Long-Term Effect of Elevated CO₂ on the Development and Nutrition Contents of the Pea Aphid (Acyrthosiphon pisum)

Chunchun Li¹, Qian Sun¹, Yuping Gou¹, Kexin Zhang¹, Qiangyan Zhang¹, Jing-Jiang Zhou¹,² and Changzhong Liu*¹

¹ College of Plant Protection, Gansu Agricultural University, Lanzhou, China, ² State Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Guiyang, China

It is predicted that the current atmospheric CO₂ level will be doubled by the end of this century. Here, we investigate the impacts of elevated CO₂ (550 and 750 µL/L) on the development and nutrition status of the green pea aphid for six generations, which is longer than previous studies. All seven examined physiological parameters were not affected over six generations under the ambient CO₂ level (380 µL/L). However, the elevated CO₂ levels (550 and 750 µL/L) prolonged nymph duration, decreased adult longevity, female fecundity and protein content, and increased the contents of total lipid, soluble sugar and glycogen. There was a significant interaction between the effect of CO₂ levels and the effect of generations on nymph duration, female fecundity and adult longevity. The elevated CO₂ had immediate effects on the female fecundity and the contents of total protein, total lipid and soluble sugar, starting within F₀ generation. The adult longevity decreased, and the glycogen content increased from the F₁ generation. However, the significant effect on the nymph development was only observed after three generations. Our study indicates that the elevated CO₂ levels first influence the reproduction, the nutrition and the energy supply, then initiate aphid emergency responses by shortening lifespan and increasing glucose metabolism, and finally result in the slow development under further persistent elevated CO₂ conditions after three generations, possibly leading to population decline under elevated CO₂ conditions. Our results will guide further field experiments under climate change conditions to evaluate the effects of elevated CO₂ on the development of the pea aphids and other insects, and to predict the population dynamics of the green pea aphid.

Keywords: Acyrthosiphon pisum, elevated CO₂, generation, development, nutrition

INTRODUCTION

A rise in atmospheric carbon dioxide (CO₂) concentration is the most conspicuous characteristics of global climate change in this century (Michael, 2019). CO₂ concentration is predicted to continue to increase from the current 400 ppm to between 750 and 1,300 ppm by the end of this century (IPCC, 2014). The elevated CO₂ will not only accelerate the process of global warming, sea level...
The effects of elevated atmospheric CO$_2$ concentration on plant nutritional and defensive chemistry, and their consequent effects on insect have received extensive attention (DeLucia et al., 2012; Facey et al., 2014). In particular, unprecedented increases in atmospheric CO$_2$ have the capacity to change plant chemistry, thus, impacting plant nutrients and leading to changes in the synergistic relationship between insects and plants (Robinson et al., 2012; Zavala et al., 2013; Ode et al., 2014). For example, the effect of elevated CO$_2$ on the physiological metabolism of aphid host plant alfalfa (Medicago sativa L.) has been shown previously (Sun et al., 2019), which discovered that the concentrations of soluble protein, soluble carbohydrate and starch were higher when plants grown under elevated CO$_2$, also the concentration of flavone, total polyphenols and simple phenols increased significantly in alfalfa. The concentrations of phenolics, terpenoids, condense tannins, and gossypol were increased in Gossypium hirsutum by elevated CO$_2$ (Coviella et al., 2002). The elevated CO$_2$ mediated the decrease of plant N content resulting in a nutritional deficiency for protein-limited insect pests (Mattson, 1980; Coviella and Trumble, 1999) and reduced fecundity and fitness of most leaf-chewing insects (Coll and Hughes, 2008). The CO$_2$-mediated lower nitrogen content and higher C: N ratio and thus a decrease nutritional quality of Zea mays caused a significant decline in the survival and weight gain as well as larval food consumption of Ostrinia furnacalis (Xie et al., 2015). The growth of Lymantria dispar larvae was significantly inhibited by elevated CO$_2$ and CO$_2$-induced changes in quality of leaves of Populus pseudosimonii and Betula platyphylla (Li et al., 2011). Similarly, the population density and body mass of the vine weevils (Otiorynchus sulcatus) decreased under elevated CO$_2$ (Johnson et al., 2011). Johnson et al. (2020) reported that the elevated CO$_2$ accelerated the growth rates of the cotton bollworm (Helicoverpa armigera). Furthermore, insect responses to rising CO$_2$ are not uniform. Robinson et al. (2012) found that while Lepidoptera populations decreased with elevated CO$_2$, the populations of other groups such as Homoptera and Acari actually increased.

Wu et al. (2006) found that the elevated CO$_2$ concentration (750 µL/L) decreased the protein and total amino acid contents of Helicoverpa armigera. Similarly, the study on the larvae of Stegobium panicum and Lasioderma serricorne showed that the contents of polysaccharide, soluble protein and lipids in the larvae as well as the utilization rate of the larvae decreased with increasing CO$_2$ concentration (Cao et al., 2006). Some studies suggested that the elevated CO$_2$ concentration can increase the relative feeding rate, prolong the development time, decrease the relative growth rate and pupa weight in chewing mouthparts insects (Bidart-Bouzat and Imeth-Nathaniel, 2008; Massad and Dyer, 2010). For example, the larva-to-adult emergence survival rate of Cnaphalocrocis medinalis decreased by 44.0%, and the egg hatching rate reduced by 26.8% under the elevated CO$_2$ as compared to the ambient CO$_2$ (Li et al., 2013). Aphids are considered to be the most important group of insect pests in temperate regions because of its rapid parthenogenic reproduction and short life cycle. Hughes and Bazzaz (2001) investigated interactions between five species of phloem-feeding aphids (Homoptera: Aphididae) at elevated CO$_2$ (700 µL/L) compared to the ambient CO$_2$ (350 µL/L), and found that Acyrthosiphon pisum population were reduced by over 60% at elevated CO$_2$, in contrast, Myzus persicae populations increased by 120% at elevated CO$_2$, but the CO$_2$ treatment did not significantly affect the populations of the remaining three species (Aphis nerii, Aphis oenotherae, Aulacorthum solani). Also, under elevated CO$_2$, winged aphid adults were produced in all the aphid species except Myzus persicae, but the proportion of winged to wingless was not affected by CO$_2$ levels. The pea aphid, A. pisum Harris, is a worldwide pest that feeds on leguminous crops and forages grasses such as peas, dry beans, alfalfa, clover and fresh beans (Harrington, 1945). Its rapid parthenogenic reproduction and short life cycle enable them to very quickly cause serious economic and production losses of host crops (Loisy and Eubanks, 2000; Ryalls et al., 2013). In addition to the direct feeding damage, the pea aphids also transmit 25 types of viruses, including Alfalfa mosaic virus and Pea enation mosaic virus (Makkouk, 1988). In previous studies, many factors that affect the growth and development of the pea aphid were investigated from different perspectives, including temperature (Hubbacher et al., 2018), photoperiod (Miquel et al., 2019), symbiont (Lv et al., 2018), genetics (Caillaud and Losey, 2010; Wang et al., 2012), and host plant quality (Kordan et al., 2018). Among them rising global CO$_2$ concentrations has become an important climatic factor affecting pea aphid growth, for example, elevated CO$_2$ concentrations altered plant phenolics and thus the performance of aphids (Hong et al., 2020). Studies found that the aphids grown at 380 ppm CO$_2$ had the longest pre-reproductive period and the aphids grown at 1,050 ppm CO$_2$ had the highest intrinsic rate of natural increase (Amiri-jami et al., 2012). Li and Liu (2017) found that developmental duration of three consecutive generations of green pea aphids was shortened, while larval weight, adult weight, weight difference and mean daily relative growth rate all displayed increasing trends under elevated (750 µL/L) CO$_2$. However, the biochemistry and genetic bases of the long-term effect of elevated CO$_2$ on aphid development and nutrients are not investigated.

The objectives of this study are to analyze the independent and interactive effects of two elevated CO$_2$ levels on the development and nutrition content of the pea aphids over six generations. The aim of this study is to provide new and comprehensive insights into the function of CO$_2$ as well as its potential role in control strategies of pea aphids in agricultural systems.

**MATERIALS AND METHODS**

**Insects and CO$_2$ Treatment**

The stock colony of the green pea aphid Acyrthosiphon pisum Harris was from a single parthenogenetic female collected from the alfalfa (Medicago sativa L) field in Experimental Station of
the Gansu Agricultural University, Lanzhou, Gansu Province, China. Fresh and clean alfalfa leaves were placed on the filter paper in 9 cm petri dishes with the back facing up and moist absorbent cotton around the petioles and leaf edges to ensure the leaves fresh. Several small holes were drilled on the lid of the petri dish to facilitate the passage of CO₂. Three levels of CO₂, i.e., ambient CO₂ (~380 µL/L) and elevated CO₂ (550 and 750 µL/L), were applied continuously to the petri dishes with the aphids in separate rearing chambers. The CO₂ levels were chosen based on the current and predicted levels of CO₂ in future years, respectively (Watson et al., 1996).

**Determination of Aphid Development for Six Generations**

Twenty newly emerged (<6 h) nymph from the aphid stocks were individually placed into a previously prepared petri dish and treated with one of the three CO₂ levels for more than 30 days in a rearing chamber. The broad alfalfa leaves were changed every 3 days during the experiment. The observations were made daily at 8 h intervals until the adults died. Then another 20 nymphs (<6 h) in the petri dishes were individually transferred to new petri dishes with fresh leaves and kept for next run of the treatments and observations. This procedure was repeated six times thus six generations (F₀–F₅) were examined under each CO₂ level. This made 20 replicates for each treatment at each generation. Each treatment was repeated three times for each CO₂ level. A total of 1,080 nymphs (20 nymphs × 3 treatments × 6 generations × 3 replicates) was used.

The duration of each nymphal stage and the molting during the nymph stages in each petri dish were recorded daily at 8 h intervals until the adults died. The number of nymphs produced per aphid and the adult longevity were recorded when the adults died. Nymphal duration was between hatching and adult emergence.

**Sample Preparation for Biochemistry Measurements**

A total of 135 adult aphids was collected from each generation for the detections of total protein, soluble sugar, glycogen and lipid under the influence of CO₂. Briefly, five adult aphids from each generation were placed into a 1.5 mL centrifuge tube containing 200 µL of the lysis buffer solution (100 mM KH₂PO₄, 1 mM ethylenediaminetetraacetic acid, and 1 mM dithiothreitol, pH 7.4). The aphids were homogenized and then centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was then used as one biological sample for the nutritional detections of total protein, sugar, glycogen and lipid. 20 µL of the biological sample was used for the protein content measurement and another 160 µL was used for the measurements of lipids, soluble sugar and glycogen content. This biological sample preparation of biochemistry measurements was repeated for nine times for each of three CO₂ treatments (380, 550, and 750 µL/L).

**Determination of Total Protein Content**

The total protein content of the aphids was detected according to the method of Lowry et al. (1951). About 20 µL of the biological sample was transferred into a 96-well microplate and mixed with of 200 µL Coomassie brilliant blue G-250 for 15 min at room temperature. The optical density (OD) values were measured at 595 nm. Bovine serum albumin was dissolved in the same buffer and diluted into a series of concentrations, which was used as the standard. The total protein contents were calculated based on the standard curve of bovine serum albumin. The experiment was repeated nine times.

**Sample Preparation for the Measurements of Total Lipid, Soluble Sugar, and Glycogen Content**

Briefly, 160 µL of the biological sample was transferred into a 2 mL centrifuge tube, and 20 µL of 20% sodium sulfate solution (Na₂SO₄; Sigma) was added, then mixed with 1,500 µL of a chloroform–methanol solution (1:2v/v). This mixture was then centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was used for the nutritional detection of lipids and soluble sugar, and the precipitant was used for the glycogen content measurement. The preparation was prepared nine times for each CO₂ treatment.

**Determination of Total Lipids Content**

The total lipid content was detected according to the methods of Handel (1985). Briefly, about 100 µL of the supernatant was transferred into a 1 mL centrifuge tube and heated at 90°C until solvent was completely evaporated. 10 µL of 98% concentrated sulfuric acid was then added to the tube and incubated at 90°C for 2 min in a water bath. After cooling the samples on ice, 190 µL of 1.2 g/L vanillin reagent was added and reacted for 15 min at room temperature. The OD value was measured at 525 nm, and the total lipid content was calculated based on the standard curve of triolein. The experiment was repeated nine times.

**Determination of Soluble Sugar**

The soluble sugar and glycogen were determined according to the methods of Handel (1965) and Handel and Day (1988). To detect the soluble sugar, 150 µL of the supernatant was transferred into a 1.5 mL centrifuge tube and completely evaporated it at room temperature. 10 µL of distilled water and 240 µL of anthrone reagent were added, kept for 15 min at room temperature and then incubated in boiling water for a further 15 min, followed by cooling at room temperature, then added to a 96-well microplate. The OD value at the 630 nm wavelength was measured. The soluble sugar content was calculated based on the standard curve of D-glucose. The experiment was repeated nine times.

**Determination of Glycogen Content**

The precipitant from pre-preparation was mixed with 1,200 µL of the biological sample was transferred into a 96-well microplate and mixed with of 200 µL Coomassie brilliant blue G-250 for 15 min at room temperature. The optical density (OD) values were measured at 595 nm. Bovine serum albumin was dissolved in the same buffer and diluted into a series of concentrations, which was used as the standard. The total protein contents were calculated based on the standard curve of bovine serum albumin. The experiment was repeated nine times.
was calculated using D-glucose as standard. The experiment was repeated nine times.

**Statistical Analyses**

All data were analyzed with the IBM SPSS Statistics version 23.0 for Windows (Chicago, IL, United States). A two-way ANOVA was conducted to examine the effects of CO$_2$ level, generation and the interaction between the CO$_2$ level and the generation on the physiological parameters (nymphal duration, adult longevity, female fecundity and nutrition contents) followed by Tukey’s HSD test was applied to determine differences specific treatments. The general linear model (GML) procedure of SAS was used to compare the means there is a significant interaction effects between generation and CO$_2$ level is identified. Duncan’s Multiple Range Test to compare the means of the physiological parameters if the interaction effect is not significant.

**RESULTS**

**There Is a Significant Interaction Between CO$_2$ Levels and Generation**

There was a statistically significant interaction between the effects of CO$_2$ level and generation on the nymph duration ($F = 36.3; df = 10; p < 0.001$; Table 1). This indicates that the effect CO$_2$ levels on nymph duration is dependent on the generation, and the nymph duration at each generation is dependent on the CO$_2$ levels. Similarly, significant interactions were found for the measurements of adult longevity ($F = 13.1; df = 10; p < 0.001$), female fecundity ($F = 17.3; df = 10; p < 0.001$), protein content ($F = 20.3; df = 10; p < 0.001$) and sugar content ($F = 9.5; df = 10; p < 0.001$; Table 1). So, to interpret the effect of CO$_2$ levels and generations without inference of each other, one-way ANOVA followed by Tukey’s HSD test was used to separately analyze the effect of CO$_2$ level at each generation and the effect of generation under each CO$_2$ level on each of the performance indices.

**A. pisum Nymph Duration Is Increased by Elevated CO$_2$**

Figure 1A shows the Tukey’s HSD test results of the effect of CO$_2$ levels on the nymph duration at each generation. It revealed that the nymph duration was shortened at F$_0$ generation by the elevated CO$_2$ levels [$F(2, 6) = 9.5, p = 0.014$; Figure 1A]. The nymph duration at F$_1$ generation was not significantly affected by the elevated CO$_2$ levels. It was significantly increased by the elevated CO$_2$ levels only after F$_3$ generation [$F(2, 6) = 12.0, p = 0.008$; Figure 1A]. At F$_5$ generation, the nymph duration was increased significantly by the elevated CO$_2$ levels from 7.3 ± 0.2 days at 380 µL/L to 10.7 ± 0.5 days at 550 µL/L and 13.1 ± 0.7 days at 750 µL/L [$F(2, 6) = 91.5, p < 0.001$; Figure 1A].

Figure 1B shows the Tukey’s HSD test results of the effect of generations on the nymph duration at each CO$_2$ level. Under the ambient CO$_2$ level 380 µL/L, the nymph duration was not changed and kept a similar length of 7.4 ± 0.3 days throughout 6 generations (Figure 1B). Under elevated CO$_2$, the nymph duration had a significant increase at F$_4$ and F$_5$ generations [$F(5, 12) = 38.3, under 550 µL/L CO$_2$ level, p < 0.001; F(5, 12) = 135.5 under 750 µL/L CO$_2$ level, p < 0.001].

**A. pisum Adult Longevity Is Decreased by Elevated CO$_2$**

The adult longevity was not affected by CO$_2$ levels and remained constant of 20.6 ± 0.8 days at F$_0$ generation (Figure 2A). It was decreased significantly from F$_1$ generation by elevated CO$_2$ levels and reduced from 21.8 ± 0.2 days at 380 µL/L to 15.7 ± 0.6 days at 550 µL/L and 12.3 ± 1.2 days at 750 µL/L at F$_3$ generation [$F(2, 6) = 111.3, p < 0.001$; Figure 2A]. Similarly, the adult longevity also remained constant days under the ambient CO$_2$ level (380µL/L) over the generations (Figure 2B). However, it started to decrease significantly under the elevated CO$_2$ levels. Under the elevated CO$_2$ level of 750 µL/L, the adult longevity was significantly reduced to 12.3 ± 1.2 days by F$_5$ generation [$F(5, 12) = 22.8, p < 0.001$; Figure 2B].

**A. pisum Fecundity Is Significantly Reduced by Elevated CO$_2$**

At F$_0$ generation, the number of nymphs per female was not affected significantly by 550 µL/L CO$_2$ level but significantly reduced by 750 µL/L CO$_2$ level (Figure 3A). At F$_1$ generation, the reduction of the number of nymphs per female became more obvious under the elevated CO$_2$ levels (Figure 3A). By F$_5$ generation, the number of nymphs per female was reduced significantly from 68.4 ± 2.4 under the ambient CO$_2$ level to 15.5 ± 0.5 under 550 µL/L CO$_2$ level and to 8.5 ± 3.3 under 750 µL/L CO$_2$ level [$F(2, 6) = 558.2, p < 0.001$; Figure 3A]. There was no reduction in the number of nymphs per female between generations under the ambient CO$_2$ level 380 µL/L (Figure 3B). Significant reduction of the number of nymphs per female was observed between generations under elevated CO$_2$ levels [$F(5, 12) = 58.9 under 550 µL/L CO$_2$ level, p < 0.001; F(5, 12) = 30.9 under 750 µL/L CO$_2$ level, p < 0.001] when the effect of CO$_2$ levels was analyzed (Figure 3B).

**A. pisum Total Protein Content Is Reduced Under Elevated CO$_2$**

The elevated CO$_2$ levels significantly reduced the total protein contents even at F$_0$ generation [$F(2, 24) = 127.6, p < 0.001$; Table 2 and Supplementary Figure 1A]. The total protein content under the ambient CO$_2$ was not affected throughout the generations (Table 2 and Supplementary Figure 1B). Under the elevated CO$_2$ level of 550µL/L, the total protein contents were significantly reduced from 19.7 ± 0.6 µg/aphid at F$_0$ generation to 13.6 ± 0.5 µg/aphid at F$_3$ generation [$F(5, 48) = 176.9, p < 0.001$]. Under the elevated CO$_2$ level of 750 µL/L, it was reduced from 16.6 ± 0.6 µg/aphid at F$_0$ generation to 12.5 ± 0.8 µg/aphid at F$_3$ generation and reached 11.4 ± 0.9 µg/aphid at F$_5$ generation [$F(5, 48) = 61.9, p < 0.001$; Table 2 and Supplementary Figure 1B].

**A. pisum Total Lipid Content Is Not Affected Under Elevated CO$_2$**

The total content of total lipids was significantly increased even at F$_0$ generation by both elevated CO$_2$ levels [$F(2, 24) = 5.4,
| Measurement          | Type III sum of squares | df | Mean square | F-value | p-value |
|----------------------|-------------------------|----|-------------|---------|---------|
| Nymph duration       | 42.4                    | 10 | 4.2         | 36.3    | 0.000   |
| Adult longevity      | 80.9                    | 10 | 8.1         | 13.1    | 0.000   |
| Fecundity            | 2,322.6                 | 10 | 232.3       | 17.3    | 0.000   |
| Protein              | 134.5                   | 10 | 13.5        | 20.3    | 0.000   |
| Total lipid          | 2.3                     | 10 | 0.2         | 0.6     | 0.787   |
| Soluble sugar        | 72.5                    | 10 | 7.4         | 9.5     | 0.000   |
| Glycogen             | 1.1                     | 10 | 0.1         | 1.2     | 0.308   |

**FIGURE 1** | Effects of CO$_2$ and generations on the nymph duration of *Acyrthosiphon pisum*. (A) Comparison of the effect of CO$_2$ levels (380, 550, and 750 µL/L) at each of six generations (F$_0$–F$_5$). (B) Comparison of the effect of generations under each of CO$_2$ levels (380 µL/L, elevated 550 and 750 µL/L). Different lowercase letters indicate significant differences between CO$_2$ levels at each generation (A) and between generations at each CO$_2$ level (B) as determined by one-way ANOVA Tukey's HSD test at $p < 0.05$.

$p = 0.012$; **Table 2** and **Supplementary Figure 2A**. At F$_5$ generation, it was increased from 6.4 ± 0.7 µg/aphid under 380 µL/L level to 7.9 ± 0.5 µg/aphid under 550 µL/L level and to 8.0 ± 0.6 µg/aphid under 750 µL/L level [$F(2, 24) = 17.2$, $p < 0.001$]. The total content of lipids was unchanged over the generations under the ambient and elevated CO$_2$ levels (**Table 2** and **Supplementary Figure 2B**). The total lipid contents increased by 14.9–23.4% and 13.8–25.0% across generations under the elevated CO$_2$ levels of 550 and 750 µL/L, respectively, relative to those under the ambient CO$_2$ level.
**A. pisum Sugar Content Is Increased Under Elevated CO₂**

The content of soluble sugar and glycogen were significantly increased at each generation (Table 2 and Supplementary Figures 3A, 4A) but were not affected by the elevated CO₂ levels over generations (Table 2 and Supplementary Figures 3B, 4B). The significant increase was observed at F₀ generation for the soluble sugar content from 25.4 ± 0.6 µg/aphid at 380 µL/L level to 33.7 ± 1.0 µg/aphid by 550 µL/L level and to 36.8 ± 0.5 µg/aphid by 750 µL/L level \([F(2, 24) = 557.8, p < 0.001]\). At F₅ generation, the soluble sugar was increased from 25.6 ± 0.4 µg/aphid under the ambient CO₂ to approximately 40.3 ± 0.5 µg/aphid by the elevated CO₂ 750 µL/L (Table 2).

Under both elevated CO₂ levels, the content of soluble sugar was slightly and significantly increased after F₂ generation under 550 µL/L level \([F(5, 48) = 24.8, p < 0.001]\) and after F₄ generation under 750 µL/L level \([F(5, 48) = 26.9, p < 0.001; \text{ Table 2 and Supplementary Figure 3B}].

**A. pisum Glycogen Content Is Significantly Increased Under Elevated CO₂**

The glycogen content was significantly increased from F₁ generation (Table 2 and Supplementary Figure 4A). It was significantly increased from 2.0 ± 0.1 µg/aphid at 380 µL/L level to 2.6 ± 0.3 µg/aphid by 750 µL/L \([F(2, 24) = 5.7, p = 0.010]\). At F₅ generation, the glycogen content increased from 2.0 ± 0.1 µg/aphid under the ambient CO₂ to approximately 2.8 ± 0.1 µg/aphid by the elevated CO₂ 750 µL/L (Table 2). The glycogen content was not affected by the elevated CO₂ levels over
Li et al. CO₂ Affected on Acyrthosiphon pisum

FIGURE 3 | Effects of CO₂ and generations on the female fecundity of Acyrthosiphon pisum. (A) Comparison of the effect of CO₂ levels (380, 550, and 750 µL/L) at each of six generations (F₀–F₅). (B) Comparison of the effect of generations under each of CO₂ levels (380 µL/L, elevated 550 and 750 µL/L). Different lowercase letters indicate significant differences between CO₂ levels at each generation (A) and between generations at each CO₂ level (B) as determined by one-way ANOVA Tukey’s HSD test at p < 0.05.

generations even under the higher elevated CO₂ level 750 µL/L (Table 2 and Supplementary Figure 4B).

DISCUSSION

The current study reports the long-term effects of elevated CO₂ levels on the development and nutritional dynamics of the pea aphid A. pisum over six generations. It confirms that the pea aphid is well adapted to the current environmental CO₂ level as all seven examined physiological parameters were not affected over 6 generations under the ambient CO₂ level. However, the elevated CO₂ levels (550 and 750 µL/L) prolonged the nymph duration (Figure 1), decreased the adult longevity, the female fecundity and the protein content (Figures 2, 3 and Table 2), and increased the contents of total lipid, soluble sugar and glycogen (Table 2). The elevated CO₂ had an immediate effect on the female fecundity and the contents of total protein, total lipid and sugar, starting within F₀ generation. In the current study, only total protein and total lipid contents were analyzed. It is not known whether or not they are influenced by the changes of vitellogenin and triacylglyceride. Triacylglycerols is a major lipid in aphid fat body as energy reserves (Ward et al., 1981) and accumulated in aphid fat body under stress (Bergman et al., 1991; Chen et al., 2005). Vitellogenin is a major yolk protein (Arrese and Soulages, 2010) and directly regulates egg maturation and affect ovary development and decrease ovulation (Ge et al., 2019; Huang et al., 2019).

The adult longevity decreased, and the glycogen content increased from F₁ generation. However, the significant effect on the nymph development was only observed after three generations (Figure 1A). Furthermore, the interactions between the CO₂ levels and the generations significant influenced nymph
duration, female fecundity and adult longevity. The biggest effect of CO₂ was found on the female fecundity by the generations (Figure 3A), indicating that the elevated CO₂ may not be conducive to the reproduction of the aphids. The results are consistent with that of the study in Cnaphalocrocis medinalis, where elevated CO₂ reduced the survival rate from larva to adult emergence by 44.0% compared with ambient CO₂ (Li et al., 2013). Docherty et al. (1997) also reported a significantly reduced fecundity in Phylaliopsis fagi under the elevated CO₂ level of 600 µL/L. However, it was reported that elevated CO₂ levels could increase Myzus persicae population (Hughes and Bazzaz, 2001) and Nilaparvata lugens population (Xiao et al., 2011). It is possible that the elevated CO₂ levels first influence the reproduction, the energy supply and the nutrition status, and then initiation of shortening lifespan and increasing glycogen transition to glucose for emergency escaping, and finally result in the slow development of the pea aphid under persistent elevated CO₂ conditions, possibly leading to population decline under elevated CO₂ conditions.

Insects store nutrition in the form of proteins, carbohydrates, glycogen and lipids (Ahsaei et al., 2014). Nutrition storage in insects has significant implications for their survival and reproduction (Makkouk, 1988). Our results show a significant reduction of protein content by the elevated CO₂ levels (Table 2). This is consistent with previous reports that the fertility of the pea aphid was closely related to the protein contents in the body (Ahsaei et al., 2013). For example, the reproduction ability of the pea aphids was positively proportional to its protein content: the greater protein content, the higher the reproduction ability (Raikhel and Dhadialla, 1992). The protein content of Nilaparvata lugens declined under elevated CO₂, speculated that it would be difficult for it to obtain nutrients and energy after feeding on rice which grown under high CO₂ concentrations (Zeng et al., 2012). Similarly, elevated CO₂ decreased the protein content of Helicoverpa armigera larvae by 14.16% (Wu et al., 2006). Therefore, we propose that the elevated CO₂ inhibited protein synthesis in the aphids, and directly led to the reduction of protein contents, thus further caused the longer nymph duration and lower female fecundity of the pea aphids in elevated CO₂ levels than those of the aphids under the ambient CO₂.

The total lipid and soluble sugar contents were significantly increased by the elevated CO₂ levels at F₀ generation (Table 2). Interestingly, over the generations, they were not significantly changed under the elevated levels of CO₂, neither generation nor its interaction with CO₂ level had a significant effect on them (Table 2 and Supplementary Figures 2B, 3B). However, the total lipid and soluble sugar contents were significantly higher under the elevated CO₂ levels than those under the ambient CO₂ condition at each generation (Table 2 and Supplementary Figures 2A, 3A). The function of soluble sugar is to offer nutrition for the energy requirement of the muscles when an insect is walking or escaping (Hansford and Johnson, 1975). These increased responses to elevated CO₂ could be the initiation for their escaping from unsuitable environment. Our study results show that glycogen content increased with the elevated CO₂ concentration in the pea aphid, which could reduce the feeding rate of the aphids, thus longer nymph duration and reduced adult fecundity under elevated CO₂ than that under the ambient CO₂.

On the other hand, elevated CO₂ can lead to the changes of plant secondary metabolism, thus affect the insect physiology indirectly (Bidart-Bouzat and Imeth-Nathaniel, 2008; Todgham and Stillman, 2013). The soluble sugars in the aphid body are mainly from the sap of the phloem of the host plant (Li et al., 2008). Elevated CO₂ could enhance plant biomass and leaf area, so soluble sugar levels increase, leading to enhance the behavior of the pea aphids (Curtis and Wang, 1998). The reduced metabolic efficiency of plants under elevated CO₂ could weaken the feeding of insect herbivores and slow the growth of insects (Vuurinen et al., 2004). Elevated CO₂ could also increase the concentrations of various phenolic compounds, which would act to reduce the pest population and affect natural conditions.
enemies (Koricheva et al., 1998). For example, the content of flavone, phenolics and condensed tannins and gossypol were higher in the aphid host plant the alfalfa, *Medicago sativa* (L.) grown under elevated CO₂ (Sun et al., 2019). Carbohydrate content in plants increased with elevated atmospheric CO₂ due to higher photosynthetic rates (Reddy et al., 2010; Kimball, 2016). Elevated CO₂ increased carbon to nitrogen ratio of plant tissues (Wilsey, 1996). This causes lower nitrogen concentrations in plants and leads to less amino acids available to aphids (Guo et al., 2013b; Ziska et al., 2016). It is widely recognized that the reproductive ability and abundance of aphids are largely determined by the availability of amino acids in their diet (Janson and Smilowitz, 1986), and the nutritional quality and resistance of host plants (Guo et al., 2014).

In the current study, the significant effects of the elevated CO₂ on the growth of the aphids fed with alfalfa leaves were studied for over 6 generations, which is longer than previous studies of 3 generations. Our study clearly demonstrates that the elevated CO₂ affect some parameters such as nymph developmental duration after 3 generations and shows that the final consequence of the elevated CO₂ on aphid population dynamics is combinatory and long time. The results will guide further field experiments to evaluate the effects of the elevated CO₂ conditions on the development of the pea aphids and other insects under climate change conditions.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

**ETHICS STATEMENT**

Informed consent was obtained from all individual participants included in the study. The research project was conducted on invertebrate species that are not subjected to any specific ethical issue and legislation. 

**AUTHOR CONTRIBUTIONS**

CuL, QS, and CaL conceived and designed research. CuL, QS, and J-JZ conducted the experiments. YG and KZ contributed new reagents and analytical tools. CuL and J-JZ analyzed the data. CuL and CaL wrote the manuscript. J-JZ made critical revision, proofreading, and replying comments. All authors read and approved manuscript.

**FUNDING**

This work was financially supported by the National Science Foundation of China (31960351, 31660522, and 31960227) and Program of Introducing Talents to Chinese Universities (111 Program No. D20023) to J-JZ.

**ACKNOWLEDGMENTS**

We would like to thank all of the participants who volunteered their time in the study. We appreciate very much the valuable comments and suggestion by the reviewers and editors.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.688220/full#supplementary-material

**REFERENCES**

Ahsaei, S. M., Hosseininaveh, V., Talaei-Hassanlouei, R., and Bigham, M. (2014). Changes in energy content of *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) in response to different diets. *Arthropods* 3, 166–173.

Ahsaei, S. M., Tabadkani, S. M., Hosseininaveh, V., Allahyari, H., and Bigham, M. (2013). Differential accumulation of energy by the colour morphs of the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae) mirrors their ecological adaptations. *Eur. J. Entomol.* 110, 241–245. doi: 10.14411/eje.2013.035

Amiri-jami, A. R., Sadeghi, H., and Shoore, M. (2012). The performance of *Brevicoryne brassicae* on ornamental cabbages grown in CO₂-enriched atmospheres. *J. Asia Pac. Entomol.* 15, 249–253. doi: 10.1016/j.aspen.2012.01.001

Arrese, E. L., and Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.* 55, 207–225. doi: 10.1146/annurev-ento-112408-085356

Bergman, D. K., Dillwith, J. W., and Berberet, R. C. (1991). Spotted alfalfa aphid, *Theroaphis maculata*, fatty acids relative to the condition and susceptibility of its host. *Arch. Insect Biochem. Physiol.* 18, 1–12. doi: 10.1002/arch.940180102
transgenic cotton plants. J. Exp. Bot. 53, 323–331. doi: 10.1093/jexbot/53.367.323

Coviella, C. E., and Trumble, J. T. (1999). Effects of elevated atmospheric carbon dioxide on insect-plant interactions. Conserv. Biol. 13, 700–712. doi: 10.1046/j.1523-1739.1999.98267.x

Curts, P. S., and Wang, X. (1998). A meta-analysis of elevated CO2 effects on woody plant mass, form, and physiology. Oecologia 113, 299–313. doi: 10.1007/s004420050381

DeLucia, E. H., Nabit, P. D., Zavala, J. A., and Berenbaum, M. R. (2012). Climatic change: resetting plant–insect interactions. Plant Physiol. 160, 1677–1685. doi: 10.1104/pp.112.204750

Docherty, M., Wade, F., Hurst, D., Whitaker, J., and Lea, P. (1997). Responses of tree sap-feeding herbivores to elevated CO2. Glob. Change Biol. 3, 51–59. doi: 10.1046/j.1365-2486.1997.00096.x

Dury, S. J., Good, J. E. G., Perrins, C. M., Buse, A., and Kaye, T. (1998). The effects of increasing CO2 and temperature on oak leaf palatability and the implications for herbivorous insects. Glob. Change Biol. 4, 55–61. doi: 10.1046/j.1365-2486.1998.00102.x

Facy, S. L., Ellsworth, D. S., Staley, W. T., and Johnson, S. N. (2014). Upsetting the order: how climate and atmospheric change affects herbivore–enemy interactions. Curr. Opin. Insect Sci. 5, 66–74. doi: 10.1016/j.cois.2014.09.015

Ge, L. Q., Zheng, S., Gu, H. T., Zhou, Y. K., Zhou, Z., Song, Q. S., et al. (2019). Jingjiangmycin-induced UDP-glycosyltransferase 1-2 is a positive regulator of fecundity and population growth in Nilaparvata lugens (Stål) (Hemiptera: Delphacidae). Front. Physiol. 10:1747. doi: 10.3389/fphys.2019.00747

Guo, H., Liu, X. M., Guo, X. L., Liu, Z., Ma, M. M., Lyu, B., et al. (2013a). A preliminary analysis on the relationship between CO2 concentrations and global climate change. J. Subtrop. Resour. Environ. 8, 1–13

Guo, H., Sun, Y., Li, Y., Liu, X., Zhang, W., and Ge, F. (2014). Elevated CO2 decreases the response of the ethylene signaling pathway in Medicago truncatula and increases the abundance of the pea aphid. New Phytol. 201, 279–291. doi: 10.1111/nph.12484

Guo, H. Y., Sun, Y., Li, B., Tong, M., Harris, K., Zhu-Salzman, K., et al. (2013b). Pea aphid promotes amino acid metabolism both in Medicago truncatula and bacteriocyes to favor aphid population growth under elevated CO2. Glob. Change Biol. 19, 3210–3223. doi: 10.1111/gcb.12260

Handel, V. E. (1965). Microseparation of glycogen, sugars, and lipids. Anal. Biochem. 11, 266–271. doi: 10.1016/0003-9861(65)90014-X

Handel, V. E. (1985). Rapid determination of total lipids in mosquitoes. J. Am. Mosq. Control Assoc. 1, 302–304.

Handel, V. E., and Day, J. (1987). Relationships of lipids, glycogen and sugars in individual mosquitoes: correlations with wing length in field-collected Aedes vexans. J. Am. Mosq. Control Assoc. 4, 549–550.

Hansford, R. G., and Johnson, R. N. (1975). The nature and control of the tricarboxylic cycle in beetle flight muscle. Biochem. J. 148, 389–401. doi: 10.1042/bj1480389

Harrington, C. D. (1945). Biological races of the pea aphid. J. Econ. Entomol. 38, 12–22. doi: 10.1093/jee/38.1.12

Hong, Y. Y., Hong, G. G., Yu, C. S., and Feng, G. (2020). Plant phenolics modulate bottom-up effects of elevated CO2 on Acyrthosiphon pisum and its parasitoid Aphidius ervi. Insect Syst. Ecol. 77, 174–197. doi: 10.1111/1744-7917.13247

Huang, B., Shi, Z. H., and Hou, Y. M. (2019). Tradeoff between triglyceride synthesis and oocytes. J. Integr. Agric. 18, 865–872. doi: 10.1016/j.jia.2019.03.005

Hubbahren, Z., Madden, R. D., and Dillworth, J. W. (2018). Influence of rearing temperature on triacylglycerol storage in the pea aphid, Acyrthosiphum pisum. Arch. Insect Biochem. Physiol. 99:e21495. doi: 10.1002/arch.21495

Hughes, L., and Bazzaz, F. A. (2001). Effects of elevated CO2 on five plant-aphid interactions. Entomol. Exp. Appl. 99, 87–96. doi: 10.1046/j.1570-7458.2001.00805.x

IPCC (2014). Contribution of Working Groups I, II and III to the 5th Assessment Report of the Intergovernmental Panel on Climate Change. Climate Change 2014: Synthesis Report. Geneva: IPCC.
Reddy, A. R., Rasineni, G. K., and Raghavendra, A. S. (2010). The impact of global elevated CO$_2$ concentration on photosynthesis and plant productivity. *Curr. Sci.* 99, 46–57.

Robinson, E. A., Ryan, G. D., and Newman, J. A. (2012). A meta-analytical review of the effects of elevated CO$_2$ on plant-arthropod interactions highlights the importance of interacting environmental and biological variables. *New Phytol.* 194, 321–336. doi: 10.1111/j.1469-8137.2012.04074.x

Rylls, J. M. W., Riegler, M., Moore, B. D., and Johnson, S. N. (2013). Biology and trophic interactions of lucerne aphids. *Agric. For. Entomol.* 15, 335–350. doi: 10.1111/afe.12024

Sun, Q., Zhang, T. W., Wei, J. Y., Lyu, Y. Q., and Liu, C. Z. (2019). Effects of elevated CO$_2$ concentration on nutrients and secondary metabolites in *Medicago sativa* leaf under different damage degrees of pea aphid (*Acyrthosiphon pisum*, Hemiptera: Aphididae). *Chin. J. Eco Agric.* 27, 92–99.

Ward, J. P., Candy, D. J., and Smith, S. N. (1981). Lipid storage and changes during flight by triatomine bugs (*Rhodnius prolixus* and *Triatoma infestans*). *J. Insect Physiol.* 28, 527–534. doi: 10.1016/0022-1910(82)90033-6

Wu, G., Chen, F. J., and Ge, F. (2006). Direct effects of elevated CO$_2$ on growth, development and reproduction of cotton bollworm *Helicoverpa armigera* Hübner. *Acta Ecol. Sin.* 26, 1732–1738.

Xiao, N. C., Wei, H., Neng, W. X., Jun, S. L., Lan, Z. H., and Fa, J. C. (2011). Effects of elevated CO$_2$ and transgenic Bt rice on yeast-like endosymbiote and its host brown planthopper. *J. Appl. Entomol.* 135, 333–342. doi: 10.1111/j.1439-0418.2010.01558.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.