

Indirect Effect of Elevated CO₂ on Population Parameters and Growth of *Agasicles hygrophila* (Coleoptera: Chrysomelidae), a Biocontrol Agent of Alligatorweed (Amaranthaceae)

Meng-Zhu Shi,^{1,2,3,4} Jian-Yu Li,^{1,2,3,4} Bo Ding,¹ Jian-Wei Fu,^{1,2,3,4,5} Li-Zhen Zheng,¹ and Hsin Chi^{4,6}

¹Institute of Plant Protection, Fujian Academy of Agriculture Sciences, Fuzhou 350013, China, ²Fujian Key Laboratory for Monitoring and Integrated Management of Crop Pests, Fuzhou 350003, China, ³Fuzhou Scientific Observing and Experimental Station of Crop Pests, Ministry of Agriculture, Fuzhou 350003, China, ⁴State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou 350002, China and ⁵Corresponding author, e-mail: fjw9238@163.com

The first three authors, Meng-Zhu Shi, Jian-Yu Li and Bo Ding, contributed equally to this article.

Subject Editor: Eric Riddick

Received 9 October 2018; Editorial decision 9 January 2019

Abstract

Alligatorweed, *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae) is an invasive weed in China that is often kept under control by the alligatorweed flea beetle, *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae) introduced into China from Argentina in the 1980s. Elevated CO₂ levels have been shown to have a direct effect on *Ag. hygrophila*. In order to fully evaluate the indirect effects of three different atmospheric concentrations of CO₂ (420, 550, and 750 ppm) on the population parameters of *Ag. hygrophila* reared on *Al. philoxeroides*, we collected life table data for *Ag. hygrophila* using the age-stage, two-sex life table method. In general, there were no significant differences in the lengths of the preadult parameters among the three treatments. The adult duration and total longevity of males, however, did increase as CO₂ increased in concentration. Although the adult preoviposition and total preoviposition periods decreased, the fecundity, oviposition days, eggs per oviposition day, net reproductive rate, intrinsic rate of increase, and finite rate of increase all increased significantly at the high CO₂ concentration. Consequently, we determined that the *Ag. hygrophila* population size will potentially increase rapidly over a short period of time at elevated CO₂ concentrations. Our results suggest that 550 and 750 ppm CO₂ may also cause physiological changes in *Al. philoxeroides* that, in turn, provide enhanced nutrition for increasing reproduction in *Ag. hygrophila* by accelerating maturation of their reproductive system. These results indicate that the efficacy of *Ag. hygrophila* as a biological control agent against *Al. philoxeroides* will likely be increased at 550 and 750 ppm CO₂.

Key words: *Agasicles hygrophila*, *Alternanthera philoxeroides*, elevated CO₂, two-sex life table, indirect effect

Alligatorweed, *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae), is an important, invasive, aquatic weed originating from the Parana River region of South America (Vogt et al. 1979) that has subsequently been introduced into many countries. Following its introduction into China in the 1930s, *Al. philoxeroides* has spread rapidly through 16 provinces, and is now extensively distributed between latitudes 18° and 35° North and longitudes 100° and 121° East (Ma 2001). In 2003, *Al. philoxeroides* was listed as one of the 16 most important alien invasive species by China's Ministry of Environmental Protection (MEP and CAS 2003).

The primary biological control agent that has been successfully used against *Al. philoxeroides* in Australia is the flea beetle, *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae) (Sainty et al. 1998). In 1986, this agent was introduced into China and was found to be effective in the control of populations of *Al. philoxeroides* within that country (Ma 2001). The larval and adult flea beetles feed directly on the emergent foliage of *Al. philoxeroides*; afterward, the mature larvae bore into the hollow stems in which they subsequently pupate. Their burrowing weakens the plant stems, eventually leading to their collapse (Lu et al. 2010).

A major factor likely to affect the efficacy of pest management measures is climate change and associated environmental changes, including increases in the levels of greenhouse gases. Concentrations of the principal greenhouse gases, carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O), have been increasing in the atmosphere since 1750 owing to human activities (Stocker et al. 2014). Of these, CO₂ is responsible for 50–60% of global warming (Fuwa 1994). In 2011, the atmospheric concentration of 391 ppm CO₂ exceeded preindustrial levels by approximately 40%, and is predicted to increase to 550 ppm by the year 2050 and 770 ppm by 2200 (Solomon et al. 2007).

A number of studies have shown that elevated levels of CO₂ have considerable effects on various species of the fauna and flora, including plants (Huang et al. 2017, Mateos-Naranjo et al. 2010), insects (Chen et al. 2005, Shi et al. 2014), and aquatic organisms (Parra et al. 2016). Some of these reports have demonstrated that these effects may often be indirect. For example, it has been shown that the direct effects of elevated CO₂ on the cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) are weak (Yin et al. 2010) and that it mainly affects these insects indirectly via a cascade effect through their plant hosts (Wu et al. 2006, Sun et al. 2011). Assessment of the direct effects of elevated CO₂ via an examination of the life table and population dynamics of *Ag. hygrophila* has shown that the intrinsic rate of increase (*r*), finite rate of increase (*λ*), and net reproduction rate (*R*₀) are higher, and that the mean generation time (*T*) is shorter under 750 μl/liter CO₂ than it is at ambient CO₂ conditions (420 μl/liter) (Fu et al. 2016).

In the present study, we assessed the indirect effect of elevated CO₂ on *Ag. hygrophila* when the host plant, *Al. philoxeroides*, was initially cultivated under three different concentrations of CO₂ (420, 550, and 750 ppm) and then used to rear the beetles. The objectives of this study were to 1) determine the changes, if any, in the population parameters of *Ag. hygrophila* reared on *Al. philoxeroides* that had been cultivated under different CO₂ conditions, 2) compare the life table parameters, using the comprehensive age-stage, two-sex life table theory, with values reported in previous life table studies (Fu et al. 2016), and 3) predict the population growth of *Ag. hygrophila*

and its potential for the control of *Al. philoxeroides* under conditions of increasing CO₂ concentrations.

Materials and Methods

Closed-Dynamics CO₂ Chambers

All experiments were conducted in growth chambers (PRX-450D; Haishu Safe Apparatus, Ningbo, China) under controlled environmental conditions, i.e., a photoperiod of 14:10 (L:D) h, temperatures of 26 ± 1°C during the light period and 24 ± 1°C during the dark period, and relative humidity (RH) of 75 ± 5%. Three CO₂ concentrations were used in this study: 420 ± 20.1 ppm (ambient CO₂ condition), 550 ± 24.4 ppm, and 750 ± 28.8 ppm. The CO₂ concentrations in the growth chambers were maintained using CO₂ controllers designed by the Institute of Plant Protection, Fujian Academy of Agricultural Sciences (IPP, FAAS), Fujian Province, and constructed by Foshan Shunyidong Electronic Science & Technology Co. Ltd, Guangdong Province, China (new pattern patent No. ZL 201320567450.X). The controllers continuously monitored CO₂ concentrations and recorded the generated data. At the end of the experiment, the CO₂ concentration data were exported, and the mean averages were calculated.

Cultivation of *Al. philoxeroides*

Al. philoxeroides, originally collected from a river in Nantong Town, Minhou County, Fuzhou, China, was grown in a greenhouse at IPP, FAAS. For the purposes of this study, *Al. philoxeroides* plants were propagated from stem cuttings. Stems of approximately 5 cm in length with a single node were inserted into planting soil in pots (length: 47.5 cm, width: 34 cm, height: 15 cm). The pots were kept in growth chambers under one of the three CO₂ conditions for 30 d. Plants 20–30 cm high were used in the experiments.

Rearing of *Ag. hygrophila*

Ag. hygrophila colonies were originally collected from an experimental field at the IPP, FAAS and reared in a growth chamber at

Table 1. Developmental time, longevity, adult preoviposition period (APOP), total preoviposition period (TPOP), oviposition days (*O*_d), eggs per oviposition day (*E*_d), fecundity of all females (*F*), and fecundity of reproductive females (*F*_r) of *Agasicles hygrophila* reared on *Alternanthera philoxeroides* cultivated at three different CO₂ concentrations

Parameters	420 ppm		550 ppm		750 ppm	
	<i>n</i>	Mean ± SE	<i>n</i>	Mean ± SE	<i>n</i>	Mean ± SE
Egg duration (days)	179	4.1 ± 0.02b	204	4.0 ± 0.01c	239	4.2 ± 0.03a
First instar duration (days)	153	3.1 ± 0.02a	171	3.1 ± 0.02a	227	2.9 ± 0.03b
Second instar duration (days)	142	2.3 ± 0.03b	161	2.1 ± 0.02c	224	2.4 ± 0.03a
Third instar duration (days)	127	2.9 ± 0.05b	143	3.1 ± 0.04a	210	2.7 ± 0.05c
Total larval duration (days)	127	8.2 ± 0.06a	143	8.3 ± 0.06a	210	8.0 ± 0.05b
Pupal duration (days)	88	6.7 ± 0.09a	104	6.3 ± 0.08b	167	6.7 ± 0.05a
Pre-adult duration (days)	88	18.9 ± 0.10a	104	18.6 ± 0.07a	167	18.9 ± 0.05a
Adult female duration (days)	45	8.5 ± 0.63a	57	9.4 ± 0.61a	91	10.0 ± 0.52a
Adult male duration (days)	43	9.0 ± 0.64b	47	9.3 ± 0.69b	76	11.5 ± 0.75a
Overall female life span (days)	45	27.4 ± 0.64a	57	27.9 ± 0.63a	91	28.8 ± 0.53a
Overall male life span (days)	43	27.9 ± 0.66b	47	28.0 ± 0.71b	76	30.4 ± 0.74a
APOP (days)	28	5.4 ± 0.26a	39	4.5 ± 0.12b	69	3.9 ± 0.08c
TPOP (days)	28	24.1 ± 0.26a	39	22.9 ± 0.12b	69	22.7 ± 0.10b
Oviposition days (<i>O</i> _d ; days)	28	1.9 ± 0.27c	39	4.0 ± 0.37b	69	5.4 ± 0.39a
Eggs per oviposition day (<i>E</i> _d) (eggs/day)	28	23.2 ± 1.2c	39	29.3 ± 0.9b	69	31.6 ± 0.7a
Fecundity (<i>F</i> _r) (eggs/reproductive female)	28	44.7 ± 7.04c	39	115.8 ± 13.15b	69	169.8 ± 12.58a
Fecundity (<i>F</i>) (eggs/female)	45	27.8 ± 5.44c	57	79.2 ± 11.49b	91	128.7 ± 12.22a

Means in the same row followed by the same letter are not significantly different (*P* < 0.05), as determined by the paired bootstrap test.

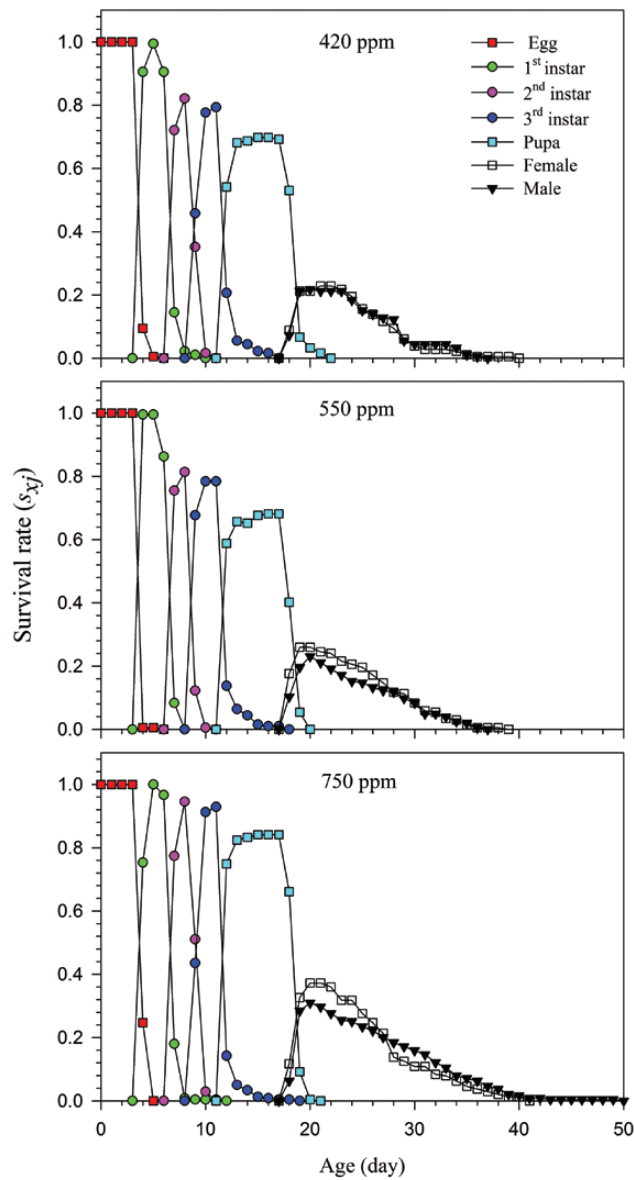


Fig. 1. Age-stage-specific survival rate (s_{xj}) of *Agasicles hygrophila* reared on *Alternanthera philoxeroides* grown under three different CO₂ concentrations.

Table 2. Population parameters and proportions of females, reproductive females, males, and N-type individuals (those that died in preadult stages) of *Agasicles hygrophila* reared on *Alternanthera philoxeroides* grown under three different CO₂ concentrations

Population parameters	CO ₂ level		
	420 ppm	550 ppm	750 ppm
Preadult survival rate (%)	49.2 ± 0.37b	51.0 ± 0.03b	69.9 ± 2.96a
First age of survival rate <50% (day)	19.0 ± 1.3b	20.0 ± 1.1b	26.0 ± 0.8a
First age of survival rate <5% (day)	35.0 ± 2.2b	35.0 ± 1.7b	39.0 ± 1.2a
Net reproductive rate (R_0 , offspring)	6.99 ± 1.62c	22.11 ± 4.05b	49.02 ± 6.14a
Intrinsic rate of increase (r /day)	0.0715 ± 0.0085c	0.1180 ± 0.0067b	0.1476 ± 0.0046a
Finite rate of increase (λ /day)	1.0741 ± 0.0091c	1.1253 ± 0.0076b	1.1591 ± 0.0053a
Mean generation time (T /day)	26.8 ± 0.50a	26.1 ± 0.30a	26.3 ± 0.23a
Proportion of female individuals (N_f/N)	0.251 ± 0.032b	0.279 ± 0.032b	0.381 ± 0.031a
Proportion of male individuals (N_m/N)	0.240 ± 0.031ab	0.230 ± 0.030b	0.318 ± 0.030a
Proportion of N-type individuals (N_n/N)	0.508 ± 0.037b	0.490 ± 0.035b	0.301 ± 0.030a
Proportion of reproductive females (N_{fr}/N_f)	0.622 ± 0.073a	0.684 ± 0.062a	0.758 ± 0.045a

Means in the same row followed by different letters are significantly different ($P < 0.05$), as determined by the paired bootstrap test.

25 ± 1°C, 75% ± 5% RH, and a photoperiod of 14:10 (L:D) h. In this study, different treatment groups of *Ag. hygrophila* were reared under ambient CO₂ condition on *Al. philoxeroides* grown under one of the three CO₂ conditions described earlier. Egg masses laid on leaves within a 24-h period were randomly selected and transferred to glass Petri dishes (diameter: 11 cm). After eclosion, the first instar larvae were transferred to a fresh glass Petri dish (diameter: 9 cm) containing a premoistened filter paper and reared individually. The larvae were supplied with fresh *Al. philoxeroides* leaves as needed until pupation. A section of *Al. philoxeroides* tip consisting of two stem nodes (length: 4–5 cm) without leaves was provided for pupation. Newly emerged adult males and females were paired and maintained in individual glass rearing containers (diameter: 5 cm, height: 8 cm) for oviposition. When there was an excess of one sex or one of the paired beetles died earlier than its mate, they were paired with adults of the opposite sex from the colonies mass-reared on *Al. philoxeroides* grown under the same CO₂ concentration. The hatching, development, survival, and fecundity of each beetle were recorded daily until the death of all individuals.

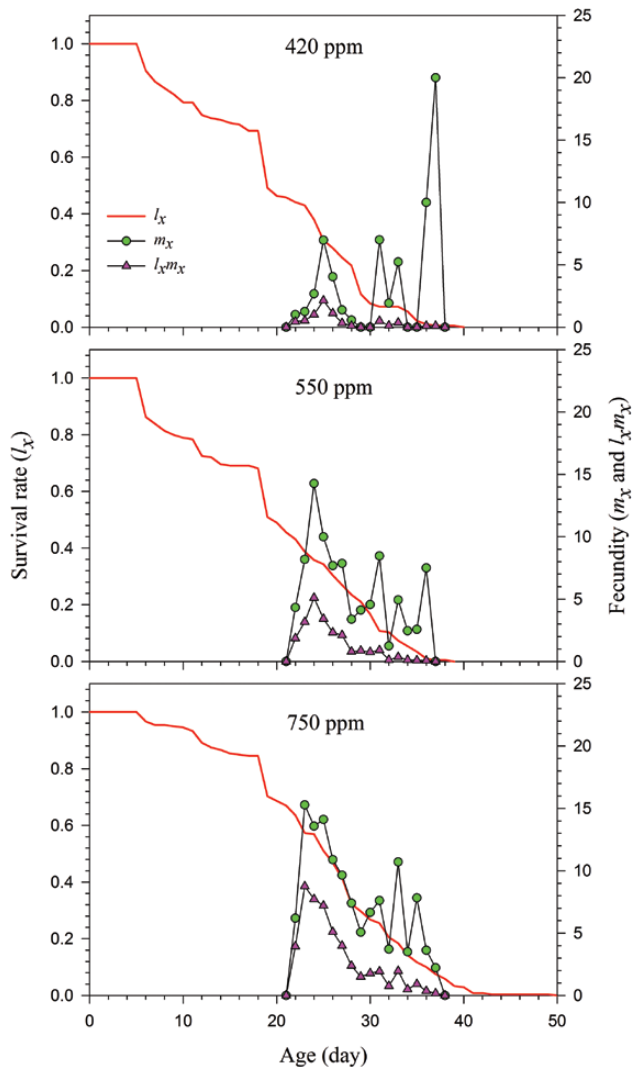


Fig. 2. Age-specific-survival rate (l_x), fecundity (m_x), and net maternity ($l_x m_x$) of *Agasicles hygrophila* reared on *Alternanthera philoxeroides* grown under three different CO₂ concentrations.

Life Table Data Analysis

The life history data of *Ag. hygrophila*, including developmental duration, lifespan, female daily fecundity, life expectancy, and population parameters, were analyzed using the program TWSEX-MSChart (Chi 2018b) based on the theory of the age-stage, two-sex life table described by Chi and Liu (1985) and Chi (1988). According to Mou et al. (2015) and Chi et al. (2016), any egg batch sampled for life table study will differentially influence the population parameters by its hatch rate. Furthermore, they derived a mathematical proof demonstrating the importance of using only the viable, hatched eggs when calculating survival, development data, and daily fecundity to correctly estimate the population parameters. Based on their findings, we used only hatched eggs in the following life table analysis. The age-stage-specific survival rate (s_{xj} , where x = age and j = stage), age-specific survival rate (l_x), age-stage-specific fecundity (f_{xj}), age-specific fecundity (m_x), and population parameters, including intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), and mean generation time (T), were calculated according to Chi and Liu (1985) by using the following equation:

$$l_x = \sum_{j=1}^k s_{xj} \quad (1)$$

where k is the number of stages.

$$m_x = \frac{\sum_{j=1}^k s_{xj} f_{xj}}{\sum_{j=1}^k s_{xj}} \quad (2)$$

The value for r was estimated from the Euler-Lotka equation with age indexed from 0 as follows:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \quad (3)$$

The finite rate of increase (λ), the net reproductive rate (R_0), and the mean generation time (T) were calculated as follows:

$$\lambda = e^r \quad (4)$$

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \quad (5)$$

$$T = \frac{\ln R_0}{r} \quad (6)$$

The age- and stage-specific life expectancy (e_{xj}), which is the length of time an individual of age x and stage j is expected to live, was calculated according to Chi and Su (2006) as follows:

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^k s'_{iy} \quad (7)$$

where s'_{iy} is the probability that an individual of age x and stage j will survive to age i and stage y and was calculated by assuming $s'_{xj} = 1$.

Oviposition days (O_d) is the mean number of days on which adult females actually lay eggs and was calculated as follows:

$$O_d = \frac{\sum_{x=1}^{N_{fr}} D_x}{N_{fr}} \quad (8)$$

where N_{fr} is the number of reproductive females, i.e., females that laid at least one egg, and D_x is the number of oviposition days of the x th reproductive female (Chen et al. 2018). The number of eggs per oviposition day (E_d) was calculated as follows:

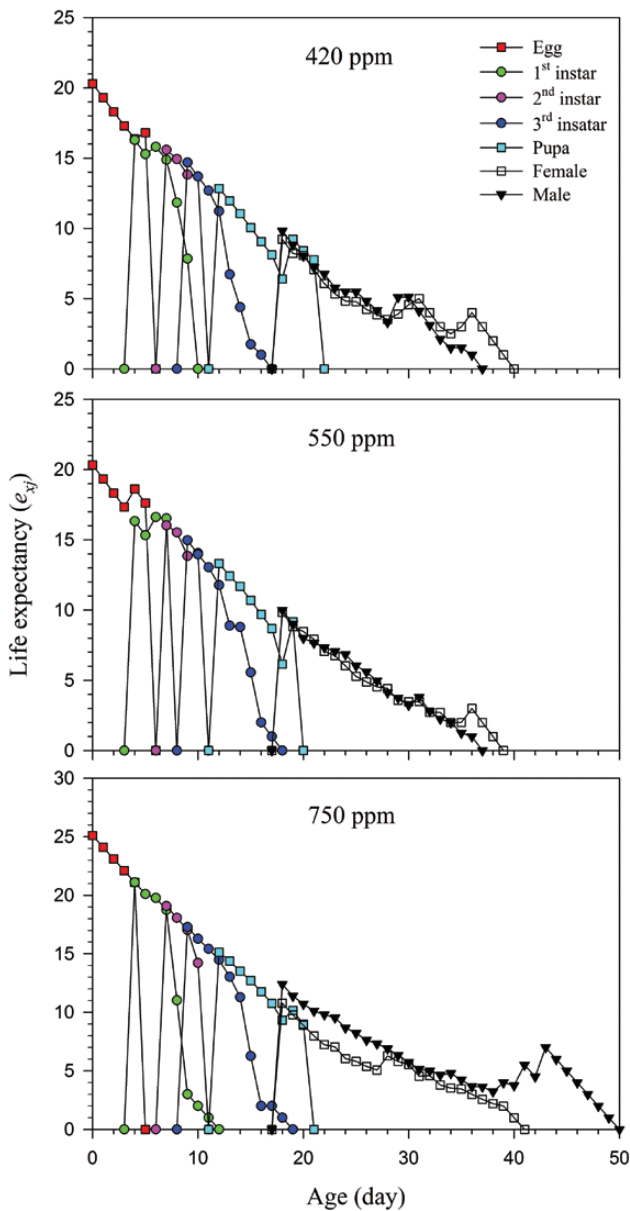


Fig. 3. Age-stage-specific survival expectancy (e_{xj}) of *Agasicles hygrophila* reared on *Alternanthera philoxeroides* grown under three different CO_2 concentrations.

$$E_d = \frac{\sum_{x=1}^{N_{fr}} E_x}{\sum_{x=1}^{N_{fr}} D_x} = \frac{\sum_{x=1}^{N_{fr}} E_x}{O_d \times N_{fr}} = \frac{F_r}{O_d} \quad (9)$$

where E_x is the total number of eggs produced by a reproductive female, and F_r is the mean fecundity based on reproductive females (Chen et al. 2018).

With reference to Fisher (1993), the age-stage reproductive value (v_{xj}) is defined as the contribution an individual of age x and stage j has to the future population. According to Huang and Chi (2011) and Tuan et al. (2014a, b), v_{xj} in the age-stage, two-sex life table is calculated as follows:

$$v_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^k s'_{iy} f_y \quad (10)$$

The variances and standard errors were estimated using the bootstrap method with resampling 100,000 times (Efron and Tibshirani 1993, Huang and Chi 2012). Although the Weibull function has often been used to fit and compare survival curves (l_x) of different treatments, Tuan et al. (2017) discussed some of the problems associated with using it. Because the number of surviving individuals usually does not decrease in a consistent manner, the survival rate may decrease from 55% at age x to 48% at age $x+1$, making it impractical to find the age of an exact survival rate. By using the bootstrap technique, however, an age where a survival rate is lower than a specific percentage (e.g., 50 or 5%) can be accessed and compared without unrealistic assumptions. In this study, we accessed and compared the ages where the survival rate was <50 and <5%.

Population Projection

Because the intrinsic rate of increase (r) is estimated based on the assumption that a population reaches a stable age-stage structure as time approaches infinity, it is inappropriate to use r to project population growth before that assumption is fulfilled. To predict and compare the population growth and age-stage structure of *Ag. hygrophila* reared on *Al. philoxeroides* grown under the three different CO_2 concentrations, we used life table data, i.e., the developmental rate, survival rate, and fecundity, to simulate population growth using the program TIMING-MSChart (Chi 2018a). An initial population of 10 newly laid eggs was used for the simulation. Given that age-stage, two-sex life tables can be used to describe stage differentiation during population growth, we calculated the increase rate of stage j from time t to $t+1$ by using a common logarithm:

$$\varphi_{j,t} = \log \left(\frac{n_{j,t+1} + 1}{n_{j,t} + 1} \right) \quad (11)$$

where $n_{j,t}$ is the number of individuals in stage j at time t . We also used a natural logarithm to calculate the increase rate of stage j from time t to $t+1$:

$$r_{j,t} = \ln \left(\frac{n_{j,t+1} + 1}{n_{j,t} + 1} \right) = \ln(n_{j,t+1} + 1) - \ln(n_{j,t} + 1) \quad (12)$$

Because the number of individuals in a stage can be 0 ($n_{j,t} = 0$ or $n_{j,t+1} = 0$), but the logarithm of zero cannot be defined, we used $n_{j,t} + 1$ and $n_{j,t+1} + 1$ in the calculation of $\varphi_{j,t}$ and $r_{j,t}$ (Akca et al. 2015, Huang et al. 2018).

Results

Age-Stage, Two-Sex Life Tables of *Ag. Hygrophila*

The developmental times of the eggs, first through third instar larvae, and pupae differed under the different CO_2 conditions. The developmental times for all stages were significantly different between the 550 ppm and 750 ppm treatments, as was that (with the exception of the first instar larvae) between the ambient CO_2 condition (420 ppm) and 550 ppm. However, the total developmental time for the cumulative preadult stages (18.9, 18.6, and 18.9 d at 420, 550, and 750 ppm, respectively) showed no significant difference among the three CO_2 treatments. Although we noted prolonged adult male longevity and an extended overall male lifespan at higher CO_2 concentrations, no significant differences among treatments were detected for adult female longevity or overall female lifespan. An evident reduction was noted in the adult preoviposition period (APOP) of females reared on *Al. philoxeroides* grown under high CO_2 concentration. The total preoviposition period (TPOP) under ambient CO_2 conditions was longer than that under the 550 and

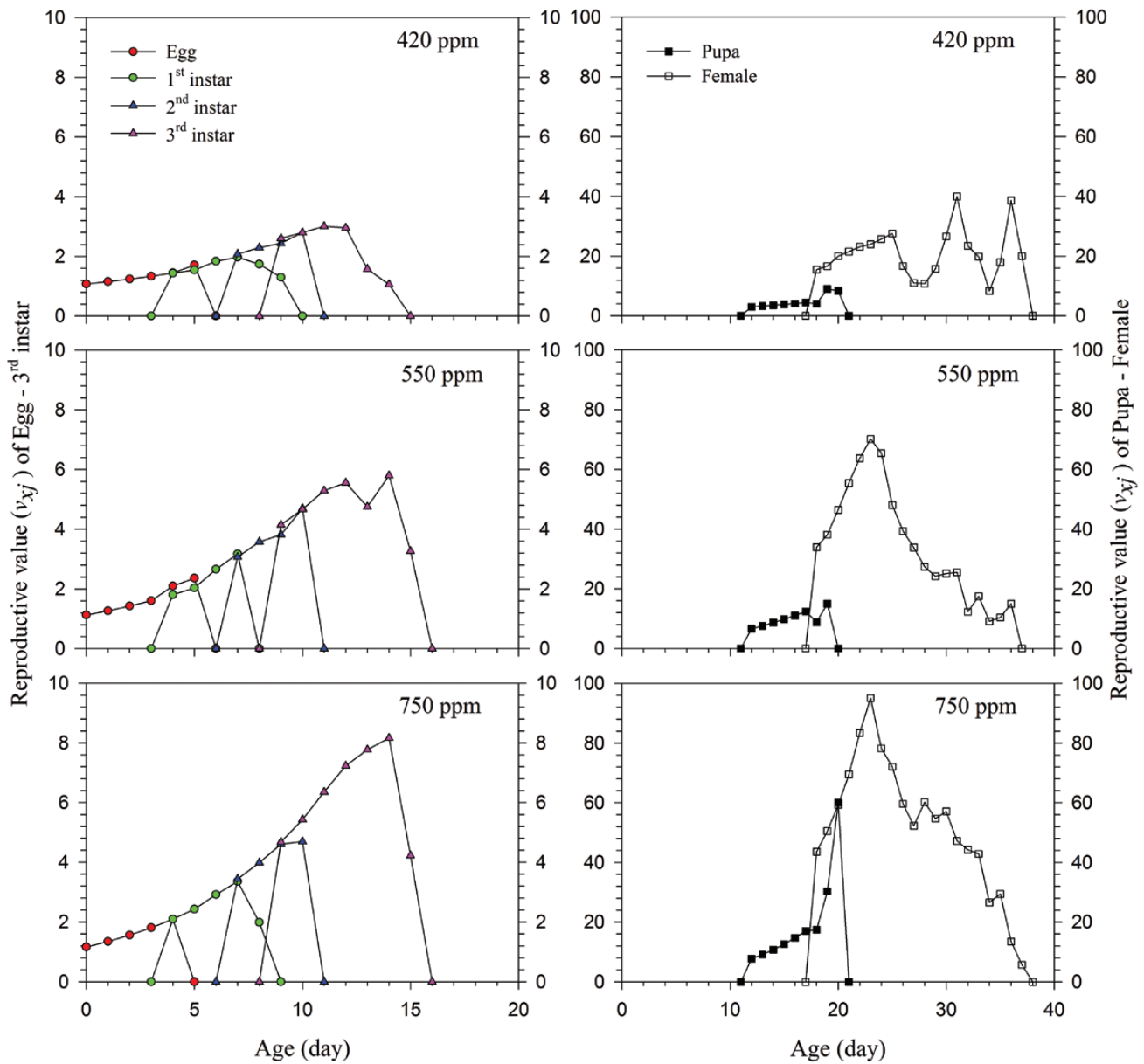


Fig. 4. Age-stage-specific reproductive value (v_{xj}) of *Agasicles hygrophila* reared on *Alternanthera philoxeroides* grown under three different CO₂ concentrations.

750 ppm CO₂ conditions. Moreover, the number of oviposition days (O_d), number of eggs per oviposition day (E_d), and female lifetime fecundity, i.e., F (mean fecundity of all females) and F_r (mean fecundity of reproductive females), showed apparent increases with increasing CO₂ concentration (Table 1).

The age-stage-specific survival rate curves (s_{xj}) which demonstrate the survival probability of an egg to age x and stage j , showed stage differentiation and stage overlapping due to the variable developmental time among individuals (Fig. 1). The overall lifespan of male *Ag. hygrophila* was longer for those individuals reared on *Al. philoxeroides* grown under 750 ppm CO₂ concentration than under 420 ppm and 550 ppm (Fig. 1). The first ages of survival rate <50% and <5% in 750 ppm CO₂ treatment were significantly later than those in 550 and 420 ppm (Table 2, Fig. 2). The m_x and net maternity ($l_x m_x$) tended to increase with increasing CO₂ concentration. The highest peak values of $l_x m_x$ were 2.14 on day 25, 5.11 on day 24, and 8.76 on day 23 for *Ag. hygrophila* reared on *Al. philoxeroides* grown

under 420, 550, and 750 ppm CO₂, respectively (Fig. 2). Although a high peak (20 eggs/individual) was observed on day 37 for the 420 ppm CO₂ treatment, the $l_x m_x$ was low (0.06 eggs/individual) because of the low survival rate at that time. At CO₂ concentrations of 420, 550, and 750 ppm, the e_{xj} of newly laid eggs were 20.3, 20.3, and 25.1 d, respectively. In general, high peak e_{xj} values were observed in the 750 ppm CO₂ treatment for each stage, with the life expectancy of adult males being distinctly longer than that of adult females (Fig. 3). The age-stage reproductive values (v_{xj}) of *Ag. hygrophila* increased with increasing CO₂ concentration, with the highest female v_{xj} peaks observed on day 23 (v_{xj} values of 70.17 and 95.04 for 550 and 750 ppm CO₂, respectively) (Fig. 4).

Population Parameters of *Ag. hygrophila*

The net reproductive rate (R_0), intrinsic rate of increase (r), and finite rate of increase (λ) of *Ag. hygrophila*, reared on *Al. philoxeroides* grown under different CO₂ concentrations showed significant

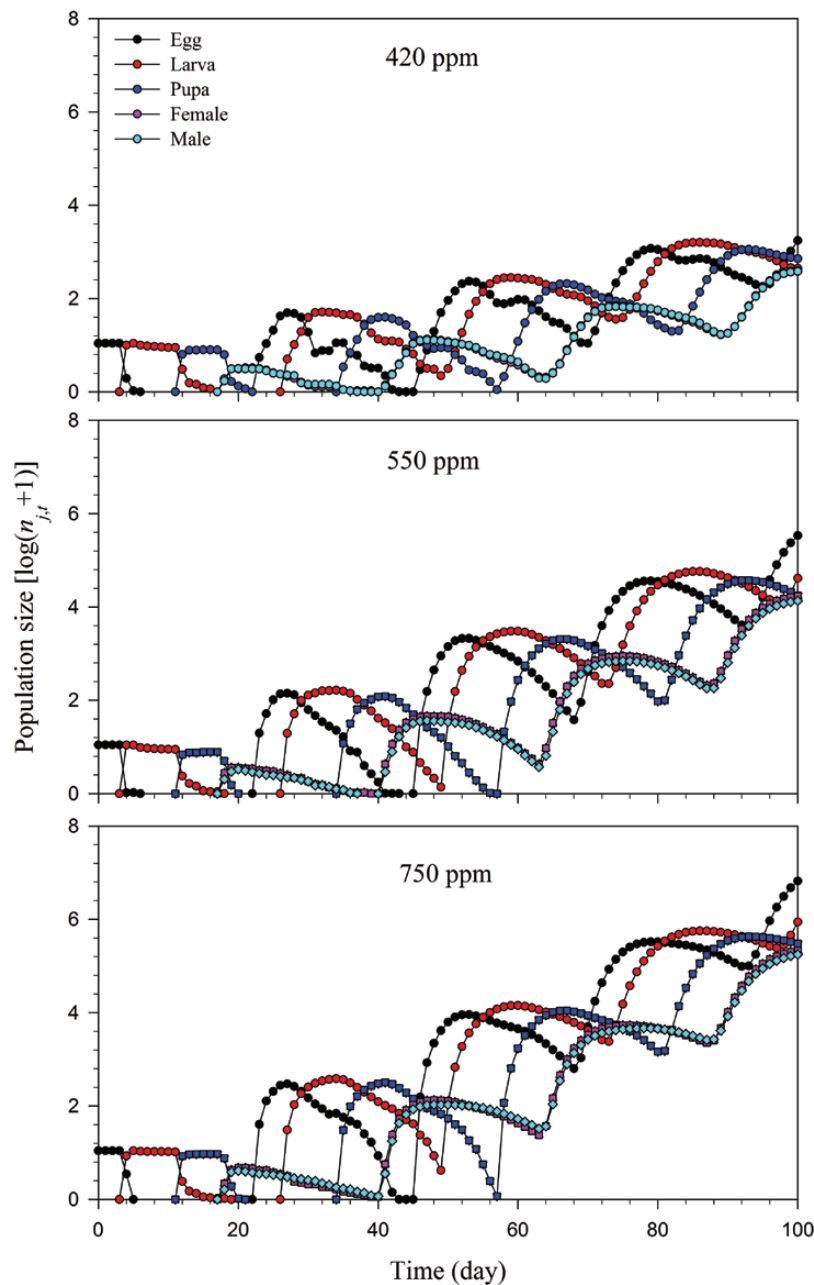


Fig. 5. Population growth of *Agasicles hygrophila* reared on *Alternanthera philoxeroides* grown under three different CO₂ concentrations.

differences and increased significantly with increasing CO₂ concentrations. However, the mean generation times (T) and N_r/N_f (the number of reproductive females/total females) showed no significant differences among the three treatments. The preadult survival rate in the 750 ppm CO₂ treatment was 69.9% and substantially higher than in the other two treatments (49.2 and 51.0% at 420 and 550 ppm, respectively) (Table 2). The highest female and male proportions in cohorts were observed in the 750 ppm CO₂ treatment.

Ag. hygrophila Population Projection

We plotted the simulated population growths of *Ag. hygrophila* reared on *Al. philoxeroides* grown under the three CO₂ concentrations based on the calculated life tables (Fig. 5). Beginning with 10 newly laid eggs, *Ag. hygrophila* is capable of producing up to

four generations within 100 d. In the 420, 550, and 750 ppm CO₂ treatments, the final population sizes were 3,692, 431,382, and 8,190,155 individuals, respectively. The population size of *Ag. hygrophila* reared on *Al. philoxeroides* grown under 750 ppm CO₂ was significantly larger than it was in either of the other two treatments. The growth rates ($r_{i,t}$) of all stages fluctuated greatly (Fig. 6).

Discussion

In this study, the initial numbers of eggs used in the life table studies for the three CO₂ treatments were 272, 287, and 292, respectively, of which 179, 204, and 239 subsequently hatched (Table 1), with respective hatch rates of 65.8, 71.0, and 81.8%. Because the eggs used for the different CO₂ treatments were randomly selected from our laboratory colony reared at ambient CO₂, they could not have

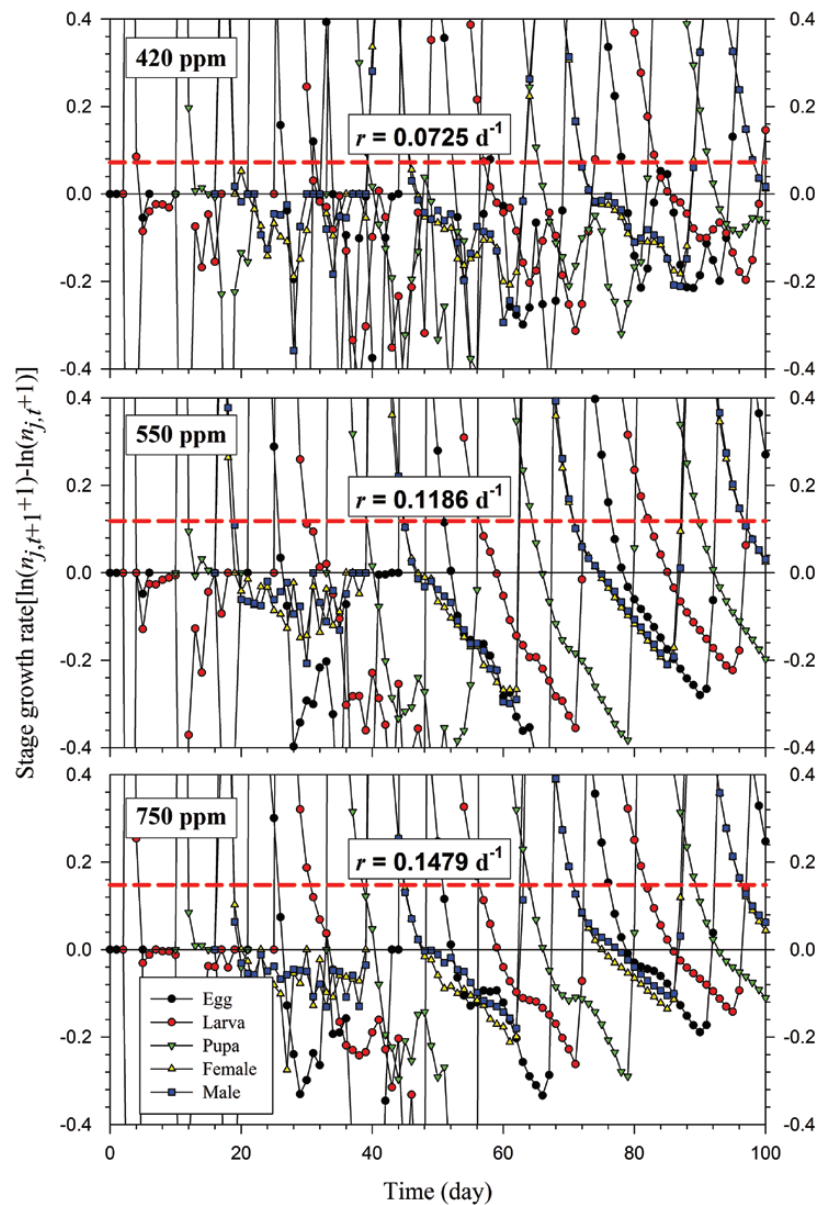


Fig. 6. Fluctuation of growth rate of each life stage of *Agasicles hygrophila* reared on *Alternanthera philoxeroides* grown under three different CO₂ concentrations. The red dashed line is the intrinsic rate of increase. We set the scale range of y-axis from -0.4 to 0.4 to reveal the fluctuation of growth rate around the intrinsic rate.

been influenced by exposure to different CO₂ concentrations, indicating that the higher hatch rate noted in the 750 ppm group was by chance. Jha et al. (2012) and Mou et al. (2015) presented convincing arguments for using only hatched (=viable) eggs to accurately estimate the population parameters. Following their advice, we used only hatched eggs when analyzing the life table raw data and calculating the survival rate and daily fecundity.

Numerous studies have demonstrated the variable effects of elevated CO₂ concentrations on the development, survival, and fecundity of insect populations. The developmental time and total lifespan of different insect species fed on host plants in elevated CO₂ environments were found to be prolonged (Wu et al. 2007, Murray et al. 2013, Rao et al. 2014, Wang et al. 2014). In our studies, the developmental durations of the pre-adult stages of *Ag. hygrophila* (a mandibulate insect) were not significantly affected when they fed on *Al. philoxeroides* plants that had been grown in elevated

CO₂ environment (Table 1). Fu et al. (2016) noted that when *Ag. hygrophila* individuals were reared directly under conditions of elevated CO₂ concentrations, the developmental durations of the egg, first instar larval and pupal stages, and adult longevity were all reduced. Our findings, therefore, indicate that there may be differences in the indirect versus direct effects of increasing CO₂ concentration on *Ag. hygrophila*. Higher fecundity under elevated CO₂ conditions has been demonstrated by Chen et al. (2005) for *Aphis gossypii* Glover (Hemiptera: Aphididae), and by Shi et al. (2014) for *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). In this study, we found that the preadult survival rate and fecundity of *Ag. hygrophila* were significantly higher in those beetles reared on *Al. philoxeroides* grown under a CO₂ concentration of 750 ppm than in beetles fed on plants grown under CO₂ concentrations of 420 and 550 ppm.

For comparison purposes, the Weibull function has often been used to fit age-specific survival curves (l_x). However, Tuan et al.

(2017) discussed some of the problems with applying the Weibull function. We compared the first ages when survival rates were <50 and <5% in different treatments based on the results of 100,000 bootstraps. This new methodology can be used to compare survival curves at any critical age.

In the present study, the lengths of the APOPs and TPOPs associated with the elevated CO₂ level were distinctly lower than those in the other two treatments, although the fecundity was higher. Based on these results, i.e., the shorter APOPs and TPOPs, the higher fecundities (F and F_p), the greater number of oviposition days (O_d), and the number of eggs laid per oviposition day (E_d), *Al. philoxeroides* plants grown under an elevated CO₂ environment (550 and 750 ppm) may have enhanced nutritive value which would be capable of stimulating the development of *Ag. hygrophila*'s reproductive system leading to earlier copulation, oviposition, and other reproductive behaviors. Given that the O_d value provides a realistic measure of the number of days that females actually produced eggs, it is a better statistic than the commonly used parameter 'oviposition period', which does not delineate the number of days the females actually lay eggs. Furthermore, because E_d indicates the number of eggs laid per day during O_d , it is, likewise, a better measure than the 'eggs per day' value derived from the entire oviposition period. Because F_r excludes females that are nonreproductive, it more accurately represents the actual fecundity of the reproductive females. We found that the R_0 , r , and λ values distinctly increased with increasing CO₂ concentration, and that the mean generation time (T) was shorter. Because elevated CO₂ levels were beneficial in terms of both female oviposition and survival, and increased male longevity, the higher fitness of *Ag. hygrophila* at elevated CO₂ concentrations was clearly demonstrated by these population parameters. The detailed physiological mechanisms underlying these characteristics merit further research.

If all biotic and abiotic conditions remain constant, the growth rate of all stages will theoretically approach the intrinsic rates of increase (r), i.e., 0.0715, 0.1180, and 0.1476/day in the 420, 550, and 750 ppm CO₂ treatments, respectively, as time approaches infinity and the population settles down to the stable age-stage distribution (Huang and Chi 2012). In Figs. 5 and 6, we demonstrated the advantage of population projection based on the age-stage, two-sex life table over the traditional female age-specific life table in revealing the stage structure and fluctuation of growth rate of different stages. Moreover, our projections show that it would be inappropriate to use a single parameter, e.g., the intrinsic rate of increase or net reproductive rate, to predict the population growth before it settles down to the stable age-stage distribution.

Although biological control is often an effective means of managing invasive plants, the probable variability in the responses of plant-insect interactions to climate change make it difficult to predict the future effectiveness of biological control programs (Reeves et al. 2015). Life tables can provide a comprehensive description of the fitness of an insect population on a given host plant (Reddy et al. 2015). In this regard, population growth can be projected using age-stage two-sex life tables, which potentially offer abundant information that can be utilized for effective biological control and other management strategies (Akköprü-Polat et al. 2015). Unlike traditional age-specific life tables, which deal only with female populations, neglect variations in the development rate among individuals, and cannot reveal stage differentiation (Lotka 1907, Birch 1948, Chi 1988, Carey 1993), the age-stage, two-sex life table theory developed by Chi and Liu (1985) incorporates both sexes and accounts for individual variations in developmental rate. Stage differentiation is critical to understanding population ecology in order to schedule pest management against a specific stage of an insect; especially

since, significant stage overlapping phenomena have frequently been observed in many insect and mite species (Chi and Liu 1985). Age-stage, two-sex life tables have, therefore, been increasingly utilized in many studies in recent years (Yu et al. 2013, Fu et al. 2016, Liu et al. 2017), and should be considered more often in assessing the effectiveness of biological control programs.

The present study demonstrated that elevated CO₂ levels resulted in causing significant effects on the majority of the population parameters of *Ag. hygrophila*, i.e., the preadult survival, fecundity, O_d , E_d , APOP, TPOP, R_0 , r , and λ . Elevated CO₂ levels, consequently, benefited the reproduction of the beetles indirectly and favorably affected the control of *Al. philoxeroides*. The increased control efficacy was also demonstrated by using population projection. Future studies should examine the simultaneous effects of elevated CO₂ on both *Ag. hygrophila* and *Al. philoxeroides*. Although we are currently undertaking research on this topic, the present study and newly initiated projects have been strictly laboratory based. Additional studies under more relevant field conditions will also be necessary.

Acknowledgments

We thank Dr. Cecil L. Smith (University of Georgia, USA) for language editing of this manuscript. The authors sincerely thank the many companies and students who provided assistance during the study, particularly in the culture of *Al. philoxeroides* and the collection of data. We are grateful to the anonymous reviewers and the editor for their valuable comments and suggestions, all of which greatly helped us in improving this article. We are grateful for the support of the National Key Research and Development Program of China (2016YFC1202101-4), the Science and Technology Major Project of Fujian (2017NZ0003-1-1), the General Program of the National Natural Science Foundation of China (31572065) on the effects of CO₂ on *Ag. hygrophila*, the Science and Technology Innovation Team Construction Project of FAAS (STIT2017-1-12, AC2017-12), and the Basic Scientific Research for Public Welfare Research Institutes of Fujian Province (2015R1024-8, 2016R1023-10).

References Cited

- Akca, I., T. Ayvaz, E. Yazici, C. L. Smith, and H. Chi. 2015. Demography and population projection of *Aphis fabae* (Hemiptera: Aphididae): with additional comments on life table research criteria. *J. Econ. Entomol.* 108: 1466–1478.
- Akköprü, E. P., R. Atlihan, H. Okut, and H. Chi. 2015. Demographic assessment of plant cultivar resistance to insect pests: a case study of the dusky-veined walnut aphid (Hemiptera: callaphididae) on five walnut cultivars. *J. Econ. Entomol.* 108: 378–387.
- Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. *J. Anim. Ecol.* 17: 15–26.
- Carey, J. R. 1993. Applied demography for biologists with special emphasis on insects. Oxford University Press, Inc., New York, NY.
- Chen, F. J., F. Ge, and M. N. Parajulee. 2005. Impact of elevated CO₂ on tritrophic interaction of *Gossypium hirsutum*, *Aphis gossypii*, and *Leis axyridis*. *Environ. Entomol.* 34: 37–46.
- Chen, G. M., H. Chi, R. C. Wang, Y. P. Wang, Y. Y. Xu, X. D. Li, P. Yin, and F. Q. Zheng. 2018. Demography and uncertainty of population growth of *Conogethes punctiferalis* (Lepidoptera: Crambidae) reared on five host plants with discussion on some life history statistics. *J. Econ. Entomol.* 111: 2143–2152.
- Chi, H. 1988. Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ. Entomol.* 17: 26–34.
- Chi, H. 2018a. TIMING-MSChart: a computer program for the population projection based on age-stage, two-sex life table. <http://140.120.197.173/Ecology/Download/Timing-MSChart.rar> (last accessed 1 August 2018).
- Chi, H. 2018b. TWSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. <http://140.120.197.173/ecology/Download/Twosex-MSChart-exe-B100000.rar> (last accessed 1 August 2018).

- Chi, H., and H. Liu. 1985. Two new methods for the study of insect population ecology. *Bull. Inst. Zool. Acad. Sin.* 24: 225–240.
- Chi, H., and H. Y. Su. 2006. Age-stage, two-sex life tables of *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with mathematical proof of the relationship between female fecundity and the net reproductive rate. *Environ. Entomol.* 35: 10–21.
- Chi, H., D. F. Mou, C. C. Lee, and C. L. Smith. 2016. Comments on the paper of Hernandez-Suarez *et al.* 'Invariance of demographic parameters using total or viable eggs.' *J. Appl. Entomol.* 140: 154–157.
- Efron, B., and R. J. Tibshirani. 1993. An introduction to the bootstrap. Chapman & Hall, New York, NY.
- Fisher, R. A. 1993. The genetical theory of natural selection: a complete variorum edition. Oxford University Press, Oxford, United Kingdom.
- Fu, J. W., M. Z. Shi, T. Wang, J. Y. Li, L. Z. Zheng, and G. Wu. 2016. Demography and population projection of flea beetle, *Agasicles hygrophila* (Coleoptera, Chrysomelidae), fed on alligator weed under elevated CO₂. *J. Econ. Entomol.* 109: 1116–1124.
- Fuwa, F. 1994. The handbook of the global environment. Asakura Publishing Co. Ltd, Tokyo.
- Huang, Y. B., and H. Chi. 2011. The age-stage, two-sex life table with an offspring sex ratio dependent on female age. *J. Agric. For.* 60: 337–345.
- Huang, Y. B., and H. Chi. 2012. Assessing the application of the jackknife and bootstrap techniques to the estimation of the variability of the net reproductive rate and gross reproductive rate: a case study in *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). *J. Agric. For.* 61: 37–45.
- Huang, H. W., H. Chi, and C. L. Smith. 2018. Linking demography and consumption of *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae) fed on *Solanum photeinocarpum*: with a new method to project the uncertainty of population growth and consumption. *J. Econ. Entomol.* 111:1–9.
- Huang, S., X. Jia, Y. Zhao, B. Bai, and Y. Chang. 2017. Elevated CO₂ benefits the soil microenvironment in the rhizosphere of *Robinia pseudoacacia* L. seedlings in Cd- and Pb-contaminated soils. *Chemosphere.* 168: 606–616.
- Jha, R. K., H. Chi, and L. C. Tang. 2012. Effects of survival rate and fecundity on the intrinsic rate of increase of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Formosan Entomol.* 32: 223–235.
- Liu, J., W. Huang, H. Chi, C. Wang, H. Hua, and G. Wu. 2017. Effects of elevated CO₂ on the fitness and potential population damage of *Helicoverpa armigera* based on two-sex life table. *Sci. Rep.* 7: 1119.
- Lotka, A. J. 1907. Studies on the mode of growth of material aggregates. *Am. J. Sci.* 24: 199–216.
- Lu, J., L. Zhao, R. Ma, P. Zhang, R. Fan, and J. Zhang. 2010. Performance of the biological control agent flea beetle *Agasicles hygrophila* (Coleoptera: Chrysomelidae), on two plant species *Alternanthera philoxeroides* (alligatorweed) and *A. sessilis* (joyweed). *Biol. Control.* 54: 9–13.
- Ma, R. Y. 2001. Ecological adaptation for introduced bio-control agent, *Agasicles hygrophila*, for alligator weed, *Alternanthera philoxeroides*, in China. Ph.D thesis, Chinese Academy of Agricultural Sciences, Beijing.
- Mateos-Naranjo, E., S. Redondo-Gómez, L. Andrades-Moreno, and A. J. Davy. 2010. Growth and photosynthetic responses of the cordgrass *Spartina maritima* to CO₂ enrichment and salinity. *Chemosphere.* 81: 725–731.
- Ministry of Environmental Protection (MEP) of the People's Republic of China. 2003. The first list of exotic invasive species of China. Document No.11. Index no. 000014672/2003-00014.
- Mou, D. F., C. C. Lee, C. L. Smith, and H. Chi. 2015. Using viable eggs to accurately determine the demographic and predation potential of *Harmonia dimidiata* (Coleoptera: Coccinellidae). *J. Appl. Entomol.* 139: 579–591.
- Murray, T. J., D. T. Tissue, D. S. Ellsworth, and M. Riegler. 2013. Interactive effects of pre-industrial, current and future [CO₂] and temperature on an insect herbivore of *Eucalyptus*. *Oecologia.* 171: 1025–1035.
- Parra, G., A. Galotti, R. Jiménez-Melero, F. Guerrero, E. Sánchez-Moyano, F. Jiménez-Gómez, and M. Conradi. 2016. Effects of experimental long-term CO₂ exposure on *Daphnia magna* (Straus 1820): from physiological effects to ecological consequences. *Chemosphere.* 156: 272–279.
- Rao, M. S., D. Manimanjari, A. C. R. Rao, P. Swathi, and M. Maheswari. 2014. Effect of climate change on *Spodoptera litura* Fab. On peanut: a life table approach. *Crop Prot.* 66: 98–106.
- Reddy, G. V. P., and H. Chi. 2015. Demographic comparison of sweet potato weevil reared on a major host, *Ipomoea batatas*, and an alternative host, *I. triloba*. *Sci. Rep.* 5: 11871. doi: 10.1038/srep11871
- Reeves, J. L., D. M. Blumenthal, J. A. Kray, and J. D. Derner. 2015. Increased seed consumption by biological control weevil tempers positive CO₂ effect on invasive plant (*Centaurea diffusa*) fitness. *Biol. Control.* 84: 36–43.
- Sainty, G., G. McCorkelle, and M. Julien. 1998. Control and spread of alligator weed, *Alternanthera philoxeroides* (Mart.) Griseb., in Australia: lessons for other regions. *Wetl. Ecol. Manag.* 5: 195–201.
- Shi, B. K., J. L. Huang, C. X. Hu, and M. L. Hou. 2014. Interactive effects of elevated CO₂ and temperature on rice planthopper, *Nilaparvata lugens*. *J. Integ. Agr.* 13: 1520–1529.
- Solomon, S., D. Qin, M. Manning, Z. Chen, M. Margquis, K. B. Averyt, M. Tignor, and H. L. Miller, eds. 2007. Climate change 2007: the physical science basis: working group I contribution to the fourth assessment report of the IPCC. Cambridge University Press, Cambridge and New York.
- Stocker, T. F., D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley, eds. 2014. Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY.
- Sun, Y. C., H. J. Guo, Z. Y. Liu, and F. Ge. 2011. The mechanism by which elevated CO₂ affects the interaction between herbivorous insects and their host plants. *Chin. J. Appl. Entomol.* 48: 1123–1129.
- Tuan, S. J., C. C. Lee, and H. Chi. 2014a. Population and damage projection of *Spodoptera litura* (F.) on peanuts (*Arachis hypogaea* L.) under different conditions using the age-stage, two-sex life table. *Pest Manag. Sci.* 70: 805–813.
- Tuan, S. J., C. C. Lee, and H. Chi. 2014b. Erratum: population and damage projection of *Spodoptera litura* (F.) on peanuts (*Arachis hypogaea* L.) under different conditions using the age-stage, two-sex life table. *Pest Manag. Sci.* 70: 1936.
- Tuan, S. J., P. W. Chang, P. Saska, R. Atlihan, and H. Chi. 2017. Hostplants mixture and fitness of *Kolla paulula*: with an evaluation of the application of Weibull function. *J. Appl. Entomol.* 141: 329–338.
- Vogt, G. B., J. U. McGuire, Jr., and A. D. Cushman. 1979. Probable evolution and morphological variation in South American disonychine flea beetles (Coleoptera: Chrysomelidae) and their amaranthaceous hosts. USDA Technical Bulletin No. 1593. United States Department of Agriculture and Mississippi Agricultural and Forestry Experiment Station.
- Wang, G. H., X. X. Wang, Y. C. Sun, and F. Ge. 2014. Impacts of elevated CO₂ on *Bemisia tabaci* infesting Bt cotton and its parasitoid *Encarsia formosa*. *Entomol. Exp. Appl.* 152: 228–237.
- Wu, G., F. Chen, and F. Ge. 2006. Response of multiple generations of cotton bollworm *Helicoverpa armigera* Hübner, feeding on spring wheat, to elevated CO₂. *J. Appl. Entomol.* 130: 2–9.
- Wu, G., F. J. Chen, Y. C. Sun, and F. Ge. 2007. Response of successive three generations of cotton bollworm, *Helicoverpa armigera* (Hübner), fed on cotton bolls under elevated CO₂. *J. Environ. Sci. (China).* 19: 1318–1325.
- Yin, J., Y. Sun, G. Wu, and F. Ge. 2010. Effects of elevated CO₂ associated with maize on multiple generations of cotton bollworms *Helicoverpa armigera*. *Entomol. Exp. Appl.* 136: 12–20.
- Yu, L. Y., Z. Z. Chen, F. Q. Zheng, A. J. Shi, T. T. Guo, B. H. Yeh, H. Chi, and Y. Y. Xu. 2013. Demographic analysis, a comparison of the jackknife and bootstrap methods, and predation projection: a case study of *Chrysopa pallens* (Neuroptera: Chrysopidae). *J. Econ. Entomol.* 106: 1–9.