



Life History Traits of *Ophraella communa* (Coleoptera: Chrysomelidae) on *Helianthus annuus* (Asteraceae)

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ABSTRACT

Sunflower (*Helianthus annuus*, Asteraceae) is an economic crop cultivated worldwide. The ragweed leaf beetle (RLB; *Ophraella communa* LeSage) has been reported as a pest of sunflower. This study investigated the life history of RLBs on sunflower under laboratory conditions at two temperatures (25°C and 30°C). The objectives of this study were to investigate the optimal temperature range for the development of RLBs on sunflower and to determine the effect of temperature on both sexes of RLBs at the immature stages and adult RLBs. The sex of RLBs at the immature stages was identified based on the emerging adult, and the data recorded were separated accordingly. The study results revealed that the developmental period was shorter at 25°C (male RLBs: 33.48 ± 0.27 days, female RLBs: 33.33 ± 0.38 days) than at 30°C (male RLBs: 28.50 ± 0.40 days, female RLBs: 26.88 ± 0.43 days), and that the period exhibited no statistical difference between the sexes at the same temperature. The survival rate per stage was higher at 25°C (26% ± 2.92%) than at 30°C (20.77% ± 2.52%), which decreased with an increase in age. The total fecundity of RLBs on sunflower was higher at 25°C (270.63 ± 20.88 eggs) than at 30°C (109.77 ± 15.89 eggs). These results demonstrated that 25°C had the most favorable effects on the life history of RLBs on sunflower.

Key words: sunflower, ragweed leaf beetle, immature stages, different sexes, temperature

Introduction

Sunflower (*Helianthus annuus*), the most common plant in the family Asteraceae, is a crop plant with high potential for the yield of seeds and edible oil such as polyunsaturated fatty

acids; this oil is used in cooking and manufacturing hydrogenated oil. In addition, sunflower meal is used as animal food protein, and its kernels can be consumed raw and toasted. Sunflower is cultivated on an average of 25 million hectares globally (Torimiro *et al.*,

2014). Sunflower ranks fourth among all oil-producing crops per year in Asia, accounting for 3.4 million tons (7.9%) of the world production (Mittaine, 2016).

The ragweed leaf beetle (RLB, *Ophraella communa*), a potential biocontrol agent against the common ragweed (*Ambrosia artemisiifolia*), is native to North America (Futuyma, 1990; Palmer and Goeden, 1991). Wang and Chiang (1998), Zhou *et al.* (2010), and Zhou *et al.* (2014) have reported that the first recorded appearance of RLB in Japan, China, Korea, and Taiwan was in 1996, 2001, 2000, and 1996, respectively. In Europe, the first recorded appearance of RLB was in Switzerland and Italy in 2013, and it was later recorded in France, Germany, Hungary, and Russia (Lommen *et al.*, 2017).

Originally, RLB fed on only one host species, *A. artemisiifolia* (Bosio *et al.*, 2014). However, RLB is now known to feed upon other members of the Ambrosiinae subtribe, namely *Parthenium hysterophorus*, *Xanthium strumarium* L., *A. psilostachya* DC (Palmer and Goeden, 1991), and sunflower (Dernovici *et al.*, 2006). Dernovici *et al.* (2006) investigated the risk of sunflower damage by RLB and concluded that the damage caused was negligible because of no egg production and because the survival rate of first instar larvae was less than 50%. Dernovici *et al.* (2006) also stated that RLBs are capable of reproducing on sunflower, but succeeding generations cannot survive by feeding on sunflower. In a field experiment, Zhou *et al.* (2011) assessed the survival rate and population density of RLB on six sunflower varieties when intercropped with the common ragweed. The results revealed that all the stages of RLB failed to be established on different sunflower varieties when intercropped with the common ragweed for 2 months. However, Watson and Teshler (2013) conducted a similar comparative study on the risk of sunflower and common ragweed damage by RLB and revealed that RLB has great potential for causing damage to sunflower.

Temperature is one of the major environmental factors affecting the biology, physiological, behavioral characteristics, and population dynamics of ectothermic organisms such as insects (Kobori and Hanboonsong, 2017). Chen *et al.* (2019) mentioned that the life history traits of RLB on bitterweed were highly affected

by temperature. They reported that the developmental period decreased with an increase in temperature. The survival rate at each stage was the highest at 25°C and the lowest at 20°C and decreased with an increase in age. The fecundity of RLB on bitterweed was the highest at 25°C and the lowest at 20°C. Thus, they concluded that a temperature of 25°C had the most favorable effects on the life history of RLBs on bitterweed. Therefore, the current study investigated the effects of temperature on the life history traits of RLBs on sunflower under laboratory conditions.

Materials and Methods

RLBs (*O. communa*)

RLBs (*O. communa*) used in this study were cultured and maintained in the greenhouse of the Department of Plant Medicine (DPM) at the National Pingtung University of Science and Technology (NPUST) following the protocol mentioned in the study by Chen *et al.* (2019).

Sunflower (*H. annuus*)

Sunflower (*H. annuus*) plants were grown and maintained in the field of DPM, NPUST. Nitrogen fertilizer was applied in the second week after planting and at a 2-week interval, and leaves for the experiment were collected after 4 weeks of planting. Other management activities included weeding at initial stages of the sunflower growth and removal of flowers to allow continuous development of leaves.

Preparation of sunflower leaves

The collection of sunflower leaves for laboratory experiments on the life history of RLB started 6 weeks after germination. Young to partially mature leaves were used in the experiment. The sunflower leaves were cut with scissors to fit into 9-cm petri dishes. Cotton wools were moistened and placed on hand tissues for 10 min for the removal of excess moisture. Then, in a 9-cm petri dish, a leaf was placed on top of the moistened cotton wools. One adult pair (male and female) of RLBs was then placed in one petri dish for mating. In total, 10 pairs of RLBs at 25°C and 13 pairs of RLBs at 30°C were allowed to mate for egg production in 23 petri dishes.

Pairing of RLBs

Adult male and female RLBs were randomly picked from the pupa container and transferred to a 9-cm petri dish for pairing on the second day. The insects copulated in petri dishes on the sunflower leaves on the second day after emergence. The petioles of the sunflower leaves were attached with moistened cotton wool placed in the petri dishes, with each petri dish containing one leaf (Xiong, 1992). The paired RLB adults were then transferred onto the leaf in the petri dish, which was then sealed with a transparent plastic wrapper. The transparent plastic wrapper covering each petri dish was then pierced with needles, creating 10 pores per petri dish. These petri dishes were then placed on trays at room temperature ($25 \pm 2^\circ\text{C}$) for mating. The number of eggs obtained at both temperatures were as follows: total initial number of adult pairs ($30^\circ\text{C} = 10$ eggs, $25^\circ\text{C} = 13$ eggs), number of eggs collected per pair ($30^\circ\text{C} = 20$ eggs, $25^\circ\text{C} = 20$ eggs), and total initial number of eggs at each temperature ($30^\circ\text{C} = 200$ eggs, $25^\circ\text{C} = 260$ eggs).

Collection of eggs for life history study

In total, 20 eggs per pair from the first oviposition of the paired RLB adults were collected and reared in a petri dish (3.5 cm) (Taylor *et al.*, 2015). Eggs collected from different pairs were reared separately (20 eggs per petri dish). Because more than 20 eggs were collected from the same pairs, some eggs were randomly excluded from each treatment. To rear the eggs, cotton wool was moistened and placed in a 3.5-cm petri dish (Ju *et al.*, 2011). The eggs were then placed in growth chambers with respective temperatures of 25°C and 30°C , $75 \pm 5\%$ relative humidity (RH%), and a photoperiod of 14 h of light and 10 h of darkness. The eggs were placed in the chambers until hatching. The egg incubation period for each temperature treatment (or trial) was the recorded.

Transferring of larvae and rearing

Each larva (< 12-h-old) was transferred into a 3.5-cm petri dish and was treated as one replication (Yadav and Chang, 2014; Lommen *et al.*, 2017). In each petri dish, a piece of leaf measuring approximately 2.5 cm^2 was prepared and placed on moistened cotton (2-mm

thickness) to prevent leaf desiccation (Bostanian *et al.*, 2009). The petri dishes were then covered with a transparent plastic wrapper, and five pores were pierced on the wrapper. Each petri dish was then labeled with the larval number, date of hatching, and rearing temperature condition. The sunflower leaf in each petri dish was replaced every 3 days for the first and second larval instar stages and every 2 days for the third instar stage. All larvae and the pupal stage were reared individually in the same petri dish, and the development time of each immature stage was recorded until adult emergence.

Pairing of adults

Newly emerged RLB adults were sexed, paired, and placed in 9-cm petri dishes on the second day after emergence for mating and egg production. The leaf in each petri dish was replaced daily.

Data collection

This study first collected data on the developmental periods of the immature stages, from eggs to adult emergence. The developmental period data of eggs were collected from the first day of production to the day of hatching. The four sides of the leaf onto which eggs were attached in each petri dish were marked with numbers 1 to 4. These numbers were written on the plastic wrapper covering the petri dish. This marking with numbers was to ensure consistency in data recording by starting from the same egg, and the same developmental period data were recorded for each egg until hatching, after which they were transferred into individual petri dishes. Data recorded for male and female eggs were separated based on the sex of the emerging adult. Data recorded for the larval and pupal stages were separated based on sex because all the insects were reared individually from hatching till adult emergence.

For all the developmental stages, data were collected according to the procedure described by Chen *et al.* (2019). The temperature effects on egg hatchability and the total survival rate of each developmental stage of RLBs on sunflower were investigated.

Daily egg production data were collected. Each old leaf with attached eggs was replaced

with a fresh leaf. The leaf with attached eggs was then placed under a microscope (ZEISS Stemi DV4, Zeiss Discovery, Wetzlar, Germany) to count the numbers of eggs. The eggs were counted using a desk tally counter (H-102E, Cosmos Precision Works, Japan). The total number of eggs per female RLB was recorded when they were alive and laying eggs.

The sex of RLBs was determined by observing the body size and abdomen (size and color). The body lengths of male and female RLBs were differentiated based on the methods described by Zhou *et al.* (2012) and Chen *et al.* (2014). When a shortage of male RLBs existed, adult male RLBs were collected from the RLB population in the greenhouse to pair-up with the available female RLBs. Moreover, whenever a shortage of females existed, newly emerged female RLBs would be paired with existing male RLBs (Zhou *et al.*, 2010; Chen *et al.*, 2016).

To compare the developmental period, survival rates, longevity, and fecundity of RLBs at different temperatures, means and standard errors were determined using SPSS software version 25 (IBM SPSS statistics V25, 2017). Significant differences ($P < 0.05$) between means were compared using the Tukey multiple range test.

Results

Developmental periods for RLBs on sunflower

The results revealed that for both sexes, the developmental periods of the same immature stage differed significantly at both temperatures (egg: $F = 20.96$, $P = 0.001$, $DF = 105$; first instar larvae: $F = 28.19$, $P = 0.001$, $DF = 105$; second instar larvae: $F = 12.77$, $P = 0.001$, $DF = 105$; third instar larvae: $F = 10.81$, $P = 0.001$, $DF = 105$; pupa: $F = 15.51$, $P = 0.001$, $DF = 105$; and egg to adult (total immature period): $F = 78.06$, $P = 0.001$, $DF = 105$). However, the developmental periods of male and female RLBs at the same temperature were not significantly different (Table 1).

The developmental period of RLBs was longer at 25°C than at 30°C for all the immature stages. Similarly, the developmental periods of the immature stages of both male and female RLB adults on sunflower were longer at 25°C

than at 30°C (Table 1).

Survival rate of RLBs

The results revealed that the egg survival rate was higher at 25°C than at 30°C ($F = 0.73$, $P = 0.04$, $DF = 23$). Same trends were observed for the survival rates of the larvae and pupae and the total survival rates from egg to adult emergence (larvae: $F = 3.91$, $P = 0.05$, $DF = 23$, and pupa: $F = 0.44$, $P = 0.04$, $DF = 23$). The total survival rate of the immature stages from egg to adult emergence was higher at 25°C than 30°C ($F = 1.86$, $P = 0.02$, $DF = 23$) (Table 2).

Preoviposition, oviposition, postposition, and adult longevity periods of RLBs on sunflower

The results demonstrated that the preoviposition and oviposition periods decreased with an increase in temperature; however, temperature had no effects on the postoviposition period (preoviposition: $F = 51.15$, $P = 0.001$, $DF = 52$; oviposition: $F = 17.06$, $P = 0.001$, $DF = 52$; and postoviposition: $F = 3.16$, $P = 0.08$, $DF = 52$).

The results revealed that the longevity of both male and female RLBs decreased with an increase in temperature. The longevity of both sexes of adult RLBs was higher at 25°C than at 30°C. The longevity of the same immature stage differed considerably between both sexes at different temperatures ($F = 23.46$, $P = 0.001$, $DF = 106$). The longevity of male RLBs and female RLBs did not differ significantly at the same temperature (Table 3).

Fecundity of RLBs

The results indicated that on sunflower, the total number of eggs laid per female RLB was significantly higher at 25°C than at 30°C ($F = 61.89$, $P = 0.001$, $DF = 55$). The number of eggs laid per female per day was also higher at 25°C than at 30°C ($F = 146.81$, $P = 0.001$, $DF = 55$) (Table 4).

Discussion

According to Lommen *et al.* (2017), the developmental period of RLB on sunflower is longer than that on the common ragweed. The long developmental period of RLB is related to their feeding preferences and the thickness of

Table 1. Duration of each developmental stage of *O. communa* on sunflower at 25°C and 30°C

Temp. (°C)	Sex	n	Egg	Development stage (days, mean ± SE) *				
				1 st Instar	2 nd Instar	3 rd Instar	Pupa	Egg-Adult
25	♂	25	7.48 ± 0.15 a	6.72 ± 0.14 a	5.76 ± 0.12 a	5.68 ± 0.16 a	7.84 ± 0.19 a	33.48 ± 0.27 a
	♀	27	7.67 ± 0.17 a	6.78 ± 0.15 a	5.12 ± 0.12 a	5.22 ± 0.18 ab	8.15 ± 0.12 a	33.33 ± 0.38 a
30	♂	28	6.32 ± 0.25 b	5.64 ± 0.15 b	4.93 ± 0.16 a	4.64 ± 0.20 b	6.96 ± 0.19 b	28.50 ± 0.40 b
	♀	26	5.96 ± 0.11 b	5.15 ± 0.16 b	4.62 ± 0.17 b	4.42 ± 0.09 b	6.73 ± 0.17 b	26.88 ± 0.43 b

* Means within the same column followed by different letters indicating significant difference at the 5% significance level according to the Tukey test.

Table 2. Survival rate of RLBs at each developmental stage that fed on sunflower leaf at two temperatures

Temp. (°C)	N	Egg	Larvae	Pupa	Egg-Adult
25	260	64.00 ± 5.13 a	47.65 ± 5.11a	90.11 ± 3.34 a	26.00 ± 2.92 a
30	200	56.92 ± 6.27 b	35.75 ± 3.46 b	83.71 ± 4.64 b	20.77 ± 2.52 b

* Means within the same column followed by different letters indicating significant difference at the 5% significance level according to the Tukey test.

Table 3. Preoviposition, oviposition, postposition, and adult longevity periods (days; mean ± standard error) of RLBs on sunflower at two temperatures

Temp. (°C)	Sex	N	(days, mean ± SE)			
			Pre-oviposition	Oviposition	Post-oviposition	Adult longevity
25	♂	25	-	-	-	29.96 ± 1.47 a
	♀	27	6.22 ± 0.27 a	20.93 ± 1.43 a	0.96 ± 0.15 a	28.15 ± 1.40 a
30	♂	28	-	-	-	20.67 ± 0.71 b
	♀	26	3.54 ± 0.26 b	14.19 ± 0.74 b	1.42 ± 0.21 a	18.88 ± 0.68 b

Mean ± standard error (SE). Means within the same column followed by different letters indicating significant difference at $P < 0.05$ according to the analysis of variance and the Tukey test.

Table 4. Fecundity of RLBs on sunflower at two temperatures

Temp. (°C)	N	Total no. eggs laid/female	No. eggs/female/day
25	27	270.63 ± 20.88a	13.52 ± 0.61a
30	26	109.77 ± 15.89b	5.94 ± 0.19b

Mean ± standard error. Means within the same column followed by different letters indicating significant difference at $P < 0.05$ according to the analysis of variance and the Tukey test.

sunflower leaf tissues to the weaker mandibles in the early developmental stages of RLB (Dernovici *et al.*, 2006).

The growth and development of ectotherms strongly depend on ambient temperature (Aguilar-Alberola and Mesquita-Joanes 2014).

When insects are subjected to lower or extreme lower temperatures, development is delayed or stopped, resulting in a prolonged developmental period. Hance *et al.* (2007) and Zhou *et al.* (2013) have further stated that lower temperature causes insects in the immature stages to undergo

diapause, resulting in a prolonged developmental period. The development time of the immature stages (egg to pupa) of insects is inversely proportional to the increase in temperature (Honek *et al.*, 2003).

The results for egg viability on sunflower obtained in this experiment contradicted the results (40% egg viability) reported by Dernovici *et al.* (2006). Egg hatchability reached 64% at 25°C and 57% at 30°C (Table 2). According to Lommen *et al.* (2017), the survival rate of RLB varies between different sunflower cultivars. The larval survival rate found in this experiment was less than 50% at both temperatures. The survival rate of pupa found in this experiment was less than that reported by Dernovici *et al.* (2006). According to Bressan and Nascimento (2001), a longer period of exposure to nonpreferable host from early to late stages of organisms can result in mortality in the late stages.

In most ectotherm species, longevity increases with a decrease in temperature and vice versa (Reznik *et al.*, 2009; Zhou *et al.*, 2010). Insect longevity is normal at optimum temperatures and is more or less symmetrically decreased at both the lower and upper limits of temperature tolerance (Piyaphongkul *et al.*, 2012).

Table 3 presents that the preoviposition, oviposition, and postoviposition periods of RLB on sunflower decreased with an increase in temperature. A similar result was reported by Dernovici *et al.* (2006), with an oviposition period of 23 days on sunflower. According to Khaliq *et al.* (2014), temperature has a great influence on the preoviposition, oviposition, and postoviposition periods of the insects. Khaliq *et al.* (2014) stated that the preoviposition period decreases with an increase in temperature, whereas the oviposition and postoviposition periods increase with an increase in temperature up to a certain temperature and then decrease with accompanying decrease in the survival rate.

The fecundity of RLB on sunflower was found to be influenced by different temperature regimes (Table 4). Both the total number of eggs laid per female RLB and the average number of eggs per female RLB per day were higher at 25°C. An adult female RLB laid up to 270 eggs on sunflower, which was higher than that (126

eggs) reported by Dernovici *et al.* (2006). Changes in the oviposition period also affect the fecundity of the insect (Steinbauer, 2005). Overall, temperature affected the survival rate, longevity, and fecundity of RLB on sunflower through the reduced fitness of the adult females, reduction in female body size.

According to a review by Smith (1990), the fecundity of invertebrates is mostly affected by size, population density, food, age, temperature, geographic location, egg or offspring size, amount of male ejaculate, number of previous matings by males, and clutch interval. Temperature might influence insect fecundity at the following two levels: egg maturation and the time required for strategic ovipositioning of the egg by the adult female (Berger *et al.*, 2008). Lower temperatures result in reduced fecundity due to male sterility, which decreases the movement of both male and female adults to an extent where mating no longer occurs, or incapacitation of sperm (Danks *et al.*, 1994). In a previous study, exposure to sublethal heat stress inhibited the development of nymph, lowered fecundity, and extended the development time of egg (Piyaphongkul *et al.*, 2012). Gotthard (2004) stated that temperature affects insect fecundity by influencing insect plasticity. Furthermore, temperature influences insect fecundity through the effects on the pupal weight and adult body size, meaning that larger females are more fecund than smaller ones (Calvo and Molina, 2005).

Temperature affects nearly the rates of all processes from biochemical to kinetics in generation time (Kingsolver and Huey, 2008). According to Khaliq *et al.* (2014), both high and low temperatures have considerable effects on the insects, and they stated that higher temperature affects the life cycle stage, growth, and some internal metabolic activities, whereas lower temperature disturbs insects physiologically, mechanically, and behaviorally. Overall, temperature influences the general performance and fitness of the insects, leading to influences on the characteristics, including fecundity, of ectotherms (Calvo and Molina, 2005; Hance *et al.*, 2007; Kingsolver and Huey, 2008).

In conclusion, this study demonstrated that 25°C had more favorable effects on the life

history of RLBs on sunflower. The study suggests that if the field temperature is approximately 25°C, the chance of infestation of RLBs on sunflower will be higher.

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豬草金花蟲 (*Ophraella communa*) (鞘翅目：金花蟲科) 取食向日葵 (菊科) 的生活史特性

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摘 要

向日葵 (*Helianthus annuus*) 屬菊科植物 (Asteraceae) 為世界上重要的經濟作物。豬草金花蟲 (RLB, *Ophraella communa*) 是向日葵上有記載的潛在害蟲。本研究在兩種不同溫度 (25°C 和 30°C) 下研究豬草金花蟲取食向日葵的生活史。本研究的目的是確認豬草金花蟲在向日葵上發育的有利溫度，並確定溫度對未成熟期的生長、發育和成蟲壽命及繁殖的影響。結果顯示在 30°C 時其發育所需時間比在 25°C 短，經統計分析具差異顯著性，在相同溫度下雌雄蟲之發育時間長度則不具統計差異性。25°C 定溫下各齡期的存活率均高於 30°C；餵食向日葵的豬草金花蟲於 25°C 時總產卵量達 270.63 粒，而 30°C 時僅 109.77 粒，具統計差異顯著性。由以上結果顯示 25°C 對向日葵上的豬草金花蟲生活史具有利的影響。

關鍵詞：向日葵、豬草金花蟲、生活史、溫度