C. tujafilina populations in laboratory conditions.

2. Experimental Procedures

2.1 Aphid source
C. tujafilina were collected in Rzeszów, Poland. Their host plants were T. orientalis L. The collected females were taken to a laboratory, where they were bred on lignified shoots so that the colonies would grow large and larvae could reach maturity.

2.2 Experimental procedures
The aphids were bred on T. orientalis at five different temperatures: 10°C, 15°C, 20°C, 25°C, 28°C, 75% humidity and photoperiod of 14L:10D in a climate chamber (Sanyo MLR-351H). They were bred on 1.5 to 2.5-year old bushes. The plants were obtained from a nursery of ornamental plants and they were always the same cultivar of the species named “Aurea Nana”. The plants were put in pots (21×21×19 cm) and watered three times a week.

For each temperature 20 young apterous aphids (in the same age) were individually placed on shoots. The length of the following developmental stages was recorded: pre-reproduction, reproduction, post-reproduction, the total life span and female fecundity. The observations were carried out five times a week. Spatial separation of aphids was achieved using 27×20 cm sheer gauze cover secured at the base of the branch, enabling movement of the specimens only within the area isolated by the cover.

To determine the demographic parameters of populations, 100 larvae were observed. At each temperature about 50 young adults were introduced onto each bush. The larvae they bore within 24 h were then used in the experiment. A hundred of the first larvae were isolated in each temperature on a twig in a gauze cover and observed five times a week. In order to calculate the total fecundity of the population and the mean fecundity of the female, newly bred larvae were taken with a thin brush and counted. Demographic parameters for the aphid populations at different temperatures were determined according to the methods described by Birch [23]. The net reproduction rates – R0, mean generation time – T, intrinsic rate of increase – r, finite rate of increase – λ, time necessary for the population to double – DT, were calculated. The intrinsic rate of increase (r), calculated with the Wyatt and White formula [24], relates the fecundity of an individual aphid to its development time: r = (lnM) / D. D means the developmental period from birth to the beginning of the first reproduction (pre-reproductive period), while Mj is the number of nymphs produced by the adult in the first D days of reproduction after the adult moult. The net reproduction rates Ro=Σ(mj-1) (where mj and mi were cumulative daily survival and fertility respectively); mean generation time T=lnR0/r, finite rate of increase λ=e^r and doubling time DT=ln2/r.

2.3 Statistical analysis
Statistical analyses, conducted with a difference test (proportion test), evaluated the data on particular developmental stages of generations, the total lifespan and fecundity in different temperatures. The linear regression analysis was used to estimate the lower developmental threshold for the aphids by using the developmental rate data obtained at constant temperatures from 10°C to 25°C. Developmental rates obtained at 28°C were outside the growth curve and not included in the linear regression. On the basis of regression equation the number of degree-days necessary for development at lower developmental threshold was calculated. The data were analysed using ANOVA, StatSoft, Inc., STATISTICA, version 9.0, www.statsoft.com.

3. Results
Developmental times for the whole development at five different temperatures are presented in Figure 1. The pre-reproduction stage took 7.2 to 19.2 days. The longest development phase was recorded for the specimens developing at 10°C, and the shortest for those at 25°C.
Influence of temperature on the biological parameters of the anholocyclic species Cinara tujafilina (Hemiptera: Aphidoidea) (Figure 1). The pre-reproduction stage depended on the temperature. The rate of nymphal development was higher at 25°C than at any other temperature (Figure 2). The developmental rate was positively and linearly related to rearing temperature. Based on the regression equation y=0.0058x-0.0192, R²=0.8418, the threshold temperature for development is 3.5°C. Thus, the degree-day requirement was found to be 172.4 dd, which is the period from birth to becoming an adult.

When aphids develop at 10°C, the pre-reproduction period takes up as much as 48% of the lifespan. At 25°C, the same period lasts only 18% of the lifespan (Table 1). Statistically significant differences in lifespan were indicated between the lifespan of aphids at 10°C and 25°C (Proportion tests, P≤0.01) as well as between 15°C and 25°C (Proportion tests, P≤0.01).

The reproduction period took 7.8 to 33.2 days (Figure 1, Table 1). The longest reproduction period was recorded for the aphids developing at 25°C, while the shortest occurred at 10°C. As many as 83% of the lifespan at 25°C and 70.2% at 20°C are taken up by the reproduction period, while for lower temperatures the values are only 19.5% at 10°C, 25.5% at 28°C and about 30% at 15°C. These are statistically significant differences: between 10°C and 20°C (Proportion tests, P≤0.001); 10°C and 25°C (Proportion tests, P≤0.001); 15°C and 20°C (Proportion tests, P≤0.001); 15°C and 25°C (Proportion tests, P≤0.001); 20°C and 28°C (Proportion tests, P≤0.001); 25°C and 28°C (Proportion tests, P≤0.001). The post-reproduction period was recorded only for the specimens bred at 10°C, 15°C and 28°C and for them it took 1.1 to 4.2 days (Figure 1).

Aphid lifespans were between 25.1 and 40 days, depending on temperature. The specimens bred at 20°C and 25°C lived the longest, while those at 28°C – the shortest (Figure 1, Table 1). The total lifespan at various temperatures was statistically significantly different between the temperatures of 10°C and 20°C (Proportion tests, P≤0.001); 10°C and 25°C (Proportion tests, P≤0.001) as well as between 15°C and 20°C (Proportion tests, P≤0.001); 15°C and 25°C (Proportion tests, P≤0.001); 28°C and 20°C (Proportion tests, P≤0.001); 28°C and 25°C (Proportion tests, P≤0.001). The aphid survival rate (lx) changed with the temperature (Figure 3). Even 100% of C. tujafilina larvae reached maturity on the 9th day of life at 25°C, while for 10°C the value was 80% on the 21st day of development (Figure 3).

The temperature also influenced the total fecundity of aphid populations, which ranged between 688 and 2626 larvae and increased as the temperature grew from 10°C to 25°C and decreased at temperatures higher than 25°C (Table 1). Thus, at 10°C and 28°C females bred only 26.2% and 21.3% respectively, compared to those reared at 25°C. The differences in fecundity

Figure 1. Developmental time of apterous Cinara tujafilina as a function of temperature a) prereproduction, b) reproduction, c) postreproduction.
Table 1. Mean developmental time, longevity, total fecundity and rate of development of *Cinara tujafilina* at different temperature regimes (n=20).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Prereproduction</th>
<th>Reproduction</th>
<th>Postreproduction</th>
<th>Longevity</th>
<th>Fecundity n=100</th>
<th>Rate of development</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Days</td>
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<td>Days</td>
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<td>%</td>
</tr>
<tr>
<td>10</td>
<td>19.2±0.4</td>
<td>48</td>
<td>7.8±0.6</td>
<td>19.5</td>
<td>1.1±0.3</td>
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</tr>
<tr>
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<td>18.3±0.4</td>
<td>45.7</td>
<td>13.5±0.8</td>
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<td>1±0.0</td>
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</tr>
<tr>
<td>20</td>
<td>12.1±0.3</td>
<td>30.2</td>
<td>28.1±0.3</td>
<td>70.2</td>
<td>0±0.0</td>
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<td>25</td>
<td>7.2±0.4</td>
<td>18</td>
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<td>83</td>
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<tr>
<td>28</td>
<td>10.7±0.9</td>
<td>26.7</td>
<td>10.2±0.4</td>
<td>25.5</td>
<td>4.2±1.3</td>
<td>10.5</td>
</tr>
</tbody>
</table>

**Figure 2.** Rate of development (1/days) of larval stage of apterous *Cinara tujafilina* as a function of temperature. $y=0.0058x-0.0192$, $R^2=0.8418$; ANOVA, $F=202.24$, d.f. =1.38, $P<0.000$. 

**Figure 3.** Survival rates of apterous *Cinara tujafilina* at different temperatures.
of the females reared at different temperatures were statistically significant between all the temperatures: 10°C and 15°C, 10°C and 20°C, 10°C and 25°C, 15°C and 20°C, 15°C and 25°C, 20°C and 25°C, 28°C and 25°C, 28°C and 20°C, 28°C and 15°C, all at P≤0.0001 by proportion tests. The highest mean daily fecundity was 3.97 larvae per female on the 22nd day of life at 20°C (Figure 4).

The intrinsic rate ($r_m$) was the highest (0.17) and the lowest (0.09) at 25°C and 10°C, respectively (Table 2). The calculated demographic parameters of the population indicate that during the day the population grows between (La) 1.09 and 1.19 times along with temperature increase and decrease at 28°C. The mean development time for one generation (T) is from 21.88 days to 17.22 and in that time the population increases 6.88 to 26.24 times (Ro) along with temperature growth and decrease at 28°C (Table 2).

4. Discussion

The study confirms that the temperature is an important factor that can influence many aspects of aphid life. Several main consequences of temperature growth have been recorded for C. tujafilina. Firstly, it was established that as the temperature increases to 25°C, the pre-reproduction period, i.e. larvae developmental time, shortens, while at the same time the rate of development increases. Similar relations were observed for other aphid species, eg. T.salignus [25].

The developmental threshold was found to be 3.5°C, which means that at this temperature the development time for the species from the 1st instar to an adult is long and equals 172 dd. This indicates that the population developing in Poland differs in terms of developmental threshold from the population in Japan, for which the temperature was established at 9.2°C [22] and which can develop in lower temperatures. This corroborates earlier observations that aphid populations separated geographically can differ in terms of temperature tolerance [26,27]. Estimated development rate at the temperature constituting the developmental threshold of C. tujafilina is, however, shorter than for other Lachnidae species, eg. C. cupressi, for which it is 239 dd [20] and

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
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</tr>
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<tr>
<td>10</td>
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</tr>
<tr>
<td>15</td>
<td>17.19</td>
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<td>20.07</td>
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<tr>
<td>25</td>
<td>26.24</td>
</tr>
<tr>
<td>28</td>
<td>5.60</td>
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<table>
<thead>
<tr>
<th>$R_o$</th>
<th>$r_m$</th>
<th>$\lambda$</th>
<th>$T$</th>
<th>$DT$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.88</td>
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<td>1.09</td>
<td>21.43</td>
<td>7.70</td>
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<td>1.14</td>
<td>21.88</td>
<td>5.33</td>
</tr>
<tr>
<td>20.07</td>
<td>0.15</td>
<td>1.16</td>
<td>19.87</td>
<td>4.62</td>
</tr>
<tr>
<td>26.24</td>
<td>0.17</td>
<td>1.19</td>
<td>19.22</td>
<td>4.08</td>
</tr>
<tr>
<td>5.60</td>
<td>0.10</td>
<td>1.11</td>
<td>17.22</td>
<td>6.93</td>
</tr>
</tbody>
</table>

Table 2. Life table parameters describing Cinara tujafilina as a function of temperature.

$R_o$, net reproductive rate; $r_m$, intrinsic rate of increase; $\lambda$, finite rate of increase; $T$, generation time; $DT$, doubling time.
T. salignus, for which it is 196 [25]. This number of degree-days which a female needs to develop at developmental threshold is similar to the one for species considered to be economically important, such as B. brassicae (142.9 dd) [28] or R. padi (149.2 dd) [29].

Particularly interesting is the influence of temperature on the reproduction stage of C. tujafilina. It was shown that as the temperature grows, the reproduction period lengthens, reaching its highest value at 25°C. As a result the species can breed during over 80% of its lifespan. This indicates a different development model from T. salignus, for which the reproduction period shortens as the temperature grows [25]. Along with the lengthening of reproduction period, fecundity also grows and reaches its peak value at 25°C, at which temperature females start breeding larvae the soonest and breed the longest. The number of larvae at 25°C is highly statistically significant, almost four times as high as the one at 10°C. On the other hand, T. salignus and M. rosae show the highest fecundity at about 20°C, and then the fecundity falls along with the temperature growth [25,27].

Mortality and survival rates (lx) depend on temperature just like for other aphid species. However, for T. tujafilina the temperature increase definitely favours the aphid survival rate. The same is true for C. cupressi [20] and Metopolophium dirhodum [30], for which low temperatures result in prolonging the prereproduction and delay the reaching of maturity. But in C. tujafilina, the growth of temperature by lengthening aphid reproduction period, however, resulted in lengthening of the total lifespan and a growth in aphid survival rate. Thus the formula for the reaction to temperature differs from those described for C. cupressi, T. salignus, M. rosae and others [20,25,27].

Another proof of temperature’s impact on C. tujafilina is an increase in the intrinsic reproduction rate that corresponds to a temperature increase. This value is highest at 25°C. It is interesting that all the rm values obtained are higher than those for C. cupressi [20]. Intrinsic rate of increase (rm) can be used as a factor informing on the positive or negative impact of a factor. Also, the growth of reproduction rate was observed with a shortening mean time of one generation development at the same time. The demographic parameters for the species are higher than those for C. cupressi [20] and only slightly lower than those for T. salignus, the species known to create very large colonies and damage Salix spp, particularly in commercial willow plantings [25]. It is worth noting the lack of post-reproduction stage for specimens developing at higher temperatures. This is unlike T. salignus, which underwent this stage regardless of the temperature [25].

The study indicates that the optimal temperature for C. tujafilina development is 25°C. At such temperature the species is characterised by the shortest prereproduction period, the highest fecundity and the highest demographic parameters, particularly rm.

It is acknowledged that the optimal temperature for aphids is 20-22°C. This is the case for Macrosiphum rosae, M. euphorbiae, A. solani, U. ambrosiae and others [27,31]. On the other hand, A. gossypii, A. glycines, Brevicoryne brassicae develop best at their optimal temperature of 25°C [28,32,33]. Temperatures close to 28-30°C are considered lethal for aphids [3].

These analyses indicate that an increase in ambient temperature is favourable for C. tujafilina and provides good conditions to develop and breed. Thus it is a species which in favourable temperatures can become an important pest, comparable to C. cupressi. Its advantage over the other species is its anholocyclic development, which means that it can infest plants not only in spring and summer, but throughout the year. Anholocyclic specimens will be the first to infest new host plants in early spring, as they do not need time to hatch. It must also be noted that high temperatures over 20°C favourably influence also the possibility of anholocyclic development for other aphid species [14]. Our study corroborates the observation that European aphid species live at temperatures below their optimal ones. Consequently, climate warming will favour the growth of aphid populations, enabling its considerable increase, mainly through the increase in development rate and fecundity [3,4], but also by lengthening the reproduction stage and survival rates.

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