

## Research Article

# Effect of Air Humidity on Sex Ratio and Development of Ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae)

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Length of development of larvae and pupae of the invasive alien ladybird beetle *Harmonia axyridis*, their survival rates, sex ratio, and fresh mass of the emerged adults were measured at three contrasting levels of relative air humidity: 30, 60, and 90%, 25°C and photoperiod 16L:8D. Overall sex ratio was 51%, but there was a strong trend for higher proportion of males at low humidity and higher proportion of females at high humidity. Survival rate, larval developmental time, and adult mass were all differently influenced by air humidity depending on the food type. In individuals fed with aphid *Acyrtosiphon pisum* there was a trend for better survival, shorter development, and higher mass gained at higher humidity. These trends were opposite or nonsignificant in individuals fed with frozen eggs of moth *Ephestia kuehniella*.

## 1. Introduction

The multicoloured Asian lady beetle or harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is native to eastern temperate Asia. It has a long history of use as a classical biological control agent of aphids and coccids in North America and has been deliberately introduced and has subsequently spread in four continents at a very fast rate during the last 23 years [1]. A CLIMEX model has been set up [2] to show the potential geographic distribution of *H. axyridis* that has been subsequently largely proven by new records. Besides several environmental temperature parameters and conditions for diapause, the model takes into account moisture parameters for the prediction of successful establishment in new areas. However, there are some regions where the prediction was not accurate. In Greece, *H. axyridis* was temporally established on orange trees heavily infested with aphids in 1993-1994 [3], but failed to survive in following years despite continued releases [4]. Another case of a failure in establishment of *H. axyridis* was observed

[5] on the Azores islands. According to these studies, the released populations failed to establish due to ecological factors such as the maladaptation to the local conditions, mainly high temperature during diapause, and functional diversity saturation. Anyway, a conspicuous environmental trait that differs in these two regions from those where *H. axyridis* was successful is air humidity: very low in Greece and very high in Azores.

Among ambient factors controlled in laboratory experiments measuring development time and other life history traits of ectotherms, temperature and photoperiod are the most commonly and best studied, while moisture, for example, as relative air humidity, is often neglected: either not properly controlled or set to one value (e.g., [6]). More attention attracted moisture of substrate in soil arthropods [7] and of food or controlled atmosphere in stored product insects [8].

Developmental time of *Ophraella communa* (Coleoptera: Chrysomelidae) at different stages shortened along with the increasing relative air humidity (60%, 75%, and 90% RHs).

TABLE 1: Survival of developmental stages of *Harmonia axyridis* reared on two diets and in three levels of relative air humidity at 25°C and 16L:8D photoperiod. *N*: initial number of second instar larvae, S3, S4, P and A: percentage survival to particular larval instar, pupal, or adult stage relative to the initial number of individuals, M: sex ratio (percentage of males). Chi-square tests with their probabilities were calculated for three-dimensional contingency table (food  $\times$  humidity  $\times$  survival/sex).

Food	RH %	<i>N</i>	S3 %	S4 %	P %	A %	M %
<i>Acyrtosiphon</i>	30	100	74	55	26	24	71
<i>Acyrtosiphon</i>	60	80	84	70	36	36	48
<i>Acyrtosiphon</i>	90	90	83	67	36	34	39
<i>Ephestia</i>	30	50	84	72	60	58	62
<i>Ephestia</i>	60	50	82	70	50	50	52
<i>Ephestia</i>	90	40	75	60	28	28	18
$\chi^2$ ( $d = 2$ )			6.59	9.22	23.58	24.25	18.21
<i>P</i>			0.037	0.010	$<10^{-4}$	$<10^{-4}$	$10^{-4}$

The survival rates during the egg, larva, and entire immature stage were significantly higher at 75% RH and 90% RH than at 60% RH [9]. Low RH (20%) prolonged developmental duration of eggs and larvae reduced egg hatching and larval survival and reduced the body mass and body length of pine caterpillar, *Dendrolimus tabulaeformis* (Lepidoptera: Lasiocampidae), compared to 40%, 60%, and 80% RH [10]. As vapour pressure deficit decreased from 2.8 to 0.009 kPa, median life expectancy increased from 1.1 to 9.0 days for plum curculio *Conotrachelus nenuphar* (Coleoptera: Curculionidae) without food supply [11]. Complete development of *Ahasverus advena* (Coleoptera: Silvanidae) at 70% RH took 67, 58, and 48 days at 20, 22.5, and 25°C, respectively, while it was shorter (46, 31, and 26 days) at 90% RH [12].

The present study shows the effect of ambient humidity on some life history parameters of the ladybird *H. axyridis* that might play role in different establishment ability during its invasion to new areas.

## 2. Material and Methods

Adult beetles of the colour form *succinea* were collected outdoors and placed in laboratory, temperature 20°C, photoperiod 16L:8D, and RH 60%, and fed with aphids *Acyrtosiphon pisum*. Eggs were removed from parents and placed to the experimental incubators at 25°C, 16L:8D, and three contrasting levels of relative humidity: 30, 60, and 90%. They were checked daily for hatching. Neonate larvae were fed either with aphids *Acyrtosiphon pisum* or frozen eggs of *Ephestia kuehniella*. After moulting to the second instar, larvae were placed in groups of ten into 0.5 L glass jars covered with nylon mesh to provide air flow. They were continuously fed with either diet and provided with water in a vial that enabled limited evaporation. The food of larvae was offered in abundance to minimize their cannibalism. Survival and time of moulting to subsequent larval instars, pupa, and adult were recorded daily to calculate development time. Developmental time of individual instars was based on all individuals that survived to that particular instar even if they subsequently died. Adults were sexed one day

after emergence, and fresh mass was recorded on electronic balances with precision 0.1 mg.

Low humidity (30%) was achieved by placing dishes with sodium hydroxide to the incubator; moderate and high humidity was made thanks to small or large evaporation surface of tissue dipped to dish with tap water. Temperature and humidity were measured with electronic equipment placed in the incubator among the jars with beetles and recorded daily. Precise environmental conditions measured twice daily in the three experimental incubators were (mean  $\pm$  SD) 24.9  $\pm$  0.1°C and 29  $\pm$  6% RH, 24.9  $\pm$  0.2°C and 59  $\pm$  5% RH, and 25.0  $\pm$  0.2°C and 87  $\pm$  1% RH with no difference between morning and evening.

## 3. Results

**3.1. Survival.** Overall, survival of larvae from the second to the third instar (S3) was 80%, to the fourth instar, it was 65%, to pupa 37%, and to adult 36%. Individual treatments (food and humidity) slightly differed from each other (Table 1) in survival to the third and fourth larval instars, but there was much better survival to pupa and adult in individuals fed with *Ephestia* eggs than those fed *A. pisum* ( $\chi^2$  ( $d = 1$ ) = 9.35,  $P = 0.0022$ ). Survival to pupa and adult on *Ephestia* eggs was lower at 90% RH than in the two lower humidities ( $\chi^2$  ( $d = 2$ ) = 8.71,  $P = 0.013$ ). Survival to pupa and adult on *A. pisum* was lower at 30% RH than in the two higher humidities but this difference was not significant ( $\chi^2$  ( $d = 2$ ) = 3.81,  $P = 0.15$ ).

**3.2. Sex Ratio.** Overall, sex ratio in newly emerged adults was 51%, but there was a significant ( $\chi^2$  ( $d = 2$ ) = 10.06,  $P = 0.0065$ ) trend for higher proportion of males at low humidity and higher proportion of females at high humidity in both food treatments (Table 1).

**3.3. Developmental Time.** Development time of the first instar was two days except in larvae fed with *Acyrtosiphon* at 30% RH, where it was slightly but significantly prolonged (two-way ANOVA,  $F_{2,404} = 4.76$ ,  $P = 0.009$ ) (Table 2). There were opposite trends in the development time of the second instar, shortening with increasing humidity in larvae fed with

TABLE 2: Developmental time (days) and adult fresh mass (mg) of *Harmonia axyridis* reared on two diets and in three levels of relative air humidities at 25°C and 16L:8D photoperiod. D1 to D4, DL and DP: development time of particular larval instar, entire larval stage, and pupa, FMM: fresh mass of males, FMF: fresh mass of females. Means that do not differ significantly are marked with the same letter in each column.

Food	RH %	D1 days	D2 days	D3 days	D4 days	DL days	DP days	FMM mg	FMF mg
<i>Acyrtosiphon</i>	30	2.1 a	3.6 a	3.4 bc	7.5 a	15.1	5.0	18.2	20.7
<i>Acyrtosiphon</i>	60	2.0 b	3.3 ab	3.5 bc	6.2 b	14.1	5.0	18.3	25.1
<i>Acyrtosiphon</i>	90	2.0 b	3.1 b	3.1 c	6.4 ab	13.5	5.0	19.2	25.4
<i>Ephestia</i>	30	2.0 b	3.1 ab	3.8 ab	6.3 b	14.6	5.0	19.4	23.0
<i>Ephestia</i>	60	2.0 b	3.3 ab	4.0 a	6.9 ab	15.4	5.0	19.4	23.5
<i>Ephestia</i>	90	2.0 b	3.5 ab	3.1 c	7.3 a	14.3	5.0	14.5	21.8

*Acyrtosiphon* and prolonging with increasing humidity in larvae fed with *Ephestia* (two-way ANOVA,  $F_{2,323} = 4.01$ ,  $P = 0.019$ ). In the third instar, there was high effect of humidity (one-way ANOVA,  $F_2 = 13.4$ ,  $P = 3.10^{-6}$ ) with shorter development at high humidity and a separate effect of food (one-way ANOVA,  $F_1 = 10.4$ ,  $P = 0.001$ ) with longer development time in larvae fed with *Ephestia* eggs than with *A. pisum*. In the fourth instar, there were again opposite trends in the development time ( $F_{2,147} = 5.4$ ,  $P = 0.005$ ) with shortening of time with increasing humidity in larvae fed with *Acyrtosiphon* and vice versa. When analyzing the entire larval development, the opposite trends were still present but not significant (two-way ANOVA,  $F_{2,147} = 2.56$ ,  $P = 0.08$ ); no difference accountable to either food, humidity, or their interaction was found. Pupal development time was identical in all treatments (two-way ANOVA,  $F_{2,143} = 0.08$ ,  $P = 0.92$ ).

**3.4. Body Size.** There was a trend for increasing adult body mass with increasing humidity in larvae fed with *A. pisum* but not in those fed with *Ephestia* eggs but generally, there was overall no significant difference (two-way ANOVA,  $F_{2,143} = 2.37$ ,  $P = 0.097$ ). When males and females were included in the analysis of variance, the interaction (opposite trends) of food and humidity became significant (three-way ANOVA,  $F_{2,143} = 4.06$ ,  $P = 0.02$ ). Females were much heavier (23.8 mg) than males (18.8 mg) in all treatments (three-way ANOVA,  $F_1 = 40.6$ ,  $P < 10^{-6}$ ) but the differences were greater at 90%. The ratio of body mass of females over males was 1.26.

## 4. Discussion

**4.1. Survival.** Since we started the experiment with second instar larvae, and there is often high mortality in the first instar, our data are not fully comparable to others. Anyway, our survival rates are much lower than those of Berkvens et al. [13]. They reared each larva individually, while we started with groups of ten. Cannibalism rate is high in this ladybird species and accounted for a part of the mortality, although we have not precise data. At least, the cannibalism recorded in this study did not occur due to the lack of food. Earlier, we observed increased larval cannibalism when larvae did not have an access to liquid water for drinking, so we expected higher mortality at low humidity which was not confirmed.

**4.2. Developmental Time.** In a previous study [14], at 26°C on a diet of *Acyrtosiphon pisum*, the mean duration of each stage was as follows: egg 2.8 days, first instar 2.5 days, second instar 1.5 days, third instar 1.8 days, fourth instar 4.4 days, and pupa 4.5 days. In our experiments, the development was longer for the second through the fourth larval instars (3.3, 3.5, and 6.8 days). Similarly, we measured complete larval development time (12.7 days) on *A. pisum* diet in a previous study [15] which was shorter than the time measured here (14.5 days). The total developmental time was 20.5 days in *succinea* morph whose parents were wild-caught in Belgium and larvae fed with *A. pisum* at 23°C [16], while in our study, the total development time was estimated as 22.5 days (providing egg stage was 3 days). Part of the difference might be explained by molesting of the satiated larvae by aphids crawling over the container. In some of the previous studies, aphids were provided with their host plants and thus did not disturb resting larvae. In the present study, plants were not included because they would increase the humidity in containers, and aphids were walking all the time over the containers, disturbed resting satiated larvae which on turn also walked around the container resulting in energy expenditure and subsequently longer development.

There was generally no significant difference in the development time between the “natural” food, aphid *A. pisum* and supplement food, and frozen eggs of the flour moth *E. kuehniella*. Similarly, Berkvens et al. [16] found similar developmental times when feeding several morphs or populations by these two foods.

**4.3. Body Size.** While higher temperature shortens the development, it often decreases adult weight. Ladybird larvae reared at higher temperatures produced smaller adults than larvae reared at lower temperatures [17]. Among our treatments, there were cases when shorter developmental time resulted in larger adults but also vice versa. Fresh body mass was smaller in our experiments than in those of Berkvens et al. [16] (about 35 mg) for both foods.

**4.4. Interaction between Food Type and Humidity.** Three important life history parameters, that is, survival rate, larval developmental time, and adult mass were all differently influenced by diverse ambient humidity depending on the food type. In individuals fed with aphid *A. pisum*, there

was a trend for better survival, shorter development, and higher mass gained at higher humidity. These trends were opposite or nonsignificant in individuals fed with eggs of *E. kuehniella*. Such a difference might be explained by the food quality. While aphids survived longer and in a better condition at high air humidity when they were without their food plant, moth eggs became soon mouldy and rejected at the highest humidity. It seems that the effect of different humidities was mainly mediated through the food and did not strongly influence the life parameters directly. Since *H. axyridis* performed comparatively well in all levels of humidity, it seems that it is not a strong single limiting factor for the further spread of this alien ladybird species to new areas.

**4.5. Sex Ratio.** The only measured parameter that showed a consistent trend regardless of food treatment was the sex ratio. It was close to 1:1 (0.5) at the medium humidity, while there were more males at low humidity and more females at high humidity. It emerged as differential larval mortality, maybe different rates of larval cannibalism. The only similar case found was the sex ratio of *Attagenus fasciatus* (Coleoptera: Dermestidae) which was male biased at 30°C at 40 and 60% RH, but not at 80% RH [18].

From the evolutionary perspective, sex ratio biased towards females is better, resulting in higher population growth, so that areas with high humidity might be colonized more quickly than those with low air humidity.

## 5. Conclusion

The effect of air humidity is context dependent. In our study, we used only one constant experimental temperature and constant humidity, while in the field, these factors fluctuate on a daily basis. The effect might be different in the field where plant material or other resources provide water for drinking or higher-humidity shelter in generally dry conditions or exposure to sun may generally reduce high humidity. The only report from the field conditions was a negative correlation between *H. axyridis* abundance and air relative humidity (70–90%) in noncitric plants in Brazil [19]. We recommend to monitor the sex ratio in field populations in regions with contrasting humidity although they must be checked for the occurrence of male killing bacteria [20].

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